

## OPEN ACCESS

## EDITED BY

Yasmeen Siddiqui,  
Universiti Putra Malaysia, Malaysia

## REVIEWED BY

Bin Li,  
Zhejiang University, China  
Dixit Sharma,  
Central University of Himachal Pradesh, India

## \*CORRESPONDENCE

Chun Xiao  
✉ x.chun@gmail.com  
Lingyun Zhou  
✉ hncyszly@hunaas.cn  
Xiaoping Qin  
✉ qxp99@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 02 April 2023

ACCEPTED 15 August 2023

PUBLISHED 13 September 2023

## CITATION

Yang W, Yang H, Bao X, Hussain M, Bao Q, Zeng Z, Xiao C, Zhou L and Qin X (2023) *Brevibacillus brevis* HNCS-1: a biocontrol bacterium against tea plant diseases. *Front. Microbiol.* 14:1198747. doi: 10.3389/fmicb.2023.1198747

## COPYRIGHT

© 2023 Yang, Yang, Bao, Hussain, Bao, Zeng, Xiao, Zhou and Qin. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

# *Brevibacillus brevis* HNCS-1: a biocontrol bacterium against tea plant diseases

Wenbo Yang<sup>1,2†</sup>, Hui Yang<sup>2†</sup>, Xiaocun Bao<sup>2</sup>, Mehboob Hussain<sup>1</sup>, Qiang Bao<sup>2</sup>, Zexuan Zeng<sup>2</sup>, Chun Xiao<sup>1\*</sup>, Lingyun Zhou<sup>2\*</sup> and Xiaoping Qin<sup>1\*</sup>

<sup>1</sup>College of Plant Protection, Yunnan Agricultural University, Kunming, China, <sup>2</sup>Tea Research Institute, Hunan Academy of Agricultural Sciences, Changsha, China

As a biocontrol bacteria, *Brevibacillus* has been the subject of extensive research for agricultural applications. Antibacterial peptides (AMPs) are the main antibacterial products of *Brevibacillus*. This study isolated a strain of *Br. brevis* HNCS-1 from tea garden soil, and the strain has an antagonistic effect against five types of pathogens of tea diseases, namely *Gloeosporium theae-sinensis*, *Elsinoe leucospira*, *Phyllosticta theaeifolia*, *Fusarium* sp., and *Cercospora theae*. To determine the genetic characteristics implicated in the biocontrol mechanism, the genome sequence of the HNCS-1 strain was obtained and analyzed further, and the data are deposited in the GenBank repository (No. CP128411). Comparative genomics analyses revealed that the HNCS-1 strain and 17 public *Br. brevis* share a core genome composed of 3,742 genes. Interestingly, only one non-ribosomal peptide synthetase (NRPS) gene cluster annotated as edeine is present in the core genome. And UHPLC-MS/MS detection results showed that edeine B and edeine A were the principal antibacterial peptides in the HNCS-1 strain. This study proves that edeine is the main antibacterial peptide of *Br. brevis*, and provides a new strategy for the identification of antibacterial products from other biocontrol bacteria.

## KEYWORDS

*Brevibacillus brevis*, antagonistic activity, genome annotation, pan-genome analysis, antimicrobial peptides, edeine

## Introduction

The tea plant (*Camellia sinensis*) is an important cash crop, and its beneficial metabolites are valuable for human health (Pan et al., 2022). Similar to other plants, tea plants are vulnerable to many diseases throughout their life cycle. Among them, fungal diseases are the most serious hazards to tea plants, resulting in a decline in tea production and quality. For example, tea white scab disease occurs year-round in the Chinese tea host production area, generally resulting in a reduction of approximately 10% in production and a reduction of over 50% in the sick tea garden (Zhou et al., 2019). Tea gray blight can reduce tea production by 10–20% (Sanjay et al., 2008). For a long time, chemical control has been the main means to address tea plant diseases. However, there are fewer pesticides registered for tea plant diseases, and long-term single and excessive use of chemical pesticides has caused problems such as pathogen resistance, pesticide residues, and environmental pollution. Therefore, tea plantations urgently need a sustainable, environmentally friendly, and anti-drug-resistance plant disease control technology to replace traditional chemical control. Microbial pesticides have become one of the ideal strategies for the comprehensive control of tea plant diseases due to their persistence and environmental friendliness.

*Brevibacillus* spp. is an essential source of biocontrol microorganisms and is widespread in nature, having been discovered in soil, flora, seawater, and the intestinal tracts of animals (Ruiu, 2013). There are 20 species in the genus, including 10 *Bacillus* species discovered previously (the *Br. brevis* cluster). *Br. brevis* is the parent species of the genus *Brevibacillus* (Shida et al., 1996). It has been reported in the disciplines of biological control of plant maladies, pollution degradation, and heavy metal remediation (Samrot et al., 2015; Che et al., 2016; Wang et al., 2016). *Brevibacillus* is one of the most studied bacterial groups, and it has been one of the most important producers of antimicrobial peptides (AMPs) (Yang and Yousef, 2018). AMPs play an important role in bacteriostasis (Xu et al., 2023). AMPs can be classified according to their biosynthetic pathways as ribosomally synthesized and post-translationally modified peptides (Ripps) and non-ribosomally synthesized peptides (NRPS). Currently, the preponderance of *Brevibacillus* AMPs are produced by non-ribosomal peptide synthetases. They consist of edeine (A, B, D, and F) (Czajgucki et al., 2006), gramicidin (A-C, S) (Govaerts et al., 2001; Kessler et al., 2004), gratisin (Tamaki et al., 1983), tyrocidine (A-D) (Mootz and Marahiel, 1997), BT1583 (Wu et al., 2005), tostadin (Song et al., 2012), tauramamide (Desjardine et al., 2007), brevistin (Shoji and Kato, 1976), spergualin (Takeuchi et al., 1981), loloatin (A-D) (Gerard et al., 1999), laterocidin (Xu et al., 2010), and tridecapeptide families (Yang et al., 2017). *Brevibacillus* spp. generate numerous Ripps, such as laterosporulin (Singh et al., 2012), laterosporulin10 (Baindara et al., 2016), and Bac-GM100 (Ghadbane et al., 2013). Most *Brevibacillus* AMPs exert their antimicrobial effect via cytoplasmic membrane damage, but edeine inhibits DNA synthesis and protein translation and synthesis at varying concentrations (Szer and Kurylo-Borowska, 1972; Dinos et al., 2004).

However, the diversity of types and structures of AMPs has also led to difficulties in the purification and identification of traditional chemical analysis techniques. Fortunately, the development of next-generation sequencing technology over the past two decades has consequently stimulated research on the comparative genomics of *Br. brevis*. More biocontrol strains of *Br. brevis* have had their entire genomes sequenced in order to investigate the relationships between antibacterial mechanisms and the underlying genetic diversity of *Br. brevis* genomes. With the advancement of bioinformatics prediction, AMP gene clusters have been continuously identified. The metabolic pathway database of AMPs, which can annotate the protein gene clusters of biocontrol bacteria at the gene level and predict the results of secondary metabolites, has been perpetually improved, making the rapid identification of AMPs feasible. In addition, with the continuous publication of multiple microbial genomes and the comparison of microbial genomes between different individuals of the same species, it is gradually recognized that a single reference genome cannot represent diversity within a species (Cuellar-Gaviria et al., 2023; Wan et al., 2023).

In this study, a strain of *Br. brevis* HNCS-1 with broad-spectrum antibacterial activity was isolated from the tea garden soil. To gain a comprehensive understanding of the biocontrol potential of strain HNCS-1, we sequenced the genome of *Br. brevis* HNCS-1. A comparative genomic analysis was conducted to characterize the pan-genome structure of this biocontrol bacterium, assembled with

17 public *Br. brevis* genomes. An NRPS gene cluster was found in the core genome, which may be related to the antibacterial ability of *Br. brevis*. This study provides a genetic context for future research on AMPs and provides a scientific basis for further optimizing the field applications of the microbial biopesticide derived from *Br. brevis* HNCS-1.

## Materials and methods

### Microbial culture and preservation

The cultivation and plant protection laboratory of the Tea Research Institute of the Hunan Academy of Agricultural Sciences isolated *Br. brevis* HNCS-1 from tea garden soil and deposited it in China Center for Type Culture Collection (No. M 2022713). The cultivation and plant protection laboratory isolated and preserved *Gloeosporium theae-sinensis*, *Elsinoe leucospira*, *Phyllosticta theaeifolia*, *Fusarium* sp., and *Cercospora theae*.

The fungi were routinely cultivated at 26°C on a PDA medium (6 g potato extract, 20 g dextrose, 20 g agar, 1,000 ml H<sub>2</sub>O, pH 5.6 ± 0.2) and stored at 4°C on the same medium. Before using them in an experiment, they were activated on a PDA medium for 24 h at 26°C and then transferred by streaking. The HNCS-1 strain was stored in 20% glycerol at −80°C. It was activated by streaking at 37°C for 12 h on LB agar medium (10 g tryptone, 5 g yeast extract, 10 g NaCl, 15 g agar, 1,000 ml H<sub>2</sub>O, pH 7.0 ± 0.1).

### Antibiotics activity of *Brevibacillus brevis* HNCS-1

*Br. brevis* HNCS-1 was inoculated into 500 ml Erlenmeyer flasks containing 200 ml of the LB liquid medium and cultivated at 37°C and 200 rpm for 12 h. Then, 10 ml of the seed cultures were transferred to 500 ml Erlenmeyer flasks containing the NB medium (10 g peptone, 3 g beef extract, 5 g NaCl, 1,000 ml H<sub>2</sub>O, and pH 7.2 ± 0.2) and cultivated at 37°C and 180 rpm for 3 days. *Br. brevis* HNCS-1 fermentation broth was centrifuged at 4°C and 10,000 rpm for 10 min, and the supernatant was collected and filtered through a 0.22 μm filter.

On a PDA medium containing a 10% cell-free supernatant of *Br. brevis* HNCS-1, spore germination was used to determine the bacteriostatic activity against fungi. A fungus cake was made using a 5 mm diameter sterile cork borer and placed in the center of the plate. Plates of *P. theaeifolia*, *Fusarium* sp., *E. leucospira*, and *C. theae* were incubated at 26°C for 12 days, 11 days, 22 days, 22 days, and 7 days, respectively. The experiment was performed thrice. The antagonistic activity of *Br. brevis* HNCS-1 was evaluated by inhibition rate (IR), which is calculated using the following formula:

$$IR (\%) = \frac{(A - 5) - (B - 5)}{A - 5} \times 100$$

where *A* is the diameter of tea fungus disks in the control treatment, *B* is the diameter of tea fungus disks with *Br. brevis*

HNCS-1, and 5 is the diameter of the disks inoculated with tea fungus.

## Genome sequencing, annotation, and alignment

The entire genome of *Br. brevis* HNCS-1 was sequenced using the Illumina and Nanopore platforms. Guangdong Magigene Technology Co., Ltd. performed this study in China. Following the standard protocol provided by Oxford Nanopore Technologies (ONT), the sequencing procedure includes sample quality detection, library construction, library quality detection, and library sequencing. SMRT Link v5.1.0 was chosen for the assembly of third-generation data alone, and Unicycler was chosen for the assembly of second-generation and third-generation data. tRNAscan-SE v1.3.1, rRNAmmer v1.2, and Rfam databases briefly predicted Glimmer, tRNAs, and rRNAs, respectively. PHAST identified prophage sequences within the genome assemblies. Island viewer predicted Genomic Islands (GIs).

The complete nucleotide sequence was also searched against Non-Redundant (NR) Protein Database, Swiss-Port, Cluster of Orthologous Groups (COGs), Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), Pfam, Carbohydrate-Active enZymes (CAZy) database, Pathogen–Host Interactions (PHIs), Virulence Factor Database (VFDB), and Comprehensive Antibiotic Resistance Database (CARD) for functional annotation and further function assignment. Basic Local Alignment Search Tool (BLAST) and Diamond were used to compare the gene sequence with the reference database sequences. The finest matching results were selected based on identity, e-value, and score, which were the results of genome annotation. Circular maps of the genome were generated using Circos (Krzywinski et al., 2009).

## Phylogenetic tree and pan-genome analysis

*Br. brevis* HNCS-1 and 17 other *Br. brevis* genomes were used to construct a phylogenetic tree, with *B. subtilis* ATCC 13952 serving as an out-group. Except for *Br. brevis* HNCS-1, all sequences were obtained from the National Center for Biotechnology Information (NCBI) (Table 1). OrthoFinder software (Emms and Kelly, 2019) was used to generate a maximum likelihood (ML) phylogenetic tree, which was visualized using Figtree v1.4.4. To evaluate the phylogeny of the *Br. brevis* strains, average nucleotide identity (ANI) was calculated using the FastANI software (Jain et al., 2018). The thermal map was then visualized using the TBtools software (Chen et al., 2020).

To identify the core and strain-specific genes, a pan-genome analysis was performed on 18 *Br. brevis* isolates using the BPGA v1.3 software, which implemented functional ortholog clustering using the amino acid sequences based on Gene Family (GF) approach (Chaudhari et al., 2016). The collection of genes shared by all 18 strains was referred to as the pan-genome, while the set of shared genes within the test genomes was referred to as their

core genomes. For pan-genome genetic contexts, Heap's Law can be represented by the following formula:

$$y = k \times n^\gamma$$

where  $y$  represents the extent of the pan-genome,  $n$  represents the number of genomes, and  $k$  and  $\gamma$  are fitting parameters. According to this law,  $\gamma$  can be calculated as  $\alpha = 1 - \gamma$ , so when  $\alpha < 1$  ( $0 < \gamma < 1$ ), the extent of the pan-genome increases unboundedly with the successive addition of new genomes and is considered open. In contrast, if  $\alpha > 1$  ( $\gamma < 0$ ), the pan-genome trajectory approaches a plateau and can be considered closed as additional genomes are added (De Jesus et al., 2022).

## Prediction of genes related to antibacterial activity

The NR database was chosen as the protein sequence database for our annotation system based on a balance of quality, comprehensiveness, and our practical needs. Using the complete nucleotide sequence of the *Br. brevis* HNCS-1 genome, the online tools antiSMASH 6.0 (Blin et al., 2021) were able to identify clusters of secondary metabolite genes.

## Isolation and antibiotic activity of AMPs

One thousand milliliters of *Br. brevis* HNCS-1 supernatant was frozen at  $-80^\circ\text{C}$  for 24 h and then concentrated to 20 ml by vacuum freeze drying. The concentrated sample (2 ml) was separated by column chromatography on Sephadex G-75 using an  $\text{H}_2\text{O}-\text{CH}_3\text{OH}$  gradient [ $\text{H}_2\text{O}, \text{H}_2\text{O}-\text{CH}_3\text{OH} = 4:1$  (v/v),  $\text{H}_2\text{O}-\text{CH}_3\text{OH} = 3:2$  (v/v),  $\text{H}_2\text{O}-\text{CH}_3\text{OH} = 2:3$  (v/v),  $\text{H}_2\text{O}-\text{CH}_3\text{OH} = 1:4$  (v/v),  $\text{CH}_3\text{OH}$ ]. The eluent was collected using an EP tube, ranging from 0.7 ml to 1.0 ml per tube. The cell-free eluate's antibacterial activity against *G. thea-sinensis* was determined using agar diffusion on a PDA medium after it was filtered through a  $0.22\ \mu\text{m}$  filter membrane. In total, 1 ml of *G. thea-sinensis* spore suspension was distributed across the agar surface of the plates. Then, a sterilized cork borer was used to create 5 mm diameter wells with equal spacing in the agar. Overall, 3 days were spent incubating the dishes at  $26^\circ\text{C}$ . The experiment was performed thrice.

## UHPLC-MS/MS method

For the separation, ultra-high-performance liquid chromatography (Exion, SCIEX) was utilized. The process utilized an Agilent ZORBAX SB-C18 column (2.1 mm  $\times$  150 mm, 2.7  $\mu\text{m}$ ) with acetonitrile (A) and 0.1% formic acid in water (B) as gradient elution mobile phases at a flow rate of 0.3 ml/min. The gradient elution protocol was as follows: 5% B from 0–5 min, 5–50% B from 5–20 min, 50–100% B from 20–30 min, and 100% B from 30–35 min. The temperature of the column was set to  $35^\circ\text{C}$ .

TABLE 1 Genome features of *Brevibacillus brevis* strains used in this study.

Strain	Assembly	Size (Mb)	GC%	Level	CDS	Source	Country
Ag35	GCA_014526365.1	6.48	47.3	Contig	5808	Root nodule	America
ATCC 35690	GCA_002161835.1	6.13	47.3	Contig	5567	Soil	Poland
B011	GCA_022026395.1	6.16	47.5	Complete	5552	Tobacco roots	China
DSM 30	GCA_003385915.1	6.61	47.4	Scaffold	6224	-	-
DZQ7	GCA_001039275.2	6.44	47.4	Complete	5825	Tobacco rhizosphere soil	China
FJAT-0809-GLX	GCA_000346255.1	6.02	47.3	Contig	5527	Rhizosphere soil of watermelon	China
G25-137	GCA_015912885.1	6.34	47.1	Scaffold	5801	-	China
GZDF3.1	GCA_001649505.1	6.43	46.8	Scaffold	5924		
HK544	GCA_007725005.1	6.49	47.3	Complete	5892	Soil	South Korea
I2-B3	GCA_019749035.1	6.22	47.4	Contig	5615	Air filter	America
LABIM17	GCA_021401445.1	5.95	47.5	Chromosome	5445	Soil from rainforest	Brazil
NBRC 100599	GCA_000010165.1	6.30	47.3	Complete	5776	-	-
NBRC 110488	GCA_001748185.1	6.28	47.3	Scaffold	5847	-	-
NBRC 15304	GCA_006539845.1	6.52	47.4	Contig	6152	-	-
NCTC2611	GCA_900637055.1	6.73	47.4	Complete	6241	-	-
NRRL NRS-604	GCA_003012835.1	6.61	47.4	Contig	6208	Soil	America
X23	GCA_000296715.2	6.64	46.9	Complete	6197	Soil of vegetable field	China

The X500R Q-TOF mass spectrometer (SCIEX, Framingham, MA, USA) was used to acquire untargeted mass spectral data. The LC effluent was injected into the mass spectrometer. ESI parameters were as follows: temperature: 500°C; ion source gas 1 and 2: 50 psi; curtain gas: 35 psi; CAD gas: 7 psi. Collision-induced dissociation at 35 ± 15 V in IDA mode was utilized to capture the MS and MS/MS spectra. Maximum candidate ions: 10; threshold for intensity: 600 cps; full scan mass range: 500–1,000 Da; ion discharge voltage: 5,500 V; collision energy: 35 V; collision energy spread: 15 V.

## Results

### Biocontrol activity of *Brevibacillus brevis* HNCS-1

*Br. brevis* HNCS-1 was evaluated for its antifungal activity against pathogenic fungi. The results demonstrated that HNCS-1 inhibited the mycelia proliferation of five tea fungal diseases significantly (Figure 1). *Br. brevis* HNCS-1 was isolated from tea garden soil and demonstrated broad-spectrum antagonistic activity. The mycelial growth of *Phyllosticta theaeifolia*, *Fusarium* sp., *Cercospora theae*, and *Gloeosporium theae-sinensis* was completely inhibited by 10% *Br. brevis* HNCS-1 supernatant. Additionally, it significantly inhibits the mycelial proliferation of *Elsinoe leucospira*. *Br. brevis* HNCS-1 is a beneficial microbe with biotechnological application potential.

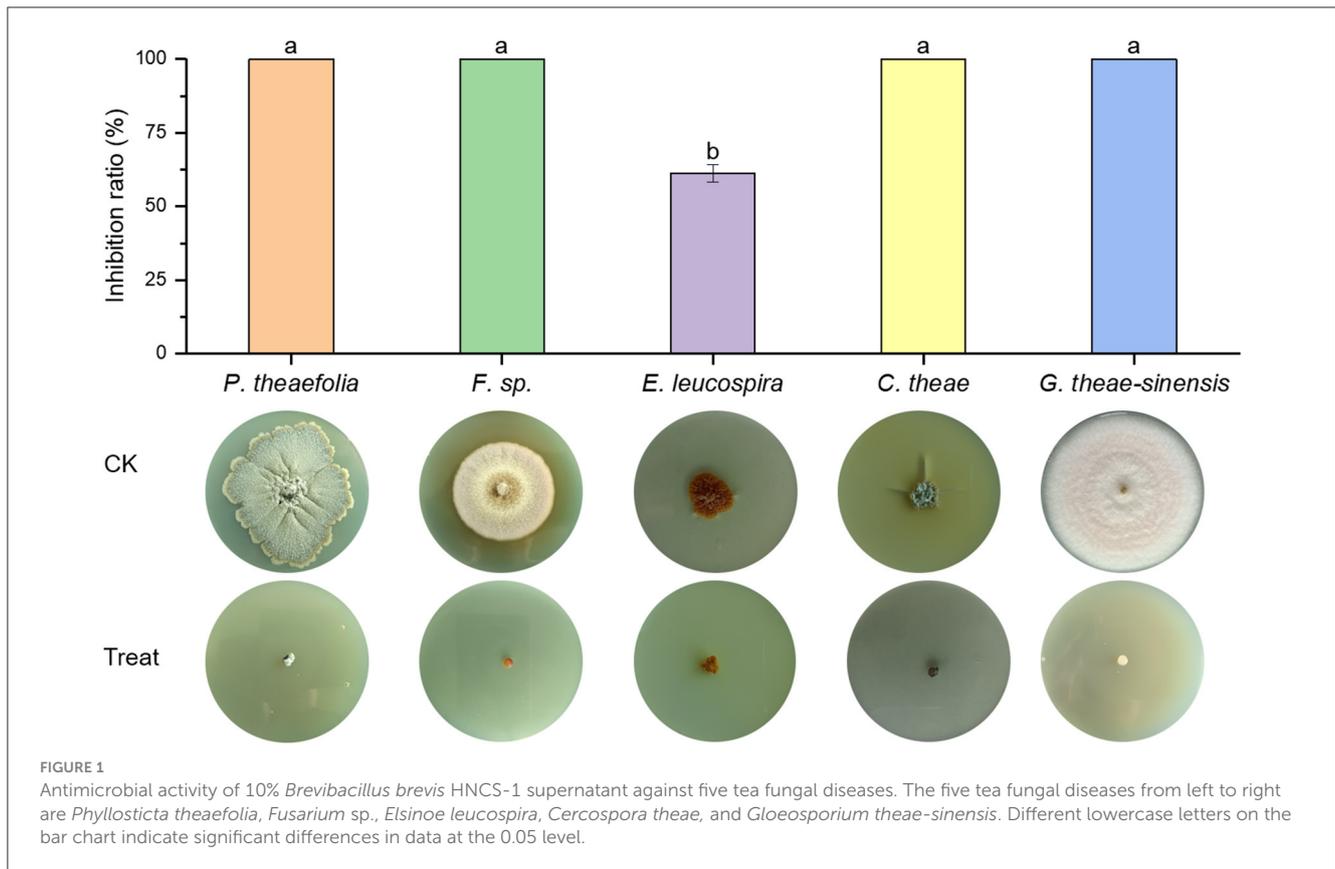
### General genome description of *Brevibacillus brevis* HNCS-1

The genome of the HNCS-1 strain consists of a single circular chromosome measuring 6,353,630 bp with a GC content of 47.15%; no plasmids were detected (GenBank CP128411). This chromosome genome consisted of 6,342 coding DNA sequences (CDS), which accounted for 87.50% of the genome. In addition, 15 sRNA, 44 rRNA, and 127 tRNA were predicted based on the chromosome sequence. In the genome, 31 Genomic Islands were identified, but no CRISPR repeat regions or prophages were found (Supplementary Figure 1).

In total, 5,796 and 4,080 identified genes were annotated as NR and Swiss-port, respectively, and 2,916 and 4,714 genes were classified into functional categories based on GO and COG designations, respectively (Supplementary Figures 2, 3). Overall, 5,810 KEGG pathways have been assigned (Supplementary Figure 4). Moreover, 4,808, 2,906, 1,190, 173, and 6 genes, respectively, were annotated in Pfm, PHI, VFDB, CAZyme, and CARD (Supplementary Figures 5, 6).

### Whole-genome phylogenetic analysis of *Brevibacillus brevis*

The phylogenetic tree of 18 *Br. brevis* genomes and *B. subtilis* ATCC 13952 was constructed using the ML method and all orthogroups, with the ATCC 13952 strain serving as the root. It was found that HNCS-1, *Br. brevis* strains 17, and ATCC 13952 formed



a large cluster, and different strains of *Br. brevis* revealed two groups (Figure 2A). In the meantime, strain HNCS-1 was a member of the same clade as strains G25-137, Ag35, ATCC 35690, GZDF3.1, NBRC100599, and LABIM17, but it was not a sister group to any other strains.

To substantiate the results of the phylogenetic analysis, we also determined the ANI values of various strains. Strains with ANI values above 95% are regarded as belonging to the same species (Richter and Rossello-Mora, 2009). The results showed that the genetic relationship between ANI and phylogenetic trees was basically consistent, but ANI was more precise, dividing 18 strains of *Br. brevis* into 5 species (Figure 2B).

According to the results, they were not distinguished by the mixed trend of geographical origin and phylogenetic clustering of strains isolated from similar environments. The genome's phylogenetic clustering differed substantially from the strains' specific habitat classification.

## The pan-genome features of *Brevibacillus brevis*

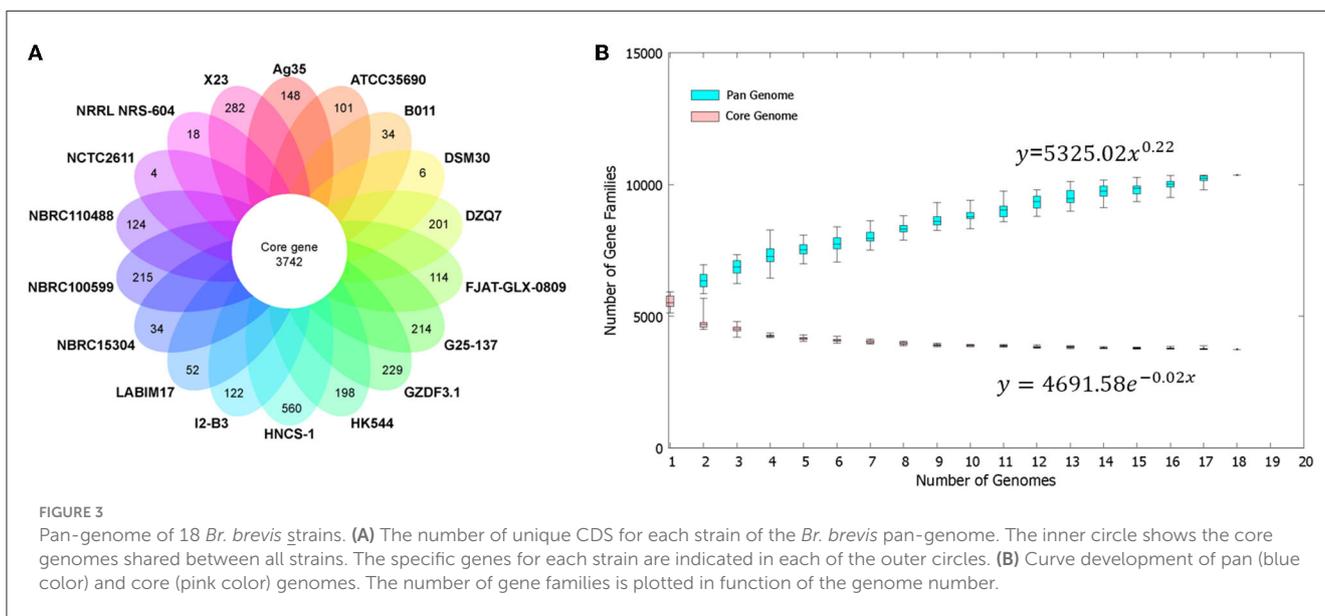
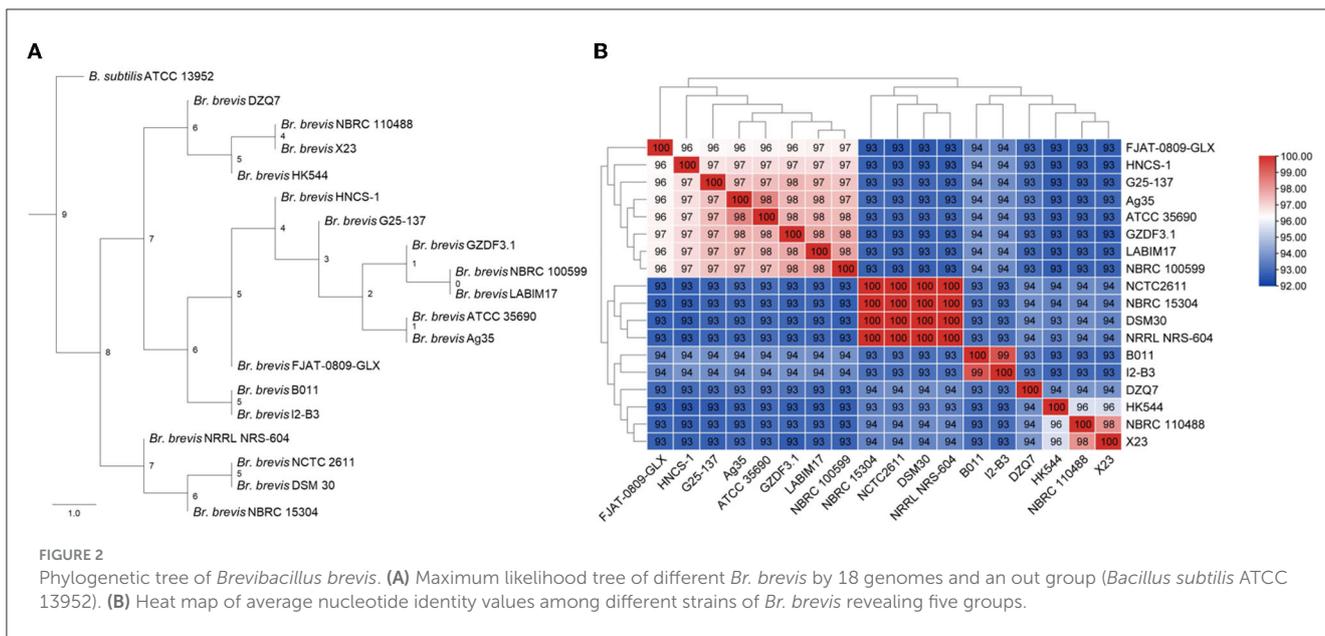
The BPGA software determined a pan-genome for the strain HNCS-1 and 17 sequenced *Br. brevis* strains by comparing pan-genome analyses of bacterial species by utilizing protein clustering data in the total pan-genome. There were 10,359 CDS in the total pan-genome of the 18 *Br. brevis* strains. Among the 10,359 protein-coding genes, 3,742 core genes represented 36.12% of the genes in the pan-genome of *Br. brevis*. The number of accessory gene

families (3,961 genes) was greater than the number of core gene families (3,742 genes). Moreover, *Br. brevis* HNCS-1 contains 1,586 accessory genes. In addition, the strain HNCS-1 also contained the greatest number of distinct transcripts (560 genes) and has greater potential for gene exploration. *Br. brevis* strains NCTC2611 and DSM30 encoded the fewest number of specific strain genes with 4 and 6, respectively (Figure 3A).

The number of *Br. brevis* genomes was plotted against the size of the pan-genome and the core genome. The pan-genome curve exhibited an asymptotic trend, indicating that 18 genomes were inadequate to characterize the complete gene repertoire of *Br. brevis*. According to the curve generated for these 18 genomes based on Heap's Law and least-square fit of exponential regression decay, the number of gene families in the pan-genome increases with the addition of each additional genome ( $\gamma = 0.22$ ), indicating that the pan-genome of *Br. brevis* strains is currently open but may be closed soon (Figure 3B).

## Analysis of genes encoding AMPs

It is noteworthy that the cell-free supernatant fermentation of *Br. brevis* inhibited numerous bacterial and fungal diseases. Additionally, the core genome sequence of *Br. brevis* was mined for AMP-encoding genes. Only one gene cluster implicated in edeine NRPS was identified in the core genome after NR annotation. The gene cluster likely contributes significantly to antimicrobial activity. The sequence similarity between the genes and the predicted *ede* BGC of *Br. brevis* Vm4 was high (Westman et al., 2013). The



identified *ede* BGC in the *Br. brevis* HNCS-1 genome was structured identically to the *Br. brevis* Vm4 genome. In brief, the *ede* BGC (43.57 kb) in *Br. brevis* HNCS-1 contained 17 open reading frames, designated *edeA* through *edeQ* based on the homologous sequences in *Br. brevis* Vm4 (44.12 kb) (Figure 4A).

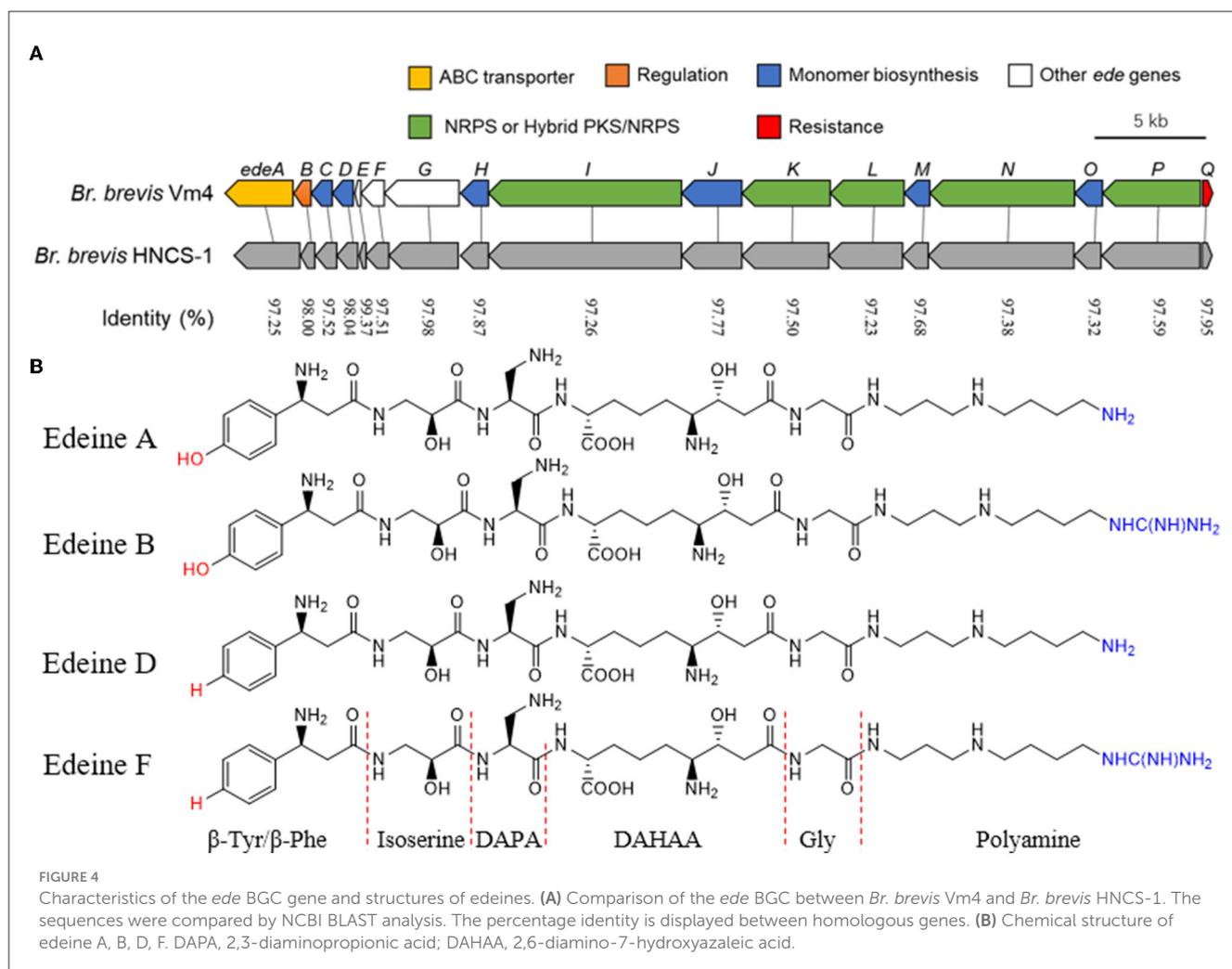
Additionally, the genome sequence of *Br. brevis* HNCS-1 was mined by antiSMASH software for the presence of gene-encoding AMPs. A total of 13 putative biosynthetic gene clusters were identified by the HNCS-1 strain genome (Supplementary Figure 7). Four putative gene clusters shared a high degree of similarity (>70% of genes shared similarity) with the petrobactin, tyrocidine, bacillopaline, and gramicidin gene clusters. Three putative gene clusters displayed modest similarity (<30% of genes displayed similarity) to the previously reported zwittermicin A, aurantinin B-D, and pacidamycin (1–7, D) gene clusters. Six

putative gene clusters were not conserved in comparison with known clusters.

### Purification and identification of AMPs

The antimicrobial peptide should be edeine, according to the results of genome annotation analysis. Edeines are a class of linear pentapeptides generated by *Brevibacillus*, a soil bacterium. Edeines A, B, D, and F exist as two isomers of  $\alpha$  and  $\beta$ ; however, only the  $\alpha$  isomer possesses remarkable antibiotic properties (Figure 4B).

The antibacterial potential of eluent extracts containing antibacterial compounds was evaluated. Using an agar well diffusion assay, the antibacterial potential of eluent extracts



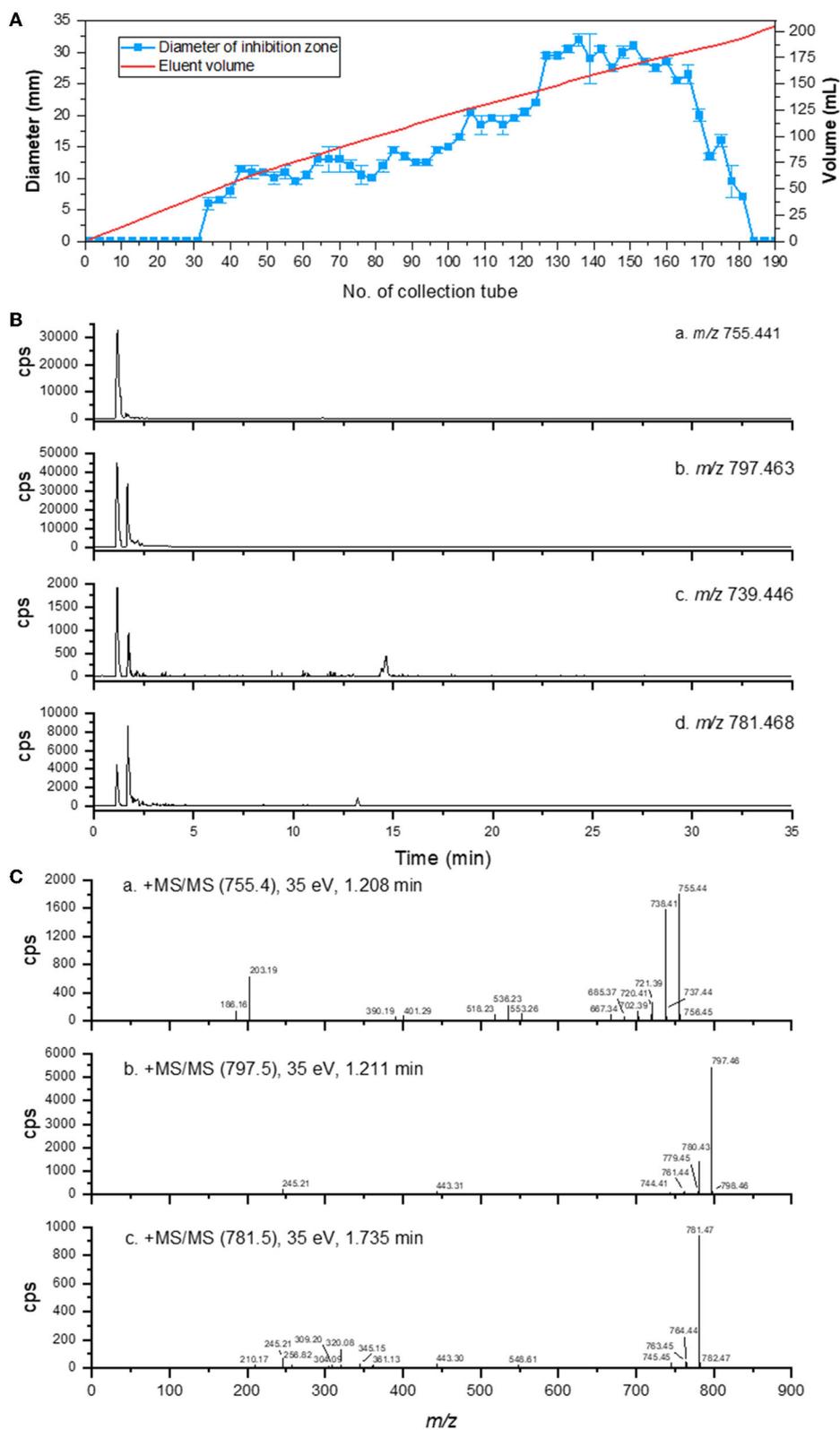
**FIGURE 4**  
 Characteristics of the *ede* BGC gene and structures of edeines. **(A)** Comparison of the *ede* BGC between *Br. brevis* Vm4 and *Br. brevis* HNCS-1. The sequences were compared by NCBI BLAST analysis. The percentage identity is displayed between homologous genes. **(B)** Chemical structure of edeine A, B, D, F. DAPA, 2,3-diaminopropionic acid; DAHAA, 2,6-diamino-7-hydroxyazaleic acid.

from distinct collection tubes against *G. theae-sinensis* was determined. With an increase in eluent volume, the bacteriostatic activity initially increased steadily and then decreased dramatically (Figure 5A). The sample with the highest antibacterial activity was analyzed by UPLC-MS/MS for additional confirmation. According to the total ion chromatogram, the majority of the ultraviolet absorption is of short wavelength.

The theoretical molar masses of edeine A ( $C_{33}H_{58}N_{10}O_{10}$ ), edeine B ( $C_{34}H_{60}N_{12}O_{10}$ ), edeine D ( $C_{33}H_{58}N_{10}O_9$ ), and edeine F ( $C_{34}H_{60}N_{12}O_9$ ) are 754.4337, 796.4555, 738.4388, and 780.4606. Compound 1 ( $m/z$  755), Compound 2 ( $m/z$  797), Compound 3 ( $m/z$  739), and Compound 4 ( $m/z$  781) were extracted from the total ion current based on the ion mass spectral ( $m/z$ ). Compound 1 ( $m/z$  755) produced a  $[M+H]^+$  ion peak  $m/z$  755.4132 (−1.7 ppm,  $C_{33}H_{58}N_{10}O_{10}$ ) at a retention time of 1.182 min (Figure 5B-a). The electrospray ionization-mass spectrometry results indicated that its fragmentation pattern was identical to that of edeine A, including  $m/z$  738, 737, 720, 667, 553, 536, 401, 390, and 203 (Figure 5C-a, Supplementary Figure 8). Compound 2 ( $m/z$  797) produced a  $[M+H]^+$  ion peak  $m/z$  797.4612 (−2.6 ppm,  $C_{34}H_{60}N_{12}O_{10}$ ) at a retention time of 1.165 min (Figure 5B-b) with observable signals. The electrospray ionization-mass spectrometry results indicated that its fragmentation pattern was identical to that

of edeine B, including  $m/z$  780, 779, 761, 443, and 245 (Figure 5C-b, Supplementary Figure 9). Compound 3 ( $m/z$  739) had faint signals at the retention time of 1.165 min (Figure 5B-c), but its peak value was too low to be analyzed further based on the available mass spectrum data. Compound 4 ( $m/z$  781) produced a  $[M+H]^+$  ion peak  $m/z$  781.4667 (−2.2 ppm,  $C_{34}H_{60}N_{12}O_9$ ) at the retention time of 1.708 min (Figure 5B-d). The electrospray ionization-mass spectrometry results indicated that its fragmentation pattern was identical to that of edeine B, including  $m/z$  780, 779, 761, 443, and 245 (Figure 5C-c, Supplementary Figure 10). To sum up, edeines were a class of highly polar AMPs with limited retention on reverse phase chromatography, which was consistent with the chromatographic retention property of the compounds discussed previously. Compounds 1, 2, and 4 were edeine A, B, and F, respectively. The chromatographic peak area suggested that edeine B had the maximum concentration, followed by edeine B and F had the lowest concentration.

In addition, to confirm the existence of secondary metabolites predicted by antiSMASH software, we extracted relevant data from the total ion current based on the theoretical molecular weight of petrobactin, tyrocidine, bacillopaline, and gramicidin, but they were not detected (Supplementary Figures 11–14).



**FIGURE 5** Purification and UHPLC-MS/MS analysis of AMPs from *Brevibacillus brevis* HNCS-1. (A) Antimicrobial activity of crude extract of HNCS-1 after elution with H<sub>2</sub>O/CH<sub>3</sub>OH. (B) The ion current of  $m/z$  755, 797, 739, and 781. (C) MS/MS spectra of  $m/z$  755, 797, and 781.

## Discussion

Allelopathy is a pervasive natural phenomenon in ecosystems, promoting and inhibiting the proliferation of diverse organisms. People have always been interested in the rational application of biocontrol's antagonistic effect on agricultural resistance and disease control. Tea plant diseases are difficult to control, resulting in substantial losses in yield and quality. Although chemical fungicides have some effect on the control of tea plant diseases, their misuse has resulted in severe environmental pollution issues. Utilizing biocontrol microorganisms to combat plant maladies is a popular topic of discussion. In certain instances, biocontrol microorganisms have been shown to be as effective as chemical fungicides in preventing plant diseases. Several bacterial species, such as *Bacillus*, *Brevibacillus*, and *Pseudomonas*, have been commercialized as biological control agents (Zhang et al., 2005; Paterson et al., 2017). Because of their extensive colonization capabilities, spore-forming *Bacillus* and *Brevibacillus* preparations are preferred for the development of commercial products. In this study, we isolated strain HNCS-1 of *Br. brevis* from tea garden soil that exhibits broad-spectrum resistance to five fungal diseases of tea plants. The HNCS-1 strain is a beneficial microorganism with prospective biotechnological application potential.

The HNCS-1 strain and 17 *Br. brevis* isolates from NCBI were subjected to a phylogenetic analysis using orthologous genes organized into four clusters. However, the strains of the same cluster originate from diverse habitats, indicating that *Br. brevis* is highly adaptable to its environment and can populate a variety of ecological niches. We examined the pan-genome of *Br. brevis* species. The results indicate that the pan-genome of *Br. brevis* is open and theoretically infinite, indicating that *Br. brevis* species tend to acquire new genes to improve adaptability. Bacteria could modify their genetic material to adapt to varying environmental conditions, resulting in increased niche diversity and larger pan-genomes (Konstantinidis and Tiedje, 2004). The HNCS-1 strain has more protein-coding genes (6,342) than other *Br. brevis* strains, making it more environmentally adaptable.

In addition, the traditional separation methods of antimicrobial substances have limitations. According to previous studies, this study tried to extract antimicrobial substances through the organic solvent extraction method, hydrochloric acid precipitation method, and ammonium sulfate precipitation method but never succeeded. Genome sequencing analysis and pan gene analysis open a new door for the identification of antimicrobial substances, which can quickly and accurately target antimicrobial substances and lay a theoretical foundation for the separation and application of antimicrobial substances.

A comparison of *Br. brevis* HNCS-1 proteins with those of other *Br. brevis* strains revealed 3,742 core genes. There is only one NRPS gene cluster annotated as edeine in the core genes. It is demonstrated that edeine is the antibacterial peptide at the center of *Br. brevis*. After a blast comparison, it was determined that the gene cluster of the HNCS-1 strain was extremely similar to the *Br. brevis* Vm4 gene cluster for edeine NRPS (identity >97%). To validate the accuracy of comprehensive genomic analysis, we used the UHPLC-MS/MS technique to identify edeine A, B, and F from the crude extract of the HNCS-1 strain, with edeine B and edeine A serving as the principal AMPs. The main species of edeine in the HNCS-1 strain differ from *Br. brevis* X23

(Du et al., 2022), which may be owing to the evolutionary tree's distant genetic relationship.

Edeine, as an AMP of the core genes of *Br. brevis*, should be abundant in the organism's secondary metabolites. Edeine was extracted from *Br. brevis* Vm4 and X23 by resin adsorption, respectively (Westman et al., 2013; Liu et al., 2022). However, ethylparaben from *Br. brevis* FJAT-0809-GLX was extracted by solvent extraction (Che et al., 2015), tostadin from *Br. brevis* XDH was extracted by ammonium sulfate precipitation (Song et al., 2012), siderophore from *Br. brevis* GZDF3 was extracted by ethanol precipitation (Sheng et al., 2020), and surfactin from *Br. brevis* KN8(2) was extracted by hydrochloric acid precipitation (Krishnan et al., 2019). The inability to extract edeine from the secondary metabolites of *Br. brevis* may have been caused by a mismatch in extraction techniques. Due to the ability of *Br. brevis* to acquire new genes from the environment, its antimicrobial peptides are diverse. The pan-genome analysis identified 560 HNCS-1 strain-specific genes. The unique genomes of the HNCS-1 strain require additional study. Additionally, genetic engineering is an excellent research direction. For instance, using *in situ* promoter engineering, the production of edeine in *Br. brevis* X23 was increased. *Br. brevis* HNCS-1 requires additional research, including the one strain many compounds approach (OSMAC) and genetic modification, to investigate novel compounds and yield enhancement.

## Data availability statement

The data presented in the study are deposited in the GenBank repository, accession number CP128411.

## Author contributions

CX, LZ, and XQ conceived and planned the experiments, funding acquisition, and revisions. WY and HY were involved in data and bioinformatics analyses, conducted experiments, and wrote the manuscript. XB and MH contributed to the interpretation of the results. QB and ZZ contributed to sample preparation. All authors contributed to the article and approved the submitted version.

## Funding

This study was supported by the National Natural Science Foundation of China (32072625 and 31660541), the Natural Science Foundation of Hunan Province (2021JJ30385), the Transformation and Industrialization Plan of Scientific and Technological Achievements of Hunan Province (2021NK1020), and the National Key Research and Development Plan (2022YFD1600800).

## Acknowledgments

The authors would like to thank the Tea Research Institute, Hunan Academy of Agricultural Sciences for the assistance with experimental materials.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2023.1198747/full#supplementary-material>

## References

- Baindara, P., Singh, N., Ranjan, M., Nallabelli, N., Chaudhry, V., Pathania, G. L., et al. (2016). Laterosporulin10: a novel defensin like Class IId bacteriocin from *Brevibacillus* sp. strain SKDU10 with inhibitory activity against microbial pathogens. *Microbiol. Res.* 162, 1286–1299. doi: 10.1099/mic.0.000316
- Blin, K., Shaw, S., Kloosterman, A. M., Charlop-Powers, Z., van Wezel, G. P., Medema, M. H., et al. (2021). AntiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res.* 49, W29–W35. doi: 10.1093/nar/gkab335
- Chaudhari, N. M., Gupta, V. K., and Dutta, C. (2016). BPGA- an ultra-fast pan-genome analysis pipeline. *Sci. Rep.* 6, 24373. doi: 10.1038/srep24373
- Che, J. M., Liu, B., Chen, Z., Shi, H., Liu, G. H., Ge, C. B., et al. (2015). Identification of ethylparaben as the antimicrobial substance produced by *Brevibacillus brevis* FJAT-0809-GLX. *Microbiol. Res.* 172, 48–56. doi: 10.1016/j.micres.11007
- Che, J. M., Ye, S. W., Liu, B., Deng, Y. Y., Chen, Q. Q., Ge, C. B., et al. (2016). Effects of *Brevibacillus brevis* FJAT-1501-BPA on growth performance, faecal microflora, faecal enzyme activities and blood parameters of weaned piglets. *Antonie Van Leeuwenhoek Int. J. General Mol. Microbiol.* 109, 1545–1553. doi: 10.1007/s10482-016-0756-8
- Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., et al. (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* 13, 1194–1202. doi: 10.1016/j.molp.06009
- Cuellar-Gaviria, T. Z., García-Botero, C., Ju, K.-S., and Villegas-Escobar, V. (2023). The genome of *Bacillus tequilensis* EA-CB0015 sheds light into its epiphytic lifestyle and potential as a biocontrol agent. *Front. Microbiol.* 14, 5487. doi: 10.3389/fmicb.2023.1135487
- Czajgucki, Z., Andruszkiewicz, R., and Kamysz, W. (2006). Structure activity relationship studies on the antimicrobial activity of novel edeine A and D analogues. *J. Pept. Sci.* 12, 653–662. doi: 10.1002/psc.775
- De Jesus, Aburjaile, L. C. L., Sousa, F. F., Felice, T. J., Soares, A. G., Alcantara, S. C., et al. (2022). Genomic characterization of *Lactobacillus delbrueckii* strains with probiotics properties. *Front. Bioinform.* 2, 912795. doi: 10.3389/fbiof.2022.912795
- Desjardine, K., Pereira, A., Wright, H., Matainaho, T., Kelly, M., Andersen, R. J., et al. (2007). Tauroamamide, a lipopeptide antibiotic produced in culture by *Brevibacillus laterosporus* isolated from a marine habitat: structure elucidation and synthesis. *J. Nat. Prod.* 70, 1850–1853. doi: 10.1021/np070209r
- Dinos, G., Wilson, D. N., Teraoka, Y., Szaflarski, W., Fucini, P., Kalpaxis, D., et al. (2004). Dissecting the ribosomal inhibition mechanisms of edeine and pactamycin: the universally conserved residues G693 and C795 regulate P-site RNA binding. *Mol. Cell* 13, 113–124. doi: 10.1016/s1097-2765(04)00002-4
- Du, J., Zhang, C., Long, Q., Zhang, L., Chen, W., Liu, Q., et al. (2022). Characterization of a pathway-specific activator of edeine biosynthesis and improved edeine production by its overexpression in *Brevibacillus brevis*. *Front. Plant Sci.* 13, 1022476. doi: 10.3389/fpls.2022.1022476
- Emms, D. M., and Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol.* 20, 238. doi: 10.1186/s13059-019-1832-y
- Gerard, J. M., Haden, P., Kelly, M. T., and Andersen, R. J. (1999). Loloatins A-D, cyclic decapeptide antibiotics produced in culture by a tropical marine bacterium. *J. Nat. Prod.* 62, 80–85. doi: 10.1021/np980219f
- Ghadbane, M., Harzallah, D., Laribi, A. I., Jaouadi, B., and Belhadj, H. (2013). Purification and biochemical characterization of a highly thermostable bacteriocin isolated from *Brevibacillus brevis* strain GM100. *Biosci. Biotechnol. Biochem.* 77, 151–160. doi: 10.1271/bbb.120681
- Govaerts, C., Orwa, J., Van Schepdael, A., Roets, E., and Hoogmartens, J. (2001). Structure elucidation of four related substances in gramicidin with liquid chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* 15, 128–134. doi: 10.1002/1097-0231(20010130)15:2<128::AID-RCM205>3.0.CO;2-O
- Jain, C., Rodriguez, R. L., Phillippy, A. M., Konstantinidis, K. T., and Aluru, S. (2018). High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat. Commun.* 9, 5114. doi: 10.1038/s41467-018-07641-9
- Kessler, N., Schuhmann, H., Morneweg, S., Linne, U., and Marahiel, M. A. (2004). The linear pentadecapeptide gramicidin is assembled by four multimodular nonribosomal peptide synthetases that comprise 16 modules with 56 catalytic domains. *J. Biol. Chem.* 279, 7413–7419. doi: 10.1074/jbc.M309658200
- Konstantinidis, K. T., and Tiedje, J. M. (2004). Trends between gene content and genome size in prokaryotic species with larger genomes. *Proc. Natl. Acad. Sci. USA.* 101, 3160–3165. doi: 10.1073/pnas.0308653100
- Krishnan, N., Velamar, B., and Velu, R. K. (2019). Investigation of antifungal activity of surfactin against mycotoxigenic phytopathogenic fungus *Fusarium moniliforme* and its impact in seed germination and mycotoxicosis. *Pestic. Biochem. Physiol.* 155, 101–107. doi: 10.1016/j.pestbp.01010
- Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., et al. (2009). Circo: an information aesthetic for comparative genomics. *Genome Res.* 19, 1639–1645. doi: 10.1101/gr.092759.109
- Liu, Q., Zhang, L., Wang, Y., Zhang, C., Liu, T., Duan, C., et al. (2022). Enhancement of edeine production in *Brevibacillus brevis* X23 via in situ promoter engineering. *Microb. Biotechnol.* 15, 577–589. doi: 10.1111/1751-7915.13825
- Mootz, H. D., and Marahiel, M. A. (1997). The tyrocidine biosynthesis operon of *Bacillus brevis*: complete nucleotide sequence and biochemical characterization of functional internal adenylation domains. *J. Bacteriol.* 179, 6843–6850. doi: 10.1128/jb.179.21.6843-6850.1997
- Pan, S. Y., Nie, Q., Tai, H. C., Song, X. L., Tong, Y. F., Zhang, L. J., et al. (2022). Tea and tea drinking: China's outstanding contributions to the mankind. *Chin. Med.* 17, 27. doi: 10.1186/s13020-022-00571-1
- Paterson, J., Jahanshah, G., Li, Y., Wang, Q., Mehnaz, S., Gross, H., et al. (2017). The contribution of genome mining strategies to the understanding of active principles of PGPR strains. *FEMS Microbiol. Ecol.* 93, 249. doi: 10.1093/femsec/fiw249
- Richter, M., and Rossello-Mora, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. USA.* 106, 19126–19131. doi: 10.1073/pnas.0906412106
- Rui, L. (2013). *Brevibacillus laterosporus*, a pathogen of invertebrates and a broad-spectrum antimicrobial species. *Insects* 4, 476–492. doi: 10.3390/insects4030476
- Samrot, A. V., Prasad, R. J., Rio, A. J., and Sneha, S. J. (2015). Bioprospecting of *Brevibacillus brevis* isolated from soil. *Recent Pat. Biotechnol.* 9, 42–49. doi: 10.2174/18722083090115111124333
- Sanjay, R., Ponmurugan, P., and Baby, U. I. (2008). Evaluation of fungicides and biocontrol agents against grey blight disease of tea in the field. *Crop Prot.* 27, 689–694. doi: 10.1016/j.cropro.09014
- Sheng, M. M., Jia, H. K., Zhang, G. Y., Zeng, L. N., Zhang, T. T., Long, Y. H., et al. (2020). Siderophore Production by rhizosphere biological control bacteria *Brevibacillus brevis* GZDF3 of *Pinellia ternata* and its antifungal effects on *Candida albicans*. *J. Microbiol. Biotechnol.* 30, 689–699. doi: 10.4014/jmb.1910.10066
- Shida, O., Takagi, H., Kadowaki, K., and Komagata, K. (1996). Proposal for two new genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. *Int. J. Syst. Bacteriol.* 46, 939–946. doi: 10.1099/00207713-46-4-939
- Shoji, J., and Kato, T. (1976). The structure of brevistin. Studies on antibiotics from the genus *Bacillus*. *X. J. Antibiot.* 29, 380–389. doi: 10.7164/antibiotics.29.380

- Singh, P. K., Chittipurna, Ashish, Sharma, V., Patil, P.B., and Korpole, S. (2012). Identification, purification and characterization of laterosporulin, a novel bacteriocin produced by *Brevibacillus* sp. strain GI-9. *PLoS ONE* 7, e31498. doi: 10.1371/journal.pone.0031498
- Song, Z., Liu, Q. X., Guo, H., Ju, R. C., Zhao, Y. H., Li, J. Y., et al. (2012). Tostadin, a novel antibacterial peptide from an antagonistic microorganism *Brevibacillus brevis* XDH. *Bioresour. Technol.* 111, 504–506. doi: 10.1016/j.biortech.02051
- Szer, W., and Kurylo-Borowska, Z. (1972). Interactions of edeine with bacterial ribosomal subunits. Selective inhibition of aminoacyl-tRNA binding sites. *Biochim Biophys Acta* 259, 357–368. doi: 10.1016/0005-2787(72)90310-3
- Takeuchi, T., Iinuma, H., Kunimoto, S., Masuda, T., Ishizuka, M., Takeuchi, M., et al. (1981). A new antitumor antibiotic, spargualin: isolation and antitumor activity. *J. Antibiot.* 34, 1619–1621. doi: 10.7164/antibiotics.34.1619
- Tamaki, M., Takimoto, M., Sofuku, S., and Muramatsu, I. (1983). Synthetic studies on graptisin. II. *J. Antibiot.* 36, 751–752. doi: 10.7164/antibiotics.36.751
- Wan, X., Takala, T. M., Huynh, V. A., Ahonen, S. L., Paulin, L., Björkroth, J., et al. (2023). Comparative genomics of 40 *Weissella paramesenteroides* strains. *Front. Microbiol.* 14, 28. doi: 10.3389/fmicb.2023.1128028
- Wang, L., Tang, L., Wang, R., Wang, X., Ye, J., Long, Y., et al. (2016). Biosorption and degradation of decabromodiphenyl ether by *Brevibacillus brevis* and the influence of decabromodiphenyl ether on cellular metabolic responses. *Environ. Sci. Pollut. Res. Int.* 23, 5166–5178. doi: 10.1007/s11356-015-5762-2
- Westman, E. L., Yan, M., Waglechner, N., Koteva, K., and Wright, G. D. (2013). Self resistance to the atypical cationic antimicrobial peptide edeine of *Brevibacillus brevis* Vm4 by the N-acetyltransferase EdeQ. *Chem. Biol.* 20, 983–990. doi: 10.1016/j.chembiol.06010
- Wu, X., Ballard, J., and Jiang, Y. W. (2005). Structure and biosynthesis of the BT peptide antibiotic from *Brevibacillus texasporus*. *Appl. Environ. Microbiol.* 71, 8519–8530. doi: 10.1128/AEM.71.12.8519-8530.2005
- Xu, C., Qin, C., Zhang, R., Niu, W., and Shang, X. (2010). Solid-phase synthesis and antibiotic activities of cyclodecapeptides on the scaffold of naturally occurring Laterocidin. *Bioorg. Med. Chem. Lett.* 20, 164–167. doi: 10.1016/j.bmcl.11009
- Xu, Y., Wang, Q., Dong, M., Song, H., Hang, B., Sun, Y., et al. (2023). Evaluation of the efficacy of the antimicrobial peptide HJH-3 in chickens infected with *Salmonella Pullorum*. *Front. Microbiol.* 14, 1102789. doi: 10.3389/fmicb.2023.1102789
- Yang, X., Huang, E., and Yousef, A. E. (2017). Brevibacillin, a cationic lipopeptide that binds to lipoteichoic acid and subsequently disrupts cytoplasmic membrane of *Staphylococcus aureus*. *Microbiol. Res.* 195, 18–23. doi: 10.1016/j.micres.11002
- Yang, X., and Yousef, A. E. (2018). Antimicrobial peptides produced by *Brevibacillus* spp.: structure, classification and bioactivity: a mini review. *World J. Microbiology and Biotechnology* 34, 4. doi: 10.1007/s11274-018-2437-4
- Zhang, X., Zhang, B. X., Zhang, Z., Shen, W. F., Yang, C. H., Yu, J. Q., et al. (2005). Survival of the biocontrol agents *Brevibacillus brevis* ZJY-1 and *Bacillus subtilis* ZJY-116 on the spikes of barley in the field. *J. Zhejiang Univ. Sci. B* 6, 770–777. doi: 10.1631/jzus.2005.B0770
- Zhou, L. Y., Li, Y. F., Ji, C. Y., Liu, H. Y., Li, W., Xiang, F., et al. (2019). Identification of the pathogen responsible for tea white scab disease. *J. Phytopathol.* 168, 28–35. doi: 10.1111/jph.12862