



Full Length Article

Characterization and Inhibitory Effects of an Antifungal Protein from the *Bacillus cereus* Strain YN917

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Abstract

In this research, a type of antifungal protein derived from the *Bacillus cereus* strain YN917, was precipitated by ammonium sulfate after fermentation. We investigated the antifungal effects and antifungal mechanism of the protein as well as its stability under different stress conditions such as the variation of temperature, pH value, exposure to ultraviolet radiation and proteases. Furthermore, the *in vitro* needle inoculation method was carried out to explore the antagonistic effects of 40% isoprothiolane Wettable powder (WP), 20% tricyclazole WP and the YN917 antifungal protein against the rice blast pathogen, *Magnaporthe oryzae*. Results showed that 20% tricyclazole WP and 40% isoprothiolane EC treatments had significant antagonistic effects on rice blast, and that the YN917 protein also had a dual inhibitory effect on mycelium growth and conidiospore germination in *M. oryzae*, which can cause this mycelium to swell and twist. The YN917 protein had a broad inhibitory spectrum with different effects on 15 plant-associated pathogenic fungi and oomycetes, such as *Botrytis cinerea*, *Bipolaris maydis* and *M. oryzae*. In addition, the physical and chemical properties of this antifungal protein were relatively stable and have potential for application in the biocontrol of fungal pathogens in plants. Overall, the results revealed that this protein has a wide tolerance to different temperatures, pH values, exposures to ultraviolet radiation and proteases, which is required for it to function as a bio-control agent against rice blast under a wide range of environmental conditions. © 2021 Friends Science Publishers

Keywords: Antifungal protein; *Bacillus cereus*; Biocontrol; Hyphal growth; *Magnaporthe oryzae*

Introduction

Rice blast is a fungal disease that seriously affects rice yield and quality and is caused by the pathogen known as *Magnaporthe oryzae*. The annual worldwide loss of rice yield accounts for about 10–30% of the total yield and has caused extreme losses to the agricultural industry sector (He *et al.* 2019). Extensive research on methods to prevent and control rice blast has been undertaken worldwide. At present, rice blast is mainly controlled by resistant varieties and by both chemical and biological control resources (Kusajima *et al.* 2018; Kong *et al.* 2018; Kimura and Fukuchi 2018; Javaid *et al.* 2019). Among these, the application of chemical fungicides is effective, but it presents challenging downsides, such as the presence of fungicide residues in rice grains, increased production costs and a harmful impact on human health and the environment (Liu *et al.* 2017; Wu *et al.* 2018). Moreover, plant pathogens can become resistant to fungicides, and cause biological

imbalances and environmental contamination (Stadnik and Borzecki 2009). In order to maintain the common goal of sustainable agriculture development, the production and utilization of bio-fungicides to control rice blast have seen a rapid growth in recent years. A variety of microorganisms such as bacteria, fungi, oomycetes and their related metabolites can be developed as biological control agents to replace chemical fungicides (Al-Reza *et al.* 2010; Hammami *et al.* 2011; Ali *et al.* 2020; Shoaib *et al.* 2020; Sharf *et al.* 2021). At present, many bio-control microorganisms exist, such as *Bacillus* spp., *Pseudomonadaceae* spp., *Agrobacterium radiobact*, *Actinomycesbovis* spp., *Coniothyrium*, *Micromonospora* spp., *Achromobacter* spp., *Serratia marcescens*, *Bipolaris* spp. (Johansson *et al.* 2014), which can be used to control rice blast.

Ongena and Jacques (2008) reported that *Bacillus* spp. could be one of the major sources of potential microbial fungicides due to their valuable characteristics. They can

prevent and control several plant diseases, including rice blast. A variety of *Bacillus* strains are currently commercialized as bio-fungicides (Bodhankar *et al.* 2017; Etesami *et al.* 2019); for example, the American AgraQuest Company developed a bactericide preparation Serenade™ with *B. subtilis* strain QST 713 and Souata AS with *B. pumilus* strain QST 2808 (Dorighello *et al.* 2015). Bayer, a German crop science company developed Kodiak® Concentrate with a *B. subtilis* strain (Dowd *et al.* 1998). China also has developed microbial preparations, Yunnan Xingyao Biological Products Factory, Yunnan Agricultural University and China Agricultural University cooperative developed the microbial fungicide Baikang with *B. subtilis* strain B908 (Ryder *et al.* 2005) and Nanjing Agricultural University made a live bactericide preparation named Maifengning with *B. subtilis* strain B3 (Zhang *et al.* 1994). *Bacillus* spp. is the main bio-control agent used against rice blast. By June 2019, there were 19 products derived from *Bacillus* spp. registered in China (including *B. subtilis*, *B. cereus*, *B. amyloliquefaciens*), 15 of which (80% of the total) were derived from *B. subtilis*.

In the early stage, a bio-control strain of *B. cereus*, named YN917, was successfully isolated from a healthy rice leaf sample of the susceptible rice cultivar ‘Xiangzaoxian No. 24’, which had a significant inhibitory effect on *M. oryzae*. Therefore, considering the priority of searching for natural alternatives to chemical fungicides, the research aim was to separate a type of antifungal protein from the *B. cereus* YN917 strain, and investigate its antifungal effects; specifically, we used ammonium sulfate for the precipitation of the YN917 antifungal protein. The stability, inhibition spectrum, characteristics and antifungal mechanism of this protein were investigated to offer a theoretical basis for the biological control of rice blast and development of natural bio-fungicides.

Materials and Methods

Experimental material

The *B. cereus* strain YN917 (16S rRNA GenBank: MT990515.1) used in this study was isolated from a healthy rice leaf sample of the susceptible rice cultivar ‘Xiangzaoxian No 24’ and stored in the Laboratory of Plant Pathogenic Microorganisms and Rice Diseases, Hunan Agricultural University, Changsha, China. Unless otherwise specified, the strain was grown on Luria-Bertani medium (10.0 g L⁻¹ tryptone, 5.0 g L⁻¹ yeast extract, 10.0 g L⁻¹ NaCl and pH 7.0) at 28°C. Stock cultures were maintained in 50% (v/v) glycerol at -80°C to provide a stable inoculum during the study period.

The tested fungus was rice blast (*M. oryzae*) and other phytopathogenic fungi such as *Botrytis cinerea*, *Bipolaris maydis*, *Ustilagoidea virens*, *Fusarium graminearum*, *Phytophthora parasitica* var. *nicotianae*, *Alternaria solani*, *Rhizoctonia solan*, *Verticillium dahliae*, *A. alternata*,

Diaporthe citri, *F. oxysporum*, *Colletotrichum gloeosporioides* and *Sclerotinia sclerotiorum*.

Indica rice ‘Yuzhenxiang’ and ‘Xiangwanxian No.12’ (Gold Nongfeng Seed Industry Technology Co., Ltd., China), and hybrid rice ‘YLiangyou 1998’ (Hunan hope Seed Technology Co., Ltd., China) were used as the test rice varieties. The tested fungicides were ‘40% isoprothiolane EC’ (500 times dilution), (Jiangsu Jiangnan agrochemical Co., Ltd., China), and ‘20% tricyclazole WP’ (750 times dilution), (Jiangsu Changqing agrochemical Co., Ltd., China).

Preparation of antifungal proteins

The antifungal proteins of strain YN917 were extracted using the ammonium sulfate fractional salting out method. After fermentation, the culture broth of the *B. cereus* strain YN917 was centrifuged at 10000 r min⁻¹ for 15 min at 4°C to remove bacterial cells, and then the cell-free supernatant was adjusted to 20, 30, 40, 50, 60, 70, 80 and 90%, in this order, with solid ammonium sulfate under ice bath conditions. It was then stored at 4°C for 48 h. The precipitate was obtained by centrifugation at 10000 r min⁻¹ for 15 min at 4°C and it was completely dissolved in phosphate buffered saline (PBS; pH 7.4). Subsequently, the precipitation was dialyzed against the same buffer for 2 d, ammonium sulfate was removed, and the antifungal activity test was then assayed. The crude extract was filtered through a 0.22 μm bacterial filter and stored at -20°C until use. Each sample was examined to determine the strongest antifungal activity, which had the optimum ammonium sulfate saturation required to prepare the antifungal protein. The experiment was repeated three times.

Antifungal activity assays

The *M. oryzae* samples were placed in the center of a potato dextrose agar medium firstly, which was then cut through with a cork borer to form a hole of 6 mm in diameter at the edge, 25 mm away from the plate center. The treated protein was prepared and 40 μL of respective treatments were added to each hole. Distilled water was used as the negative control. Each treatment was replicated three times. The dishes were incubated at 28°C until mycelial growth was reached in more than 3/4 of the culture dish, the colony diameter was measured and the inhibition rate of the antifungal YN917 protein on the growth of pathogens was calculated.

Determination of the antifungal spectrum

The dual culture method was used in this study to determine the inhibition of the YN917 antifungal protein on 15 plant pathogenic fungi and oomycetes: *M. oryzae*, two *B. cinerea* strains, *B. maydis*, *U. virens*, *F. graminearum*, *P. parasitica* var. *nicotianae*, *A. solani*, *R. solan*, *V. dahliae*, *A. alternata*, *D. citri*, *F. oxysporum*, *C. gloeosporioides*, *S. sclerotiorum*. The experiment was carried out as described in Arpita *et al.*

(2019).

Assessment of stability

High temperature tolerance: To study the thermostability of the YN917 antifungal protein, equal sample volumes were placed at -20°C , 4°C , 20°C , 30°C , 40°C , 50°C , 60°C , 70°C , 80°C , 90°C , 100°C and 121°C , for 30 min and then they were quickly cooled down to room temperature on ice.

pH tolerance: The pH values of equal sample volumes (1 mL) were adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0, by HCl (1 mol L^{-1}) or NaOH (1 mol L^{-1}). The solution was kept at 4°C for 24 h. Afterwards, the samples were readjusted to pH 7.0.

Protease tolerance: Equal sample volumes were treated with 1 mg mL^{-1} protease K, papain, pepsin, trypsin and alkaline protease, at 37°C for 1 h, and then kept in a water bath at 100°C for 5 min to inactivate the protease.

Ultraviolet radiation tolerance: Equal sample volumes were placed under 28W ultraviolet radiation at 10 cm, and were irradiated for 0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 4 h, 6 h and 12 h. A sample of untreated antifungal protein of the same concentration was used as control. The experiment was conducted twice, and three independent replicates were conducted for each treatment.

The activity of the antifungal protein against *M. oryzae* was assessed based on the experiments described above.

Effect of the antifungal protein treatment on mycelial morphology

To assess the effect of the YN917 antifungal protein on the mycelial morphology of *M. oryzae*, 1 mg mL^{-1} of protein was added to a 250 mL Erlenmeyer flask filled with 50 mL of fresh PDB medium containing *M. oryzae*. The flask was kept at 28°C and shaken at 180 r min^{-1} ; it was then observed by microscope ($\times 40$ magnification) after 1 d, 2 d, 3 d, 4 d and 5 d. All assays were conducted in triplicate, and an intact mycelium showing normal growth with an equal volume of dd- H_2O addition served as negative control.

Effect of the antifungal protein on spore germination

M. oryzae spores were mixed with the YN917 antifungal protein in a ratio of 1:1 (v/v), sterile distilled water was used as negative control. The treatments were placed at 28°C , and spore germination was observed by microscope ($\times 40$ magnification) after 2 h, 4 h, 6 h, 8 h, 10 h and 12 h. One hundred single conidia per dish were examined, the experiment was duplicated, and three independent replicates were conducted for each treatment.

In vitro control effect of the YN917 antifungal protein on M. oryzae

Control of rice blast *M. oryzae* was evaluated by *in vitro*

inoculation performed on rice leaves (Shi *et al.* 2015). In brief, same-sized healthy leaves were selected for testing at the 5-leaf stage. For the preventive treatment, the surface of the leaves was sprayed with $100\text{ }\mu\text{g mL}^{-1}$ of YN917 antifungal protein first, and after 24 h a *M. oryzae* spore suspension was inoculated. For the curative treatment, leaf surfaces were sprayed with $100\text{ }\mu\text{g mL}^{-1}$ of antifungal protein 24 h after being inoculated with the spore suspension. Negative controls were treated the same way using sterile distilled water, and the positive controls were treated with a 500 times diluent of 40% isoprothiolane EC, and a 750 times diluent of 20% tricyclazole WP, in both the preventive and curative assays. All experiments were carried out in triplicate. The samples were placed at conditions of 85–100% relative humidity and temperature of 28°C , with a 12 h photoperiod for 7 d. They were moisturized regularly with water to maintain hydration, and the antagonistic effect was analyzed.

Statistical Analysis

The data were analyzed in S.P.S.S. 20.0 and were subjected to Duncan's analysis of variance; the means were separated by Duncan's multiple range tests at $P \leq 0.05$.

Results

Optimum saturation of ammonium sulfate precipitation

Different proteins have different saturation characteristics when they are precipitated by $(\text{NH}_4)_2\text{SO}_4$. Therefore, the specific optimum $(\text{NH}_4)_2\text{SO}_4$ precipitation saturation of the YN917 protein was investigated in this study. The results (Fig. 1) showed that the protein precipitated $(\text{NH}_4)_2\text{SO}_4$ at 20–30% saturation had no inhibition on rice blast fungus, *M. oryzae*. When precipitated by $(\text{NH}_4)_2\text{SO}_4$ at 40% saturation, a slight inhibition on *M. oryzae* was observed. When precipitated by $(\text{NH}_4)_2\text{SO}_4$ at 60% saturation, the protein increased considerably, and the inhibition was significantly enhanced. The protein precipitated by $(\text{NH}_4)_2\text{SO}_4$ at 80% saturation showed a stronger antifungal activity than at other saturation levels, while the inhibition effect declined when the saturation was more than 80%. The results indicate that the optimum saturation of the precipitated antifungal crude protein from strain YN917 was 80%.

Antagonistic spectrum

The antagonistic test using common plant pathogens and oomycetes showed that the antifungal protein had a broad-spectrum antagonistic activity against the pathogens chosen for this research (Table 1). The maximum inhibition in the radial growth caused by the antifungal protein was $51.67\% \pm 1.73\%$ for grape gray-mold fungus *B. cinerea*, $46.10\% \pm 3.22\%$ for strawberry gray-mold fungus *B. cinerea*, 42.86%

Table 1: Antagonistic effects of *B. cereus* YN917 antifungal crude protein against fungal pathogens

Item	Host	Pathogen	Inhibition (%)
1	Grape	<i>Botrytis cinerea</i>	51.67 ± 1.73
2	Strawberry	<i>B. cinerea</i>	46.10 ± 3.22
3	Maize	<i>Bipolaris maydis</i>	42.86 ± 1.35
4	Rice	<i>Magnaporthe oryzae</i>	39.97 ± 1.23
5	Rice	<i>Ustilagoideia virens</i>	39.25 ± 0.43
6	Wheat	<i>Fusarium graminearum</i>	38.82 ± 2.04
7	Tobacco	<i>Phytophthora parasitica</i> var. <i>nicotianae</i>	38.02 ± 2.19
8	Potato	<i>Alternaria solani</i>	32.63 ± 1.15
9	Rice	<i>Rhizoctonia solan</i>	31.94 ± 0.09
10	Cotton	<i>Verticillium dahliae</i>	30.26 ± 1.12
11	Tobacco	<i>A. alternata</i>	29.14 ± 3.94
12	Orange	<i>Diaporthe Citri</i>	28.70 ± 1.23
13	Spicy	<i>F. oxysporum</i>	24.58 ± 0.84
14	Camphor	<i>Colletotrichum gloeosporioides</i>	22.97 ± 0.51
15	Rape	<i>Sclerotinia sclerotiorum</i>	22.48 ± 1.43

Mean ± standard deviation

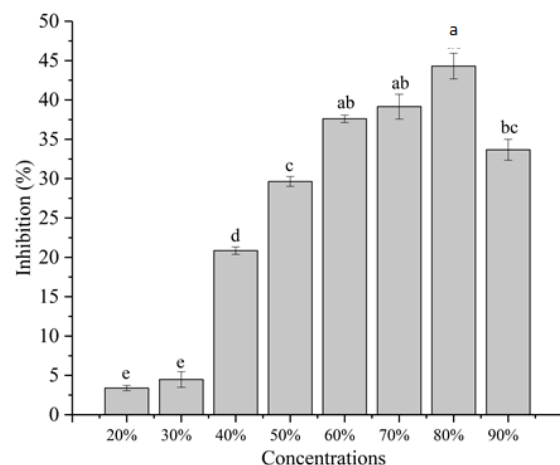
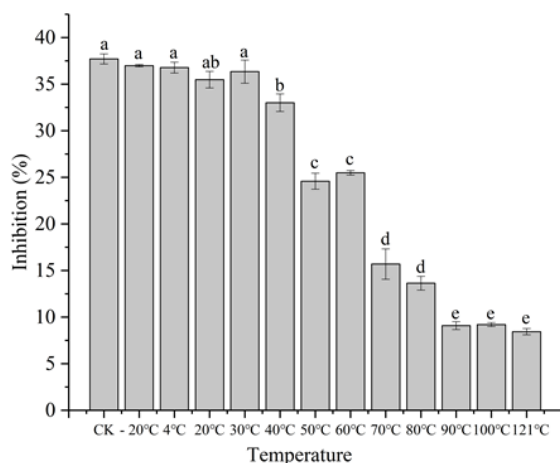
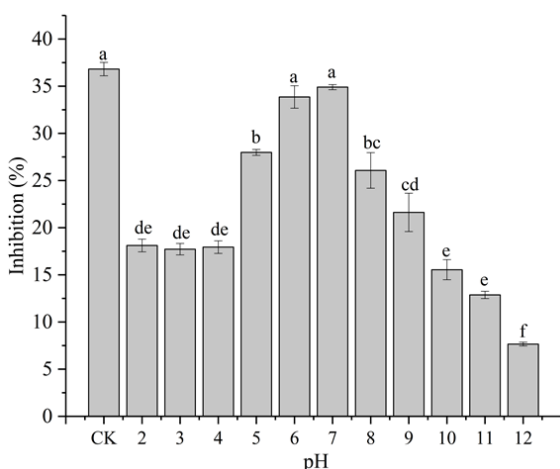
± 1.35% for southern corn-leaf blight fungus *B. maydis* and 39.97% ± 1.23% for rice blast fungus *M. oryzae*. When compared with the controls, each of the treatments showed a significant difference ($P \leq 0.05$) suggesting this protein has a potential to be successfully used in disease prevention.

Physicochemical properties

Temperature tolerance: To examine thermo-stability, the antifungal crude protein was placed at different temperatures for 1 h and antifungal activity was determined. The results (Fig. 2) showed that the inhibition rate on *M. oryzae* changed significantly after being exposed to different temperatures ($P \leq 0.05$). The antifungal activity was stable after exposure to temperatures between -20°C and 40°C . When the temperature was higher than 40°C , the inhibition rate and associated antifungal activity was significantly decreased. After exposure to 70 – 121°C , the inhibition rate continued to decrease significantly. This indicated that the crude antifungal protein has thermostability, and it can function adequately in the natural environment, showing a significant potential for its development and application.

pH tolerance: To assess pH stability, the antifungal protein was incubated at different pH values for 24 h and antifungal activity was determined. The results (Fig. 3) showed that the inhibition rate on *M. oryzae* changed significantly after different pH treatments ($P \leq 0.05$). The inhibition rate of the YN917 antifungal protein was stable at pH values between 5.0 and 8.0, and the inhibitory activity sharply decreased at pH values < 5.0 or pH values > 8 , which indicated that the protein is sensitive to overly acidic or overly alkaline conditions, but it maintains a sufficient antifungal activity against *M. oryzae* when pH is between 5.0 and 8.0.

Protease tolerance: Compared with the control, the inhibition rate of the antifungal protein did not change during protease treatments. However, when comparing protease treatments, the protein was more sensitive to protease K, alkaline protease, trypsin, pepsin and papain, and its antifungal activity was weakened after these

**Fig. 1:** Inhibitory results with different ammonium sulfate saturation**Fig. 2:** Stability of YN917 antifungal protein at different temperatures**Fig. 3:** Stability of YN917 antifungal protein at different pH values

treatments (Fig. 4). It was particularly sensitive to trypsin and pepsin, which indicated that the antifungal protein can be digested by proteases in the human digestive system and

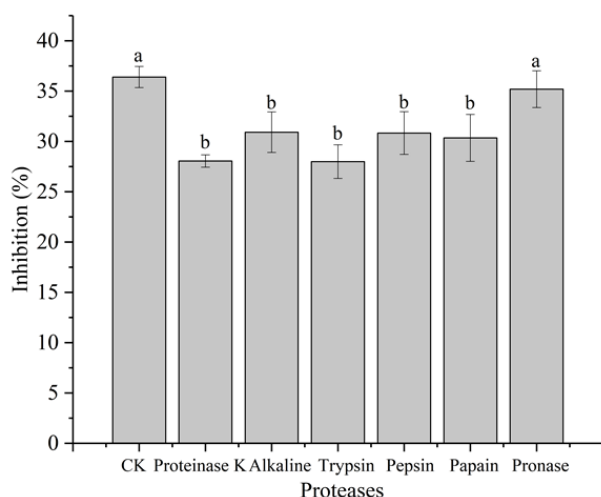


Fig. 4: Stability of YN917 antifungal protein in the presence of proteases

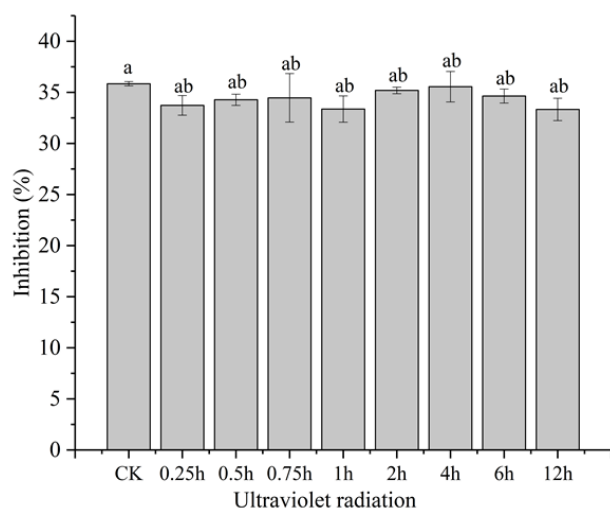


Fig. 5: Stability of YN917 antifungal protein exposed to ultraviolet radiation

it will not adversely affect human health. Therefore, this protein can be safely used in the biological control of crops.

Ultraviolet radiation tolerance: To assess UV stability, the antifungal protein was irradiated by ultraviolet radiation for 0.25–12 h and antifungal activity was determined. The activity remained stable (Fig. 5), which indicated that the protein is not sensitive to ultraviolet radiation and has good anti-ultraviolet properties.

Effect of the YN917 protein on *M. oryzae* spores

Spore production and germination of *M. oryzae* were determined in the presence of YN917. The results obtained showed the protein preparation had a significant potent inhibitory effect on the fungal spore production of *M. oryzae* at varied times. Distilled water, used as negative

control, did not inhibit spore production (Fig. 6 and Table 2). Moreover, the spores treated with the protein exhibited a swollen shape, while non-treated spores maintained their normal morphology. This suggests that the antifungal protein has a strong inhibitory effect on the spore germination of *M. oryzae*, affecting also the permeability of the conidiospore membranes.

Effect of the YN917 protein on *M. oryzae* mycelium

To assess the strength of the protein's antifungal effect, the mycelium of *M. oryzae* was microscopically observed near the zone where inhibition occurred, (Fig. 7) which revealed signs of extensive lysis of the spores; in particular abnormal distortion, slender shapes, multiple swelling and spore disintegration were observed. In contrast, the untreated negative control presented a normal mycelium and smooth surfaces. This suggests that the YN917 antifungal protein is effective in inhibiting spore formation and germination.

In vitro control effect of the YN917 antifungal protein

In order to evaluate the antifungal activity of *B. cereus* YN917 against *M. oryzae*, *in vitro* experiments were carried out on infected rice leaves of the rice cultivars 'Yuzhenxiang', 'Y-liangyou1998' and 'Xiangwanxian No.12'. The efficacy of biocontrol in the separate leaf assays is shown in Table 3. In both the preventive and curative treatments, the YN917 antifungal protein showed a significant bio-control efficacy against *M. oryzae*. In the prevention treatment, leaves treated with the protein had a lower incidence rate of disease (18.4% in Yuzhenxiang, 19.63% in Yliangyou 1998 and 14.26% in Xiangwanxian No 12) and presented smaller lesions than leaves without any treatment (97.26% in Yuzhenxiang, 97.66% in Yliangyou 1998 and 98.33% in Xiangwanxian No 12). In the curative treatment, the disease indexes of the protein were 22.53, 25.31 and 24.51%, respectively. The results were similar in both the preventive and curative samples, indicating that the YN917 antifungal protein may be effective as a potential bio-control agent against rice blast.

Discussion

This study is the first detailed investigation of the effects of temperature, pH value, ultraviolet radiation, and protease on the stability of the YN917 antifungal protein. The results describe the most favorable environmental conditions in which the protein remains active, which is particularly relevant for predicting bio-control responses. Overall, the protein can withstand a wide range of stress conditions. Stress tolerance is an important index to evaluate the applicability of a bio-control strain, based on the stability of the physical and chemical properties of its antifungal substances. Thermostability and pH-stability play a key role in the production and application of antifungal proteins. In

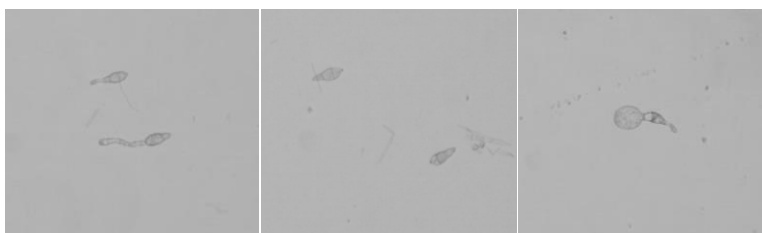
Table 2: Effect of YN917 antifungal protein on *M. oryzae* spore germination

Treatment	2 h	4 h	6 h	8 h	10 h	12 h
CK	7.69 ± 1.49	34.48 ± 3.46	55.26 ± 2.61	74.45 ± 2.32	88.19 ± 2.55	88.72 ± 3.25
YN917 protein	1.96 ± 0.64	4.00 ± 1.03	13.16 ± 1.83	12.30 ± 2.31	24.16 ± 3.07	22.72 ± 2.16

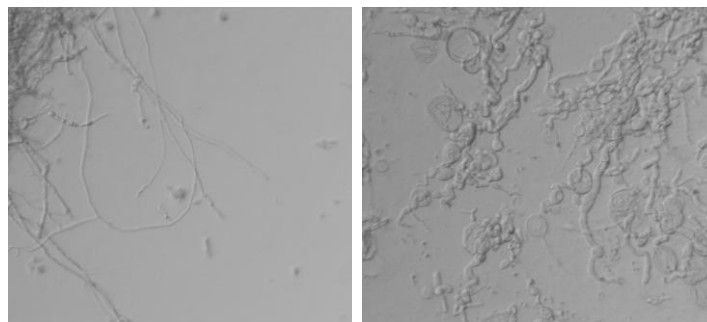
Table 3: Effect of YN917 antifungal protein on rice blast control *in vitro*

Treatments	Yuzhenxiang		Y-liangyou1998		Xiangwanxian No.12	
	-24 h	+24 h	-24 h	+24 h	-24 h	+24 h
CK	97.26 ± 2.48 a	92.41 ± 3.66 a	97.66 ± 1.66 a	94.75 ± 1.67 a	98.33 ± 2.36 a	98.30 ± 0.42 a
25% Tricyclazole WP	7.36 ± 0.55 c	54.46 ± 2.29 b	10.67 ± 0.94 c	52.96 ± 2.28 b	9.00 ± 0.82 d	56.11 ± 2.83 b
40% Isoprothiolane EC	20.15 ± 1.52 b	12.22 ± 1.38 d	22.96 ± 2.77 b	11.04 ± 0.82 d	30.99 ± 1.67 b	16.40 ± 1.85 d
100 ug/mL protein	18.40 ± 1.36 b	22.53 ± 4.16 c	19.63 ± 2.29 b	25.31 ± 1.43 c	14.26 ± 2.33 c	24.51 ± 2.00 c

Mean ± standard deviation. Values sharing same letters do not significantly differ ($P > 0.05$)

**Fig. 6:** Effect of the YN917 antifungal protein on *M. oryzae* spores

(A) Normal spores (CK); (B), (C) abnormal spores of *M. oryzae* appear bulbous and swollen

**Fig. 7:** Effect of the YN917 antifungal protein on *M. oryzae* mycelium

A negative control, without YN917 protein treatment showing normal mycelium morphology and spore germination; B mycelium treated with YN917 protein showing extensive lysis of the mycelia and destructed spores

particular it is important that antifungal proteins maintain a high antifungal activity at pH values between 8.0 and 11.0 and at temperatures below 70°C, conditions which better suit industrial production prospects (Oberoi *et al.* 2001).

In relation to the present study specifically, the results have confirmed that the YN917 protein has a wide temperature adaptation range, and can maintain a high antifungal activity after being exposed to 80°C for 30 min. This protein is considerably adapted to withstand high temperatures, providing an advantage when used in cultivation crops (specifically in the summertime). In addition, it is not sensitive to ultraviolet radiation, but acidic pH values in *in vitro* experiments seemed to significantly restrict its activity. The suitable soil pH value ensuring antifungal activity was between 5.0 and 8.0, which does correspond to the natural growth ring of common crops. Moreover, the protein is sensitive to trypsin and pepsin treatment, which indicates that it can be digested by protease

in the human digestive system and is therefore harmless to human health. In summary, this study has shown that from an ecological point of view, the YN917 protein has a wide tolerance to various stressors such as temperature, pH, ultraviolet radiation, and protease, which suggests that it could be applied to a wide range of environmental conditions.

Many antifungal proteins derived from *Bacillus* spp. have a strong inhibition activity (Gotor-Vila *et al.* 2017). For example, the antifungal protein derived from *B. subtilis* ZL2-70 was shown to strongly inhibit *V. dahliae*, *Fusarium graminearum*, *C. gloeosporioides*, and 19 other plant pathogens; the Bacisubin protein isolated from *B. subtilis* B-916 can inhibit the growth of various pathogens such as *M. oryzae*, *B. cinerea*, and *A. brassicae* (Luo *et al.* 2009). The antifungal protein YN917 derived from the *B. cereus* YN917 strain presents a broad antifungal spectrum, which can effectively inhibit the mycelial growth

of 15 pathogenic fungi and oomycetes, such as *M. oryzae*, *B. cinerea* and *B. maydis*. The experiments carried out in this particular study showed that this antifungal protein has a strong inhibitory effect on the growth and germination of *M. oryzae* spores. Furthermore, several antifungal proteins have been shown to inhibit fungal mycelium and the germination of spores (Leelasuphakul *et al.* 2008; Matar *et al.* 2009). For example, a novel protein produced by *B. subtilis* B29 (Li *et al.* 2009), can inhibit the conidial spore germination of *F. oxysporum*, suppress germ-tube elongation, and induce distortion, tumescence and rupture of a portion of the germinated spores. The Bacisubin protein, produced by *B. subtilis* B-916 (Liu *et al.* 2007) can cause the *R. solani* mycelia to enlarge and rupture, and the crude antifungal protein RY3 produced by *B. amyloliquefaciens* RY3 can inhibit the germination of *P. digitatum* spores (Chen *et al.* 2013). Finally, the protein produced by *B. cereus* HS24, can significantly inhibit conidium germination and mycelial growth in *M. oryzae* (Huang *et al.* 2019).

There is an urgent need for more efficient and sustainable alternatives to chemical fungicides to prevent or control diseases and reduce food production losses (Mnif *et al.* 2015). In this article, we have confirmed the *B. cereus* derived YN917 protein is a potent antifungal agent against *M. oryzae*. The results showed that its antifungal activity against rice blast fungus *M. oryzae* *in vitro* was like that of 25% tricyclazole WP and 40% isoprothiolane EC, suggesting that this protein can be a potential natural fungicide alternative to be used to effectively prevent or control rice blast in crop cultivations.

Bacillus spp. in nature can produce different secondary metabolites, which are related to microorganisms in different ecological environments. These secondary metabolites include antifungal and antiseptic substances, biofertilizers, as well as various active enzymes. Therefore, the determination of the antifungal components produced by the YN917 protein will be the objective of our future research.

Conclusion

In the present work, results indicate that there are natural antifungal substances in the YN917 strain of *B. cereus*. The YN917 protein showed a broad-spectrum antagonistic activity against 15 plant pathogens. Its physical and chemical properties were relatively stable and had a dual inhibitory effect on mycelium growth and conidiospore germination in *M. oryzae*. *In vitro* experiments revealed that its control effect against rice blast was considerable. Therefore, protein extracts from the YN917 strain can be considered as a promising and novel bio-control resource to be used in agricultural production.

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Author Contributions

Conceptualization, HZ; Methodology, HZ; Validation, HZ, HZ, LH, XY; Formal Analysis, HZ and HZ; Data Curation, HZ; Writing-Original HZ; Writing-Review and Editing, HZ and EL; Supervision, ZR; Funding Acquisition, ZR and EL

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

All data will be available upon reasonable request to the corresponding author.

Ethics Approval

Not applicable.

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