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Full Length Article

Fengycin Controls the Apple Blue Disease Caused by *Penicillium expansum*

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Abstract

The *fen*C gene deletion mutant of *Bacillus amyloliquefaciens* BA-16-8 was constructed to investigate the function of fengycin in controlling apple blue mold disease. The upstream and the downstream sequences of the *fen*C gene were cloned through PCR by using the genome of *B. amyloliquefaciens* BA-16-8 as a template in accordance with the homologous recombination principle. The recombinant plasmid pMAD-Δ*fen* was constructed by connecting the upstream and the downstream homologous regions of *fen*C with the spectinomycin resistance gene *spc* and then transformed into *B. amyloliquefaciens* through electroporation. The *fen*C knockout mutant strain BA-16-8-Δ*fen* was subcultured continuously at 42°C and selected though blue–white colony screening, high-temperature screening, and antibiotic resistance tests. The inhibitory effect of the fengycin-deficient mutant BA-16-8-*Δfen* on apple blue mold disease was tested via high-performance liquid chromatography, mass spectrometry, and *Penicillium expansum* growth inhibition assay *in vitro*. Compared with the wild-type *B.amyloliquefaciens* BA-16-8 strain, the *B. amyloliquefaciens* BA-16-8-*Δfen* mutant lost its fengycin-synthesizing capability and showed a significantly reduced inhibitory effect against *P. expansum*. This study concludes that Fengycin is the key component of *B. amyloliquefaciens*BA-16-8 in the control of blue mold disease. © 2020 Friends Science Publishers

Keywords: *Bacillus amyloliquefaciens*; Blue mold disease; Fengycin; Homologous recombination; Mutant

Introduction

The apple blue mold disease is the most common postharvest disease of apples, and its pathogenic fungus is *Penicillium expansum* (Palou *et al.* 2016). Collisions between fruits occur as they are stored or transported and can lead to mechanical damage. At this time, the *P. expansum* spores originally lurk on fruit surfaces and invade the fruits through wounds and produce mycelia. Mycelia cause fruit decay and produce the secondary metabolite patulin, which endangers human health and causes serious food safety problems (Al-Rawashdeh *et al.* 2015). The use of chemical fungicides, such as grim zine and flusilazole, to prevent the postharvest apple blue mold disease of apples has quick and good effects, but their long-term use can easily cause pathogenic fungus to develop drug resistance and endanger food safety. With the continuous increase in the people's awareness of environmental protection, biological fungicides are increasingly used in the prevention and treatment of plant diseases (Spadoni *et al.* 2015; Spadaro and Droby 2016). Among biological fungicides, *Bacillus* spp. is known for its high environmental tolerance and the production of various peptides and lipids. The characteristics of fungicidal substances such as lipopeptides have become the current hotspots of research on biological fungicides. *Bacillus amyloliquefaciens* can produce dozens of lipopeptide antibiotics. The lipopeptide antibiotics produced by *B. amyloliquefaciens* are divided into three families, namely, suractin, iturin, and fengycin, in accordance with their amino acid configurations. Iturin has strong antifungal activity. Fengycin can substantially inhibit the growth of filamentous fungi. Surfactin has strong surfactant activity, and its strong emulsifying and foaming capabilities can reduce the surface tension of liquids effectively. In addition, suractin has hemolytic, antiviral, antibacterial, and other biological activities (Wu *et al.* 2005; Walia and Cameotra 2015; Malmsten 2016). Given their important biological activities, many lipopeptides have been isolated and identified from *Bacillus* strains, and their biological functions have been elucidated at the genetic level.

B. amyloliquefaciens BA-16-8, an antagonistic bacterium (Fu *et al.* 2020), is effective in inhibiting *Penicillium* spp. lipopeptide antibiotics. Testing the capability of these two substances to inhibit the performance of *P. expansum* has revealed that fengycin is the main substance in *B. amyloliquefaciens* BA-16-8 that inhibits

P. expansum. This study intends to use molecular genetics technology to construct the fengycin deletion mutant of *B. amyloliquefaciens* BA-16-8 to further verify this conclusion. Moreover, this study aims to combine antigungal experiment and fruit biocontrol experiments to confirm that the fengycin in *B. amyloliquefaciens* inhibits *P. expansum*. This study could lay a foundation for exploring the antibacterial mechanism of *B. amyloliquefaciens*.

Materials and Methods

Materials

The wild-type *B. amyloliquefaciens* BA-16-8 strain was collected through laboratory breeding. The fengycin gene expression deletion mutant *B. amyloliquefaciens* BA-16-8 (*Δfen*) was constructed. The pathogenic fungus *P. expansum* was obtained from the Shaanxi Institute of Microbiology. *Escherichia coli* DH5α and pMAD were procured from Takara. pMAD-*Δfen*, which was *B. Amyloliquefaciens* with a *fenC* deletion (its promoter structure) and pMAD, was constructed in this work. Genome extraction kits, Taq DNA polymerase, Deoxynucleotides (Deoxynucleotide triphosphates (dNTPs), restriction enzymes, and DNA markers were purchased from the Neb Company. PCR product purification and plasmid extraction kits were acquired from Takara. Acetonitrile, trifluoroacetic acid, and methanol were purchased from Sigma.

The Agilent 1100 series high-performance liquid chromatography (HPLC) system was bought from Agilent. The liquid chromatography–electrospray mass spectrometer system comprised the Waters Alliance 2690 HPLC apparatus (Waters Company, U.S.A.) and the TSQ Quantum Discovery A three-stage quadrupole mass spectrometer (Thermo Fisher Scientific, U.S.A.). The primers used to amplify the upper and the lower arms of the fengycin synthase C gene (7647 bp) of *B. amyloliquefaciens* were designed on the basis of the genomic sequence of *B. amyloliquefaciens* Q426 strain in NCBI, and the primers for the resistance gene *spc* were based on the plasmid. The design of PUS19 was completed using the Primer Premier 5.0 software. The specific information is shown in Table 1. Primer synthesis and sequence determination were completed by Shanghai Shengong.

Beef extract peptone (BEP) medium was specifically formulated in reference to the literature (Afsharmanesh *et al.* 2014). The BEP agar was prepared in plate form, and the BEP broth was prepared in the form of liquid medium and used for the cultivation of antagonistic bacteria. Potato dextrose agar and broth were formulated in reference to the literature (Afsharmanesh *et al.* 2014) and used for the cultivation of pathogenic bacteria.

Shuttle plasmid pMAD constructs for the deletion mutant BA-16-8*Δfen*

A genomic DNA extraction kit was utilized to extract the genomic DNA of BA-16-8, which was used as a template, and P1/P2 were used as primers to amplify the upstream *fenC* fragment with length of 1844 bp. The amplified fragment was applied as the upstream homology arm, and P3/P4 were utilized as primers to amplify the downstream fragment with an amplification length of 1645 bp for use as downstream homology arm. In reference to the literature (Avrahami and Shai 2003; Arnaud *et al.* 2004; Ongena *et al.* 2007), the plasmid PUS19 sequence was used as a template. The P5/P6 primers of the mycin resistance gene were designed, and the spectinomycin resistance gene *spc* with an amplification length of 1150 kb was amplified. The product obtained through PCR amplification was digested with restriction enzymes and was recovered via gel electrophoresis, ligated into the relevant restriction enzyme sites of the pMAD plasmid, transferred into *E. coli* DH5α, screened to obtain pMAD-ΔfenC, and sequenced. The sequence correctness of the constructed mutant was verified. The homologous recombination process is shown in Fig. 1.

The method of Arnuad (Arnaud *et al.* 2004) was used for mutant screening to verify the correctness of the obtained pMAD-ΔfenC mutant after sequencing, and the electrical conversion method was performed under the following conditions: voltage, 2 kV; capacitance, 25 μF; and resistance, 100 Ω. The mutant was transferred into *B. amyloliquefaciens* BA-16-8, and the fengycin synthase gene deletion mutants were screened.

The gene knockout vector pMAD-Δfen was transferred into competent *E. coli* DH5α cells, placed onto a BEP plate containing 100 μg mL^{-1} X-gal, and cultured at 30°C for 24 h. At this time, the competent cells can express the *lacZ* gene if pMAD-Δfen was present or free in the cell or a single exchange had occurred. Thus, the growth of blue colonies on the plate indicated that both transformants were successful.

The selected blue colonies were transferred into BA-16-8 liquid medium, cultured at 42°C and 180 rpm, and shaken for 24 h. The bacterial cultures were cultured into fresh 50 μg mL^{-1} spectinomycin BEP liquid medium at 42°C and shaken at 180 rpm for 12 h. The temperature was reduced to 30°C, and shaking was continued for 12 h at 180 rpm. The culture broth was transferred onto a BEP plate containing 100 μg mL^{-1} X-gal and 50 μg mL^{-1} spectinomycin and incubated at 30°C for 24 h. White colonies were selected and transferred onto a BEP plate containing 3 μg mL^{-1} erythromycin. A *Mycobacterium*-sensitive strain was defined as the mutant strain BA-16-8*Δfen* with a deleted *fenC* gene.

For the identification of mutants, BA-16-8*Δfen* was used as a template, P1/P4 and P7/P8 were used as primers, and PCR was performed using BA-16-8 as a negative control. The resulting product was subjected to agarose gel electrophoresis. The size of the target band was checked for consistency with the predicted size. Bands of the same size indicated that the *spc* gene had successfully replaced the *fenC* gene, that is, the mutant was successfully constructed.

Table 1: Primer information

Primer	Primer Sequence (5′→3′)	Target fragment	Restriction site	Size (bp)
P1	CGCGGATC CGCAGATACGCCGAAGCAC	*fen*C Upstream arm	BamH I	1844
P2	CGCACGCG TCCGCAACGACGCCATTAG		Mlu I	
P3	CGC ACGCGTAAAACAGGTCTGCCGCTAT	*fen*C Downstream arm	Mlu I	1645
P4	CGCGAATTC GGTGACAAACGCAGTGAAT		EcoR I	
P5	CGCACGCG TTAGTCACTGTTTGCCACATTCG	*spc* gene	Mlu I	1146
P6	CGCGAATTC TGGTTCAGCAGTAAATGGTGG		EcoR I	
P7	TCTAATACGAATCGATACAC	*fen*C gene		7647
P8	AAAGGAGTGATTATGGCTCT			

Table 2: HPLC elution conditions

Time min	Surfactin			Fengycin		
	Acetonitrile (0.1%TFA)	Water (0.1%TFA)	Flow rate (mL/min)	Acetonitrile (0.1%TFA)	Water (0.1%TFA)	Flow rate (mL/min)
0	20	80	0.8	60	40	1
5	50	50	0.8	60	40	1
15	65	35	0.8	90	10	1
25	65	35	0.8	90	10	1

Otherwise, the mutant was unsuccessfully constructed. Finally, the PCR product was recovered and sequenced for verification.

HPLC of the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains

For the preparation of the crude extracts of strain metabolites, the fermentation broths of wild and mutant strains cultured for 24 h were centrifuged at 8000 rpm for 20 min at room temperature. The precipitate was discarded, and the resulting supernatant was placed in a sterile Erlenmeyer flask. The pH of the extract was adjusted to 2.0 by using 7 mol L^{-1} HCl. An aliquot was placed into 10 mL sterile centrifuge tubes (10 mL each) under aseptic conditions, incubated at 4°C overnight, and centrifuged at 10 000 rpm for 20 min. The precipitate was collected and mixed with 0.5 mL neutral methanol solution. This step was performed twice. The resulting extracts were combined, concentrated five times, and filtered through a 0.2 μm filter membrane to obtain the crude extract.

For the separation and purification of lipopeptide antibiotics by HPLC, the detection wavelength, column temperature, and injection volume were set to 280 nm, 30°C, and 10 μL, respectively, and the samples were analyzed through gradient elution. The elution conditions are shown in Table 2. The components were collected and concentrated using a rotary evaporator until use.

For the detection of the antibacterial activity of the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains against *P. expansum*, each concentrated solution (200 μL) of the wild and the mutant strains was purified using HPLC and detected using the Oxford cup method. The lipopeptide of the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains antagonized the ability of *P. expansum*. The culture

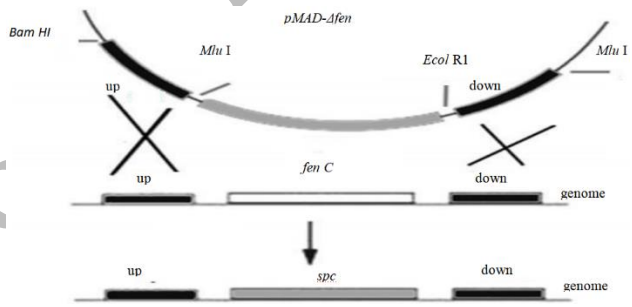

Fig. 1: Schematic diagram of homologous recombination process

temperature and time were 28°C and 5 days, respectively. The diameter of the zone of inhibition around the Oxford cup was measured and recorded. The experiment was performed using sterile water as the control, and the experiments were repeated thrice.

Control of apple blue mold disease by the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains

A *P. expansum* spore suspension was prepared in reference to the literature (Shi *et al.* 2015). The fruit biocontrol test (Fu *et al.* 2015; Zhang *et al.* 2015) was used to determine the effect of the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains on the control of apple blue mold disease. A total of 100 red Fuji apples were used as samples. The selected apples had the same size and maturity stage. The apples were sterilized with 75% ethanol and washed with water, and the surface of each apple was punched with a hole with a diameter of 6 mm and a certain depth by using a sterile punch. The apples were divided into five groups. The components in each group are shown in Table 3. The components in each group were treated into the apples.

Table 3: Components in each group

Group	Ingredient A (10 μL)	Ingredient B (10 μL)
1	*B. amyloliquefaciens* BA-16-8 bacterial suspension	*P. expansum* spore solution
2	*B. amyloliquefaciens* BA-16-8 lipopeptide Crude extract	*P. expansum* spore solution
3	*B. amyloliquefaciens* BA-16-8Δfen bacteria suspension	*P. expansum* spore solution
4	*B. amyloliquefaciens* BA -16-8*Δfen* lipopeptide crude extract	*P. expansum* spore solution
5	sterile water	*P. expansum* spore solution

Each treatment group included 20 apples. The treated apples were placed in an incubator controlled at 28°C and 95% humidity. After 96 h, apple infection was observed, and mycelial growth was quantified.

Results

Construction and screening of *B. amyloliquefaciens* BA-16-8*fenC* gene deletion mutants

Four primers were designed on the basis of the upstream and downstream sequences of the *fenC* gene of the first fengycin synthetase operon of the known strain *B. amyloliquefaciens* Q426 on NCBI, and the *B. amyloliquefaciens* BA-16-8 genome was used as template to amplify the upstream (upstream arm) and the downstream (downstream arm) sequences of *fenC*. The sequencing results showed that PCR amplification yielded an upstream sequence with a length of 1844 bp and a downstream sequence with a length of 1645 bp.

The spectinomycin resistance gene *spc* was selected to replace the fengycin synthase C gene to construct a deletion mutation vector. A 1146 bp band was obtained with the designed *spc* gene primers and PCR amplification. Each band was cut with restriction enzymes, and ligases were used for connection to the pMAD vector in the order of "upstream arm, spc, downstream arm" individually to construct the fengycin C synthase gene deletion mutation vector pMAD-Δfen. Δfen and pMAD were used as templates, and the vectors were identified through PCR using primers P1/P2, P3/P4, and P5/P6. Results showed that the PCR products with pMAD-Δfen as the template contained the upstream and the downstream sequences and the *spc* resistance gene. The absence of pMAD as a template indicated that the vector was successfully constructed.

The fengycin C synthase gene deletion mutant BA-16-8-*Δfen* was constructed in reference to the method of Arnuad and transferred into *B. amyloliquefaciens* BA-16-8 via electro transformation. Blue and white spots were screened. Positive strains were selected and subjected to plasmid extraction and enzyme digestion. After double exchange at 30°C and high-temperature plasmid loss, the fengycin synthase C gene deletion mutants were finally selected.

PCR was performed using the BA-16-8ΔfenC as the template, P1/P4 and P7/P8 as primers, and BA-16-8 as the negative control to identify whether the mutant was constructed successfully. The resulting product was subjected to agarose coagulation. The resulting gel electrophoresis bands are shown in Fig. 2. By using primers P1/P4, up–fenC–down fragments with sizes of 11 kb were obtained from BA-16-8, and up–spc–down fragments with sizes of 4.6 kb were obtained from BA-16-8ΔfenC. Using primers P7/P8, a fragment with a size of 7.6kb (fenC) was obtained from BA-16-8, and no amplified fragment was obtained from BA-16-8ΔfenC. The above results indicated that the *fenC* gene of BA-16-8ΔfenC in the mutant strain BA had been knocked out. The PCR product was purified and submitted to a company for sequencing. Results further confirmed that the *fenC* gene in BA-16-8 had been replaced by the *spc* gene.

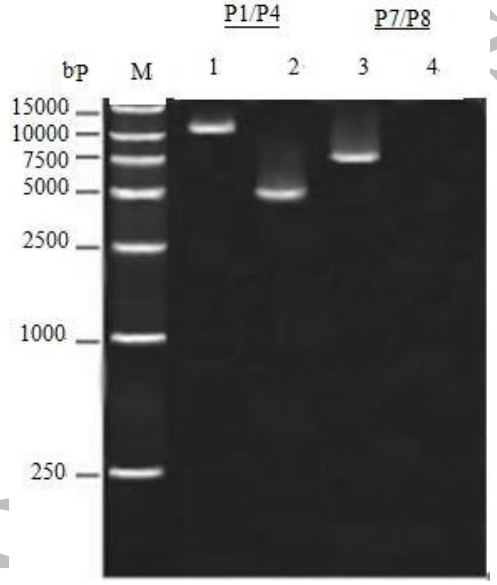

Fig. 2: PCR detection of mutant *B. amyloliquefaciens*BA-16-8*Δfen*
Note: M represent DNA marker (15000); Lane 1 represent the fragment amplified by PCR with P1/P4, taking BA-16-8 genome as template; Lane 2 represent the fragment amplified by PCR with P7/P8, taking BA-16-8 genome as template; Lane 3 represent the fragment amplified by PCR with P1/P4, taking *B. amyloliquefaciens*BA-16-8*Δfen* genome as template; Lane 4 represent the fragment amplified by PCR with P7/P8, taking *B. amyloliquefaciens*BA-16-8*Δfen* genome as template

HPLC of the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains

The crude extracts of the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains were separated and purified using HPLC, and the resulting fragments are shown in Fig. 3. Two groups of substances (a, b) were isolated from the wild-type BA-16-8 strain. The retention times of substances a

and b were 21.360 and 41.260 min, respectively. Substance b was isolated from the mutant strain, and its retention time was 21.370 min. The surfactin control and fengycin were isolated under the same elution conditions used to isolate standard samples. The substances isolated from the wild-type BA-16-8 strain were speculated to be surfactin and fengycin, and the substances isolated from the mutant BA-16-8*Δfen* strain were fengycin.

The materials for HPLC separation and purification were collected, concentrated, and made up to a volume of 1 mL, and the antibacterial activity of each fragment was measured through the Oxford cup method. Results are shown in Table 4. Only component b had significant antagonistic activity. The mutant strain that had lost the capability to synthesize fengycin showed a significantly decreased capability to inhibit *P. expansum*, and its cell-free fermentation broth almost lost its antibacterial performance. The comprehensive HPLC, mass spectrometry (MS), and antibacterial performance analysis results demonstrated that the fengycin from the 16-8 strain inhibited *P. expansum*.

MS

BA-16-8 was detected and analyzed through time-of-flight MS, and the relative molecular mass of each lipopeptide in the crude extract was obtained. The resulting mass spectrum is shown in Fig. 4, and results are shown in Table 5. The mass spectrum in Fig. 4A had two series of ion peaks. Combining the mass spectrum data with the [M + H] +, [M + Na] +, and [M + K] + ion analysis results in Table 5, the substances were identified as members of the suractin and fengycin homolog families. The series of ion peaks in Fig. 4B in combination with the [M + H] +, [M + Na] +, and [M + K] + ion analysis results in Table 5 indicated that the substances were homologs of the suractin family. Combining the PCR results with the HPLC results revealed that the antibacterial lipopeptides extracted from the fermentation broth of the wild-type BA-16-8 strain were fengycin and suractin, and the antibacterial lipids were extracted from the fermentation broth of the mutant BA-16-8*Δfen* strain. The peptide was surfactin, indicating that the mutant did not produce fengycin and that the *fenC* gene deletion mutant was successfully constructed.

***P. expansum* controls by the wild-type BA-16-8 and the mutant BA-16-8*Δfen* strains**

The results of the control effects are shown in Table 6. After 96 h, the bacterial suspension of the wild-type BA-16-8 strain and the cell-free fermentation broth can strongly inhibit the growth of *P. expansum* on the surfaces of apples. The mutant BA-16-8*Δfen* strain with *fenC* gene knockout had a significantly lower control effect than the wild-type strain. In particular, the lesion diameter under treatment with the cell-free fermentation broth (group 4) of BA-16-8*Δfen* was almost the same as that under treatment with the control. This result indicated that the cells and lipopeptide extracts of the wild-type BA-16-8 strain can effectively prevent postharvest apple blue mold disease, and the mutant BA-16-8*Δfen* strain can neither synthesize fengycin nor inhibit *P. expansum* after losing the capability to synthesize fengycin. The disease prevention capability was also significantly reduced, providing evidence that *B. amyloliquefaciens* BA-16-8 antagonized pathogenic *P. expansum* and that the main substance for the prevention and treatment of apple blue mold disease was fengycin.

Table 4: Antagonistic effect of fractions from wild type and mutant of BA-16-8 against *P. expansum*

Strain	Diameter of inhibition zone (mm)	
	a	b
*B. amyloliquefaciens*BA-16-8	0.1± 0.25	6.68 ± 0.12
*B. amyloliquefaciens*BA-16-8Δfen	0.1± 0.17	—

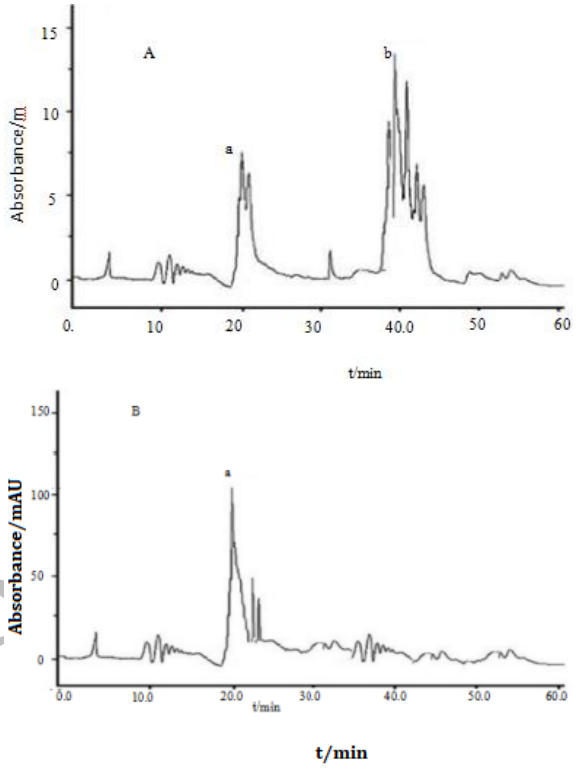

Fig. 3: HPLC spectra of wildtype strain *B. amyloliquefaciens*BA-16-8 (A) and mutant *B. amyloliquefaciens* BA-16-8Δfen (B)

Discussion

Aiming the biological control of apple blue mold disease, a strain of amyloid *Bacillus* BA-16-8, which can effectively inhibit the pathogen *P. expansum*, was bred in the laboratory, and the component conferring an effect of this strain was isolated and purified. The antifungal activities of the active substances were compared, and the inhibitory effect of fengycin on *P. expansum* was significantly higher than that

Table 5: Mass spectrometric analysis of antifungal compounds from the cell-free supernatants of BA-16-8 and BA-16-8Δfen

Strain	Fraction	Experimental charge-mass ratio (m/z)	Theoretical charge-mass ratio (m/z)	Intensity (%)	Structure assignment
BA-16-8	Surfactin	1030.54	1030.4	47	C13-Surfactin, [M+Na]+
		1044.67	1044.56	24	C14-Surfactin, [M+Na]+
		1058.78	1058.88	49	C15-Surfactin, [M+Na]+
	Fengycin	1435.61	1435.58	36	C14-FengycinA,[M+Na]+
		1449.74	144974	74	C15-FengycinA [M+Na]+
		1463.75	1463.78	56	C16-FengycinA, [M+H]+
		1477.78	1477.82	58	C17-FengycinA, [M+H]+
		1491.82	1491.83	66	C16-FengycinB [M+H]+
		1505.64	1505.65	40	C17-FengycinB, [M+H]+
		1519.91	1519.90	16	C18-FengycinB, [M+H]+
BA-16-8*Δfen*	Surfactin	1030.54	1030.4	55	C13-Surfactin, [M+Na]+
		1044.67	1044.56	45	C14-Surfactin, [M+Na]+
		1058.78	1058.88	58	C15-Surfactin, [M+Na]+

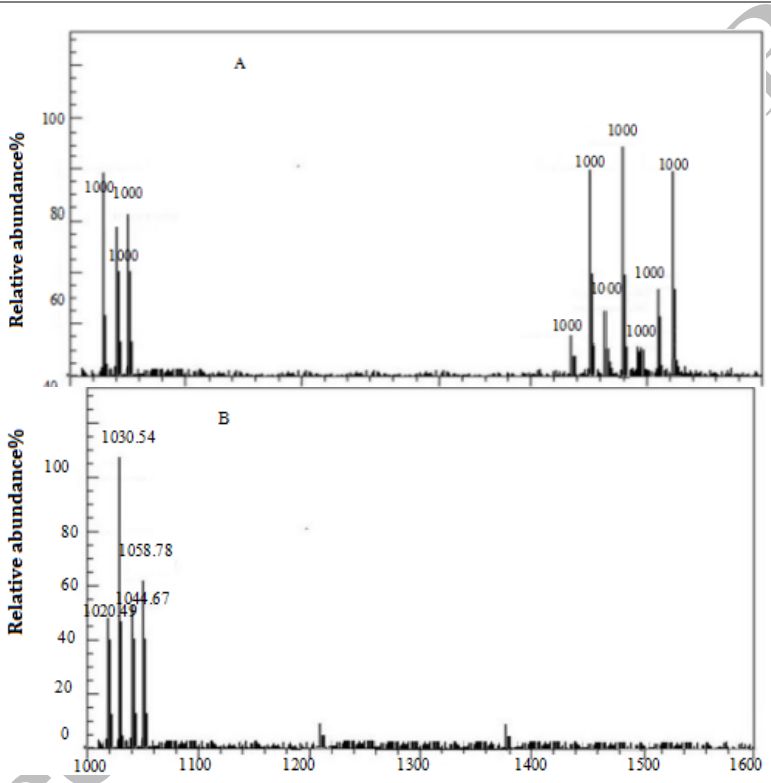

Fig. 4: Mass spectrometric result of antifungal compounds from *B. amyloliquefaciens* BA-16-8 (**A**) and *B. amyloliquefaciens* BA-16-8Δfen (**B**)

of suractin. Therefore, fengycin may be the main component produced by *B. amyloliquefaciens* to inhibit *P. expansum.*

In this study, B. *amyloliquefaciens*BA-16-8 was used as the experimental object to confirm this speculation which fengycin is the main substance to inhibit *P. expansum*. On the basis of the principle of homologous recombination, the mutant strain BA-16-8*Δfen* with a functional fengycin synthase gene defect was constructed with the help of a temperature-sensitive plasmid pMAD. After PCR, electrophoresis analysis, and sequence determination, the fengycin synthase C gene was finally determined to be knocked out successfully. Detecting the antibacterial activity of lipopeptide proteins produced by mutant and wild strains *in vitro* and *in vivo* revealed the mutation of *B. amyloliquefaciens*. The daughter BA-16-8*Δfen* lost its capability to synthesize fengycin, inhibit *P. expansum*, and control apple blue mold disease. Thus, we determined that fengycin was the main substance that inhibited *P. expansum*.

Reports have shown that fengycin can inhibit a variety of plant pathogens especially filamentous fungi. However, its specific mechanism of action remains divergent. Some reports have shown that fengycin can destroy the structure and permeability of bacterial cell membranes and the cell walls of pathogenic fungi. The lipid layer of the cell is disrupted, causing the cell structure to be destroyed (Tanaka *et al.* 2014). Fengycin also contains intracellular

Table 6: The effect of different treatments on controlling apple blue mold rot decay

Treatment	Diameter of disease decay（cm）	Growth of pathogenic fungi
Processing group 1	0.01 ± 0.002	Sterile silk
Processing group 2	0.02 ± 0.006	Sterile silk
Processing Group 3	1.36 ± 0.011	Obvious hyphae
Processing Group 4	1.48 ± 0.024	Obvious hyphae
Control group	1.50 ± 0.036	Obvious hyphae

substances, such as nucleic acids (Tao *et al.* 2011), that can act on pathogenic fungi. However, these claims have yet to be investigated and confirmed.

Reports have shown the presence of many clustered genes in the genome of *B. amyloliquefaciens* that are used to encode antibacterial peptides and other antifungal substances, including bacteriocins and lipopeptide antibiotics. However, this finding does not mean that the same bacterium is in the process of growth and metabolism. The bacterium can produce all antifungal substances at the same time, and certain genes used to encode or start antibiotic synthesis must be expressed normally under certain specific conditions or stages (Ji *et al.* 2013). The gene clusters in the genome of *B. amyloliquefaciens* contain multiple types of lipopeptide antibiotics. These clusters include *sfp*, which encodes suractin; itu, which encodes iturin; and fen, which encodes fengycin. Testing the antibacterial properties of the lipopeptide of BA-16-8 and BA-16-8 *⊿fen* revealed that fengycin had a considerable inhibitory effect on *P. expansum*.

Conclusion

This study concludes that the *B. amyloliquefaciens* BA-16-8 could inhibits the growth of *P. expansum* and could be the biocide to control apple blue mold disease. The substance that plays a key role in the process of *P. expansum* inhibition is fengycin. However, the exact mechanism of fengycin about its inhibit effect on the plant disease remain unknown. After determining the key role of fengycin in the prevention of blue mold disease caused by *P. expansum*, we will carry out research on the antagonistic mechanism of fengycin on *P. expansum* to provide a theoretical basis for the development and utilization of antibiotic lipopeptides.

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Author Contributions

Ruimin Fu planned the experiments and interpreted the results, Wei Tang made the write up and Yulian Zhang analyzed the data, Wuling Chen made the illustrations.

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