Genomics-guided isolation and identification of active secondary metabolites of *Bacillus velezensis* BA-26

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**ABSTRACT**

Here we report the discovery and identification of antibacterial substances from the secondary metabolites of a new strain *Bacillus velezensis* BA-26. The whole genome of *B. velezensis* BA-26 was sequenced. Its genome data were annotated, and four potentially unknown gene clusters and nine known secondary metabolite synthetic gene clusters were analyzed and excavated by antiSMASH software. Based on the predicted secondary metabolites from the genome of strain BA-26, the material identification of isolated and purified extracellular secondary metabolites was conducted in combination with mass spectrometry data. A total of 24 antifungal compounds were identified, namely, iturin and various fengycins. The minimum inhibitory concentration (MIC) of iturin to *Botrytis cinerea* was 62.50 µg mL⁻¹. The MICs of C₁₄ fengycin A, C₁₆ fengycin A and C₁₆ fengycin A were 62.50, 31.25 and 0.49 µg mL⁻¹, respectively, indicating that the increase in the number of carbon atoms in side chain fatty acids of fengycin improves its biological activity. Two kinds of anti-disease fungal compounds from *B. velezensis* BA-26, namely, iturin and fengycin, were purified and identified; they were found to have antibacterial activities against common pathogenic fungi. Therefore, *B. velezensis* BA-26 may be potentially used as a biological control agent. This study enriches the genome information on *B. velezensis*, elucidates the active components of *B. velezensis* BA-26 antibacterial substances, and provides a useful reference for using strain BA-26 as a biological control agent.

**INTRODUCTION**

*Bacillus* has a wide range of applications in medicine, food, agriculture, and industry because of its ability to produce a variety of extracellular bioactive substances (1). Among these, many *Bacillus* strains have been developed into commercial-grade biological control agents (2). The extracellular bacteriocin active substances produced by *Bacillus* can be divided into antibacterial substances synthesized by the ribosomal pathway: bacteriocins, antibacterial proteins, chitinase, β-1,3-glucanase, and antibacterial substances synthesized by non-ribosomal pathways such as surfactins, iturin, fengycin, and bacillaene (3). Among these, bacteriocin and surfactins, iturin, fengycins, and antibacterial proteins have been extensively characterized (4).

*B. velezensis* is a gram-positive aerobic bacillus that produces bioactive substances such as bacteriocins and lipopeptides that promote plant growth and inhibit a variety of microbial pathogens (5,6). Investigations on the molecular structure, functional characteristics and application of *B. velezensis* antibacterial substances have mainly focused on a variety of bacteriostatic active substances, whereas research on a single substance is limited (5).

Recent studies have attempted to elucidate the antibacterial mechanism of *B. velezensis* using functional genomics analysis, using FZB42 as model strain (7,8). Genome mining of *B. velezensis* has identified secondary metabolite gene clusters of lipopeptides (9). Genomic analysis and mass spectrometry have revealed extracellular secondary metabolites of *B. velezensis* 9D-6 (10). Various research studies have shown that a large number of lipopeptides appeared in the

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extracellular secondary metabolites of *B. velezensis*, which had broad-spectrum antifungal activity (11,12).

*Bacillus* lipopeptides are usually composed of three family compounds, including iturins, surfactins and fengycins (12). Since iturin was isolated from *B. subtilis*, many lipopeptides belonging to the iturin family have been identified such as iturins A, B, C, D and E; bacilomycins D, F and L; and mycosubtilin (13). Recent studies show that other strains that produce iturin include *Fusarium sambucinum*, *Alternaria solani*, *Rhizoctonia solani*, *Botrytis cinerea*, *Botryosphaeria dothidea*, *Alternaria alternate* and *Aspergillus oryzae* were very strong, but had no bacteriostatic effect on *Staphylococcus aureus* and *Escherichia coli* (21). The strain BA-26 that we used in this study was isolated from the hillside of Zhangshiyan by our laboratory. The BA-26 strain was identified as *B. velezensis* by morphological, physiological and biochemical characterization and 16S rDNA sequence analysis (22). The indicator fungi was *Botrytis cinerea*, which is maintained at the Microbiology Laboratory of the Academy of Sciences of Hebei Province, China. Strain BA-26 was cultured in NB liquid medium (containing 10 g·L⁻¹ tryptone, 5 g·L⁻¹ beef extract, 10 g·L⁻¹ glucose, and 5 g·L⁻¹ NaCl in distilled water) at 32°C for 48 h and 180 rpm continuous shaking.

**Materials and methods**

Currently, we determined that *B. velezensis* BA-26 has a strong fungal inhibitory effect, but we have not identified which secondary metabolites work. Therefore, we performed whole-genome sequencing on strain BA-26 to obtain whole-genome data, and perform gene annotation on the genome data. On the basis of genomic data, we used antiSMASHv4.2 software to analyze the secondary metabolites contained in synthetic gene clusters and predicted the antibacterial substances. Then, the antibacterial substances in its extracellular secondary metabolites were separated, purified and identified to verify the predicted antibacterial substances.

**Strain and culture conditions**

*B. velezensis* BA-26 is an antagonistic strain isolated from the hillside of Zhangshiyan by our laboratory. The BA-26 strain was identified as *B. velezensis* by morphological, physiological and biochemical characterization and 16S rDNA sequence analysis (22). The indicator fungi was *Botrytis cinerea*, which is maintained at the Microbiology Laboratory of the Academy of Sciences of Hebei Province, China. Strain BA-26 was cultured in NB liquid medium (containing 10 g·L⁻¹ tryptone, 5 g·L⁻¹ beef extract, 10 g·L⁻¹ glucose, and 5 g·L⁻¹ NaCl in distilled water) at 32°C for 48 h and 180 rpm continuous shaking.

**DNA extraction, genome sequencing and assembly**

Genomic DNA of *B. velezensis* BA-26 was extracted using a bacterial genome kit (TIANGEN, Beijing, China). A 10-kb double-stranded DNA fragment was selected to construct the SMRTbell DNA library for sequencing. DNA sequencing was performed by Suzhou Jinweizhi Biotechnology Co., Ltd., and the whole genome of *B. velezensis* BA-26 was sequenced using an Illumina HiSeq 2500 system and the third-generation high-throughput Pacbio SMRT sequencing technique. The quality statistics software cutadapt v1.9.1 was used to optimize the removal of connectors and low-quality sequences of the second-generation sequencing raw data (23). The preliminary assembly results were obtained based on the three-generation sequencing data using the software HGAP4 v4.0 and Falcon v0.3. The quality-filtered second-generation sequencing data were compared to the preliminary assembly results, and the assembly results were further corrected using the software Pilon v1.2.2 to obtain the final assembly results (23).

**Genome annotation**

Based on the predicted protein sequence, using BLAST (24); the E-value of the sequence alignment was set
to $1 \times 10^{-5}$, and the sequence alignment length to $>60\%$ of the protein length. The optimal matching results were selected as the annotation results of the gene. The NR (25), GO (26), KEGG (27), COG (28) and CAzy (29) databases were used in functional annotation of genes. Prediction and analysis of gene clusters encoding secondary metabolites in $B. velezensis$ BA-26 were performed using the online software antiSMASH v4.2 (30).

**Separation and purification of antifungal secondary metabolites**

The fermentation broth cultured with strain BA-26 for 48 h was centrifuged at 10,000 g for 30 min, and ammonium sulfate solid was added to the cell-free supernatant to reach 60% saturation, left to stand at 4°C for 12 h, and then centrifuged at 8,000 g for 30 min to collect the precipitate. Then, the precipitate was dissolved in 5 mL of sterile water, and bacteriostatic activity was assessed. Here, the precipitate represented the antibacterial crude extract, which was further separated and purified using macroporous resin XAD-7HP (31) and washed with distilled water thrice. The samples were extracted at 42°C in a rotary evaporator and stored at 4°C until use.

The sample was further purified by RP-HPLC (SHIMADZU LC-20AT, Japan). Mobile phase A was HPLC-grade water containing 0.1% (volume ratio) trifluoroacetic acid, and mobile phase B was acetonitrile containing 0.1% (volume ratio) trifluoroacetic acid. The macroporous resin XAD-7HP separation samples were loaded into a C18 column (250 × 4.6 mm, 5 µm; WONDASIL, Japan), a 10%–90% acetonitrile linear gradient was employed for elution (0–60 min), 90% acetonitrile for isometric elution (60–66 min), 90%–10% acetonitrile linear gradient for elution (66–68 min), and 10% acetonitrile for isometric elution (68–78 min) at a flow rate of 1 mL·min$^{-1}$, a detection wavelength was 214 nm, and column temperature was 30°C. The corresponding components per minute were collected, and the components with antifungal activity were further screened for structural identification.

**LC-MS/MS analysis**

The samples with antifungal activity were analyzed by mass spectrometry using an XEVO-G2-Q-TOF-MS system (Waters Company). The conditions of UPLC were as follows: mobile phase A was acetonitrile containing 0.1% (volume ratio) formic acid, and mobile phase B was HPLC-grade water containing 0.1% (volume ratio) formic acid, Waters UPLC HSS T3 (C18; 2.1 mm × 100 mm, 1.7 µm), 10%–65% acetonitrile linear gradient for elution for 3 min, and 65%–100% acetonitrile linear gradient for elution for 3 min. The injection volume is 5.0 µL, the flow rate was 0.3 mL·min$^{-1}$, the detection wavelength was 214 nm, and column temperature was 40°C. The mass scanning range was 50–4,000 M/Z, the capillary voltage was 3,000V, the desolvent temperature was 400°C, nitrogen was used in all kinds of gas paths, positive ion mode analysis was employed, and Masslynx data management software was utilized for data acquisition.

**MIC analysis**

Four kinds of high-purity antifungal compounds ($C_{15}$ iturin A, $C_{14}$ fengycin A, $C_{16}$ fengycin A, and $C_{18}$ fengycin A) were isolated and purified for MIC analysis of $B. cinerea$. Four antifungal compounds with concentrations of 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 32.50, 125.00, 250.00 and 500.00 µg·mL$^{-1}$ were prepared in sterile water using the double dilution method (32), and 100 µL each was added to the agar pore. After culturing at a constant-temperature incubator at 26°C for 48 h, the half diameter of the inhibition area was measured to determine the inhibitory effect on $B. cinerea$. We used 70% aseptic nitrile aqueous solution as control, and each group was repeated thrice.

**Results**

**Genomic characteristics of $B. velezensis$ BA-26**

The whole genome of strain BA-26 was sequenced using the Illumina HiSeq 2500 system and the PacBio SMRT sequencing technique, resulting in 11,213,428 reads for 1,682,014,200 total bases, with approximately 416-fold coverage. There are 4,035,062-bp in the complete genome circular chromosomes of strain BA-26, with a GC content of 46.41% (Table 1 and Figure 1A). The genome sequence was predicted to comprise

| Attributes         | Value  |
|--------------------|--------|
| Genome size (bp)   | 4,035,062 |
| G + C content (%)  | 46.41  |
| Plasmid            | 0      |
| Predicted genes    | 3,927  |
| Protein-coding genes (CDSs) | 3,663 |
| rRNA genes         | 89     |
| tRNA genes         | 86     |
3,927 encoding genes, of which 3,663 were protein-coding genes (CDSs), 89 were rRNA operon genes, and 86 were tRNA genes. In addition, the repeat sequences in the genome were predicted from scratch, and three long terminal repeats, 22 long scattered repeats, 5 short scattered repeats, and 83 simple repeats were detected. No plasmid sequence was identified by genome assembly. Approximately 3,433 protein-coding genes in strain BA-26 have COG numbers. They are divided into five functional classes in the COG database and are mainly related to the transport and metabolism of carbohydrates, amino acid transport, and metabolism and transcription (Figure 1B). The complete genome sequence of *B. velezensis* BA-26 has been submitted to GenBank under the accession number CP046918.

**Functional annotation of genes using KEGG**

The *B. velezensis* BA-26 genome was annotated by the KEGG database, and a histogram was obtained according to the secondary classification and statistics of KEGG. The results showed that the genome of the strain BA-26 had the highest number of metabolism-related genes (1,762), environmental information processing-related genes (291), genetic information processing-related genes (233) and cell process-related genes (87) (Figure 2). The statistical results showed that various substance metabolism pathways and energy metabolism pathways provide the necessary nutrients and energy supply for the growth of the strain. There are many genes that are related to lipid metabolism, i.e. terpenoids and polyketones metabolized by strain BA-26, and there are many genes involved in the biosynthesis of other secondary metabolites, indicating that the strain may have the potential to synthesize a variety of lipopeptide substances, although it does not rule out the possibility of discovering other novel secondary metabolites.

**Functional annotation of genes using GO**

GO is a database of annotation and classification of gene and protein functions for a variety of species. According to the properties of the encoded products (Figure 3), the functions of sequenced genes could be divided into three categories: biological process, molecular function and cellular component. Metabolic and cellular processes play a major role in the biological processes of strain BA-26. Binding and catalytic activity functions have obvious advantages in the functional category of molecular function. Cell parts and membrane gene synthesis are major aspects of the cellular component functional category. The results (Figure 3) showed that 80% of the annotated genes may be involved in the synthesis and transport of secondary metabolites. Combined with the results of functional gene annotation, we elucidated the genomic
Figure 2. Functional annotation of secondary metabolite-related genes in B. velezensis BA-26 using KEGG metabolic pathway analysis.

Figure 3. GO functional classification of B. velezensis BA-26 genes.
basis by which strain BA-26 synthesizes a variety of secondary metabolites.

**Functional annotation of genes using CAZy**

CAZy is a database of enzymes related to the degradation, synthesis and modification of carbohydrates that describes the catalytic structure of the strain as well as functional information. We used the CAZy database to annotate the genes in the *B. velezensis* BA-26 genome. The results showed (Figure 4) that the number of glycosyltransferases (GT) which was noted in the genome of strain BA-26, was 256, and there are 164 genes encoding glycoside hydrolases (GHs), and 91 genes encoding carbohydrate-binding modules (CBMs). There are 35 genes encoding carbohydrate esterases (CEs), 11 genes related to auxiliary activities (AAs), and the polysaccharin lyases (PLs) are encoded by at least three genes. The metabolism of substances in the organism is achieved by a variety of special functions. As can be seen from the above statistical data, the GTs, GHs and CBM-related enzymes occupy a larger proportion and are involved in the synthesis of the secondary metabolites of the non-ribosomal pathway.

**Prediction and identification of the gene cluster of secondary metabolites**

The presence of 13 secondary metabolite gene clusters in the genome of *B. velezensis* BA-26 was predicted by the online software antiSMASH v4.2 (Supplemental Table S1). According to the differences in gene clusters encoding secondary metabolite synthases, there are five categories: transatpks, NRPS, terpenes, PKS and Other. Six gene clusters were identical to those known in macrolactin, bacilliaene, fengycin, difficidin, bacillibactin and bacilysin, all showing 100% similarity. Clusters 11, 10 and 12 were similar to known clusters, namely, surfactin (91% similarity), rhizoctin (22% similarity) and butirosin (7% similarity). In addition, four new clusters, namely, Clusters 4, 5, 8 and 13 were predicted, and no known clusters were matched. In conclusion, there are many kinds of antibacterial substances produced by strain BA-26, especially common lipopeptide substances, which can be studied in the next step with reference to the method of separation and purification of these substances.

**Purification of antifungal compounds and LC-MS/MS analysis**

The cell-free supernatant of strain BA-26 was precipitated with ammonium sulfate and purified by macroporous resin XAD-7HP, and the crude antifungal extract was obtained. The crude antifungal extract was isolated by RP-HPLC, and 11 kinds of active components with antifungal effect were identified. The purified components had strong antifungal activity against *B. cinerea*. To determine the structure of each active substance and find new antibacterial substances, these substances were identified by LC-MS/MS (32). The results showed that there were 24 kinds of antifungal compounds in the extracellular active substances of strain BA-26, all of which were lipopeptides. Compared
with previous studies (33), they are iturins and various fengycins (Supplemental Table S2). For iturin by secondary mass spectrometry (Figure 5A), the molecular ion peaks \([\text{M} + \text{H}]^+\) (\(m/z\) 1057.5698), \([\text{M} + \text{Na}]^+\) (\(m/z\) 1079.5515) and \([\text{M} + \text{K}]^+\) (\(m/z\) 1095.5308) were detected, and their molecular weight was determined to be 1,056. These results were compared to the reported iturin data (34) and identified as C\(_{15}\) iturin A.

In this study, 23 fengycin substances were detected within the \(m/z\) range of 1,435–1,520 (Supplemental Table S2). Fengycins A and B differed in structure, and the sixth amino acids of their cyclic peptides were Ala (89.1 Da) and Val (117.1 Da), respectively (19). Therefore, in the process of mass spectrometry, different fragment ion peaks were observed. The fragment ion peaks \(m/z\) (966/1,080) and \(m/z\) (994/1,108) are usually utilized as characteristic fragment ions to identify the two lipids fengycins A and B (19). By mass spectrometry analysis, a total of five fragment ion peaks \([\text{M} + \text{H}]^+\) (1,435.7806, 1,449.7924, 1,463.8096, 1,477.8257 and 1,491.8452) were detected. They all had characteristic fragment ion peaks \(m/z\) (966/1,080), which were identified as C\(_{14}\) fengycin A, C\(_{15}\) fengycin A, C\(_{16}\) fengycin A, C\(_{17}\) fengycin A and C\(_{18}\) fengycin A (Figure 5). Similarly, fragment ion peaks \([\text{M} + \text{H}]^+\) (1,449.7905, 1,463.8070, 1,477.7845, 1,441.8416, 1,505.8545 and 1,519.8677) were detected by mass spectrometry. They all had characteristic fragment ion peaks \(m/z\) (994/1108), which were identified as C\(_{13}\) fengycin B, C\(_{14}\) fengycin B, C\(_{15}\) fengycin B, C\(_{16}\) fengycin B, C\(_{17}\) fengycin B and C\(_{18}\) fengycin B (Supplemental Figure S1). In addition, fragment ion peaks \(m/z\) (952/1,066) and \(m/z\) (980/1,094) were detected during secondary mass spectrometry as characteristic fragment ions in the identification of fengycin subtypes (fengycins A\(_2\) and B\(_2\)) (19). The characteristic fragment ion peak \(m/z\) (952/1,066) was detected at fragment ion peak \([\text{M} + \text{H}]^+\) (1,435.7767), which was identified as C\(_{14}\) fengycin A\(_2\) (Supplemental Figure S2), and the characteristic fragment ion peak \(m/z\) (980/1,094) was detected at fragment ion peak \([\text{M} + \text{H}]^+\) (1,449.7965, 1,463.8070, 1,477.8143 and 1,491.8419), which were C\(_{13}\) fengycin B\(_2\), C\(_{14}\) fengycin B\(_2\), C\(_{15}\) fengycin B\(_2\) and C\(_{16}\) fengycin B\(_2\), respectively (Supplemental Figure S3). Compared with the results of previous studies, it was found that there were also unsaturated fatty acids in the fengycin family (19, 33). Among these, C\(_{15:1}\) fengycin A, C\(_{15:1}\) fengycin A\(_2\), C\(_{17:1}\) fengycin A, C\(_{15:1}\) fengycin A, C\(_{14:1}\) fengycin B\(_2\), C\(_{15:1}\) fengycin B\(_2\), C\(_{16:1}\) fengycin B and C\(_{16:1}\) fengycin B (Supplemental Figure S4) were compared to known fengycins, their mass decreased by 2 Da, and unsaturated double bonds were detected (35).

**MIC of iturins and fengycins against B. cinerea**

MIC analysis was performed on the lipopeptide antifungal compounds iturin and fengycin that were isolated and purified from strain BA-26. The MIC of iturin was 62.50 \(\mu\text{g} \cdot \text{mL}^{-1}\) (Supplemental Table S3).
The results of MIC and determination of three different fengycin substances showed that the MIC of all fengycin substances to *B. cinerea* was ≤ 62.50 μg·mL⁻¹ (Supplemental Table S3). Comparison of the MICs of the three kinds of fengycin (C₁₄ fengycin A, C₁₆ fengycin A and C₁₈ fengycin A) against *B. cinerea* revealed a gradual increase in their antifungal activities.

**Discussion**

In recent years, the newly proposed *B. velezensis* has gradually increased in the natural environment (11,12), and in addition to its practical application as biocontrol agents, the active substances produced by *B. velezensis* strains have anti-bacterial, anti-fungal and anti-viral properties (5). As of November 2019, all or part of the gene sequencing data of 263 *B. velezensis* have been submitted to the NCBI database. The genome sequencing data of *B. velezensis* continue to increase, and genome mining has facilitated the identification of antibacterial substance synthesis-related gene clusters that may be utilized in the development of various antibacterial substances (9,10). Therefore, genome sequencing and analysis of the novel strain *B. velezensis* BA-26 is essential and may allow the identification of novel gene clusters related to the synthesis of secondary metabolites.

Whole-genome sequencing of *B. velezensis* BA-26 has identified 136 genes that are related to the biosynthesis, transport and catabolism of secondary metabolites, which is greater than that in *B. amyloliquefaciens* WS-8 (110 genes) (35) and *B. subtilis* BSD-2 (98 genes) (36). The antibacterial substance genes detected in *B. amyloliquefaciens* WY047 are *fenA*, *ituA*, *bmyD*, *hag*, *mrsA* and *tasA* (37). So far, it has been reported that most *Bacillus* strains produce not more than three or four antibacterial compounds (38). Using the online software antiSMASH v4.2, we identified nine gene clusters that may be utilized in the development of various antibacterial substances (9,10). Therefore, genome sequencing and analysis of the novel strain *B. velezensis* BA-26 is essential and may allow the identification of novel gene clusters related to the synthesis of secondary metabolites.

Eleven antifungal components were isolated by RP-HPLC, and LC-MS/MS identification showed that there were 24 kinds of antifungal compounds (Supplemental Table S1), which were largely iturins and fengycins, and both had strong antagonistic effects on *B. cinerea*. Generally speaking, the antifungal activity of fengycins is stronger than iturins, whereas its hemolytic effect is weak (39). Fengycins were first reported in *B. subtilis* (40) and have been shown to induce plant immune responses (41). A variety of fengycin variants have been identified based on the four heterogeneous sites existing in the fengycin family (19). In this study, in the fengycin family that was identified in the *m/z* 1,435–1,520 range, we detected four subtypes of fengycin, namely, A, B, A₂ and B₂. By comparing the ion peaks of protonated molecules observed by mass spectrometry, we found that the fengycin class had a molecular mass difference of 14 Da and with a chain length varying from C₁₃ to C₁₈. Similar to previous studies, the presence of unsaturated fatty acids in some fengycins with chain lengths ranging from C₁₄ to C₁₇ resulted in a 2-Da reduction in molecular weight (19, 42). It has been reported that there are also unsaturated double bonds in many fengycin derivatives (33). Liu *et al.* found that the main active substances in *B. amyloliquefaciens* WS-8 were iturin and fengycin (35). In this context, our study elucidated the active components of the antifungal substance of strain BA-26.

A previous study has shown that the antifungal compounds of strain BA-26 had a strong inhibitory effect on 12 pathogenic fungi (21). In this study, the MICs of iturin and fengycin that were isolated and purified from the strain BA-26 were determined, and both substances showed strong antifungal activity compared with other studies (32). In addition, the antifungal activity of fengycin was higher than that of iturin analogues (Supplemental Table S3), which was similar to the findings of a previous report (18). At least for surfactins, it is known that increasing the acyl chain length results in stronger bilayer solubilization and membrane activity (43). For iturins, the antifungal activity is generally enhanced with increasing number of carbon atoms on the fatty acid side chains, presumably due to stronger interactions with biomembranes (44). By comparing the MICs of C₁₄ fengycin A, C₁₆ fengycin A and C₁₈ fengycin A against *B. cinerea*, we determined that the increase in the number of side chain fatty acid carbon atoms in fengycin increases their biological activity. To our
knowledge, this is the first report on the comparison of antifungal activity of different fengycin substances. Some studies have shown that the pairwise combinations of different types of lipopeptide, such as surfactants, iturins and fengycins, also have antifungal synergistic effects, although their combined application for prevention and treatment requires further investigation (20).

In conclusion, based on the measured whole genome data of *B. velezensis* BA-26, 13 secondary metabolite synthetic gene clusters were identified by bioinformatic analysis, of which 4 belonged to potentially unknown gene clusters. In addition, 24 antifungal compounds were identified by mass spectrometry, and the structures of iturins and antifungal compounds of fengycin derivatives were revealed. In this study, by determining the MIC of iturin and different fengycin substances on *B. cinerea*, we determined that the antifungal substances derived from strain BA-26 had obvious inhibitory effects on pathogenic fungi, indicating that strain BA-26 could be potentially used as an effective biological control agent. In the future, the synergistic antifungal effects of iturins and fengycins can also be studied to provide a reference for the combined control of various antifungal substances.

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**Author contributions**

L.H.W and Z.L.P designed this experiment. W.B.Q, L.C, L.H.W, Y.X.M, W.Y.N, Z.F.Y, and C.H.C executed the experiment. W.B.Q, L.H.W and L.C performed the analysis and wrote the paper. All the authors have read and approved the final manuscript.

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**Disclosure statement**

The authors have no financial conflicts of interest to declare.

**Data availability**

All data that support the findings from this study are available from the corresponding author (...) upon reasonable request.

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