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RESEARCH ARTICLE

Ying ZHU, Shuna LI, Hongshui YUAN, Xiaojun GUO, Baocheng ZHU

Isolation and identification of the antagonistic strain DM-54 of *Bacillus amyloliquefacien* against *Verticillium dahliae*, and optimization of antifungal protein producing conditions

Abstract The strains capable of resistance against *Verticillium dahliae* Kleb were isolated and screened from the soils of cotton fields from several different provinces in China. A strain, coded DM-54, with a rather high antagonistic activity was obtained. Its morphological characteristics, physiological and biochemical properties and a 16 S rDNA sequence of this strain were further studied. The DM-54 strain was finally identified as a kind of *Bacillus amyloliquefacien.* Through a single factor experiment and an orthogonal experiment, the optimal shaking flask fermentation condition of strain DM-54 was found to be: media composed of 5% dextrin, 3% soy peptone, 0.02% $MgSO_4$, 0.01% $CaCl_2$, initial pH 7.0 and 10% inoculum volume, media volume 30/250 (mL/mL), fermentation temperature at 32°C, rotating speed $200\,r\cdot min^{-1}$, fermentation time of 48 h. Its antagonistic activity was distinguished to be elevated, at about 39.9%. Our research offers an effective means for the massive production of antagonistic proteins.

Keywords cotton verticilliumwilt, antagonistic bacterium, isolation and identification, *Bacillus amyloliquefacien*, fermentation

1 Introduction

Cotton verticillium wilt, caused by *Verticillium dahliae*, is a fungal disease that is one of the most crushing diseases in cotton worldwide and is widely distributed across the cotton-growing regions in China (Li et al., 2001). About half of the total area of about 80 million acres of cotton-producing fields in China suffers from cotton verticillium wilt. This has been the major barrier to cotton yield and quality, and has even caused huge economic losses (Hu, 2004). Currently, chemical and biological measures and the application of disease-resistant varieties are often used to control this wilt worldwide (Liang et al., 2005). Biocontrol, the screening of rival microorganisms in soil, is confirmed as the most effective among the above three methods. Such microorganisms can produce many highly active antibacterial substances against special pathogenic bacteria (Xia et al., 1996). Both the composition of medium and the culture condition are important factors affecting the production of antibacterial substances.

In this test, we screened out some strains having rival activities against *Verticillium dahliae* Kleb, and characterized one with a relatively high rivalry activity, the DM-54 strain. The composition of the medium and culture conditions were optimized. Our results offer a bright future for further studies.

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Ying ZHU (✉), Baocheng ZHU (✉)
College of Life Science, Hebei University, Baoding 071002, China
E-mail: aileenzy@126.com; zhu2222@126.com

Shuna LI, Hongshui YUAN, Xiaojun GUO, Baocheng ZHU
College of Life Science, Agricultural University of Hebei, Baoding 071001, China

2 Materials and methods

2.1 Test strain

The pathogen *Verticillium dahliae* Kleb, a causal agent of verticillium wilt disease in cotton, was preserved in the pharmaceutical engineering laboratory of Agricultural University of Hebei.

2.1.1 Reagents and medium

The reagents and medium composition for strain identification were referred from the Manual of Systematic and Determinative Bacteriology (Dong and Cai, 2001). Seed

culture medium was composed of 1% peptone, 1% glucose, 0.2% $NaH_2PO_4 \cdot 2H_2O$, 0.4% $Na_2HPO_4 \cdot 2H_2O$, and 0.05% $MgSO_4 \cdot 7H_2O$ and pH 7.0–7.2. The fermentation medium was composed of a 1% nitrogen source, 1% carbon source, 0.2% $NaH_2PO_4 \cdot 2H_2O$, 0.4% $Na_2HPO_4 \cdot 2H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.02% $CaCl_2$ and pH 7.0–7.2.

2.1.2 Method of strain screening

Soil samples were collected from cotton fields at 10–15 cm deep under the soil surface and preserved in plastic bags with the sampling time and place recorded. In our experiment, we screened 20 soil samples from Hebei, Hubei, Hunan and other provinces.

2.2 Isolation of bacteria from soil samples

One gram of soil sample was weighed and put into an 18 mL test tube with 9 mL sterilized water. After being mixed uniformly, 1 mL of the mixture was transferred into another 18 mL test tube and diluted into several concentration gradients. Thereafter, 0.1 mL diluents of $\times 10^{-4}$ and $\times 10^{-5}$ concentration gradients were separately coated on NA culture medium plates which were then put into an incubator to culture for 24 h. Different-shaped single colonies were picked out and cultured in NA culture medium at a constant temperature for 24 h. Meanwhile, the colonies on NA culture medium plates were streaked on plates to examine their purity (Cheng et al., 2000).

2.3 Preliminary screening of antagonistic bacteria against *Verticillium dahliae*

Verticillium dahliae was inoculated on PDA culture medium under 25°C for 7–10 d. Five mL sterilized water was added into a test tube covered with *Verticillium dahliae*, using a sterilized transfer needle to lightly scrape the spores. Thereafter, the 5 mL spore suspension was incorporated into the PDA culture medium which was melted and cooled down to 45°C. The medium was poured onto the plate and inoculated with the bacteria after the medium solidified. The results were observed after culturing at 24°C for 48 h (Shen et al., 1999).

2.4 Second screening of antagonistic bacteria against *Verticillium dahliae*

The method for pathogenic fungi plate preparation is the same as that mentioned in the preliminary screening above. After inoculating antagonistic bacteria into the 50 mL NB medium in a 250 mL flask and cultivating under shaking conditions of 180 $r \cdot min^{-1}$ for 48 h at 30°C, the culture fluid was centrifuged at 4°C for 10000 $r \cdot min^{-1}$ and the supernatant was filtered with a 0.2 μm filter membrane. After perforating in concretionary PDA culture medium with *Verticillium dahliae* spore suspension using a hole puncher, 80 μL fermentation broth that had filtrated bacteria was injected into the eyelet, allowed to stand for 30 min, and was cultured at 25°C stationary temperature for 72 h to measure the inhibition zone diameters; the results were recorded.

2.5 Methods of bacterial identification

2.5.1 Morphological identification

Morphological observation of the colony was conducted by picking DM-54 strains into 10 mL sterile water, imbibing 1 mL to 9 mL sterile water after sufficient mixing, and with gradual dilution, each grade concentration of the bacterial suspension was obtained, taking 0.1 mL dilution of both $\times 10^{-4}$ and $\times 10^{-5}$ concentration gradients to spray over the NA culture medium plate which was then cultured at a constant temperature of 30°C for 24 h.

2.5.2 Morphological observation of thallus

Gram staining was done to the DM-54 strain according to the methods in the Manual of Systematic and Determinative Bacteriology (Dong and Cai, 2001). The morphology of the strain was observed under a microscope.

2.5.3 Physiological and biochemical identification

We also referred to the Manual of Systematic and Determinative Bacteriology (Dong and Cai, 2001).

2.6 Extraction of DNA and amplification of 16 S ribosomal DNA gene fragments

The bacterial chromosomal DNA was extracted using the method of Sambong and Rainey (Sambong et al., 1995; Rainey et al., 1996), and detected by 1% agarose electrophoresis.

Primers were consensus primers (Lane, 1991). The forward primer was 27F:5'-AGAGTTTGATCCTG GCTCAG-3', and the reverse primer was 1495R: 5'-CTACGGCTACCTTGTTACGA-3'. The forward and reverse primers were respectively located at the 8–27 bp and 1495–1514 bp in the16 S rDNA (*Escherichia coli* 16 S rDNA).

For the PCR reaction system, conditions were as follows: DNA (70 $ng \cdot \mu L^{-1}$) template 2 μL; dNTP mixture (2.5 $mmol \cdot L^{-1}$) 2.5 μL; 27 F (20 $\mu mol \cdot L^{-1}$) 1.5 μL; 1495 F (20 $\mu mol \cdot L^{-1}$) 1.5 μL; 10 × ExTaq Buffer (Mg^{2+} plus) 5 μL; ExTaq DNA polymerase 0.2 μL; bringing up ddH_2O to 50 μL.

The PCR amplification conditions were as follows: force-degeneration at 94°C for 3 min, followed by

degeneration at 94°C for 1 min, annealing at 55°C for 1 min and at 72°C for 3 min, 30 cycles, with another extension at 72°C for 5 min. After purification, the PCR products were sent for sequencing (Sagon Company).

2.6.1 Analysis of 16 S rDNA sequences and drawing of phylogenetic tree

Resemblance analysis of the 16 S rDNA sequence was done through the GenBank database using the BLAST method. Multiple alignment was carried out among the sequences with high resemblance in the Clustal X (1.8) program. Finally, a multiple alignment array was established, with gaps instead of lines, and a phylogenetic tree was constructed using the Neighbor-Joining method (Saitou and Nei, 1987).

2.7 Extraction and identification of the antagonistic substance

The activated cells of strain DM-54 were inoculated in NB medium and shaking-cultured for 48 h under 30°C at 180 $r \cdot min^{-1}$. $(NH_4)_2SO_4$ was added to the fermentation liquid till it reached 30% degree of saturation, and the fermentation liquid was left stationary overnight at 4°C. The deposition gained from the fermentation liquid through centrifugation at 12000 $r \cdot min^{-1}$ was then dissolved at 4°C with 0.1 $mol \cdot L^{-1}$ phosphate buffer (pH 7.5), and a protein solution with dissolubility between 0–30% was obtained. $(NH_4)_2SO_4$ was added to the supernatant till it reached 60% saturation degree, and a protein solution between 30%–60% was obtained. Then a protein solution between 60%–80% was obtained using the same method. The inhibitory activities of the protein solutions of different dissolubility gradients were measured using the Cylinder-Plate Method.

2.8 Seed culture of strain DM-54

Strain DM-54 cultured in inclined medium was inoculated on seed medium then cultured on shaking tables under the condition 200 $r \cdot min^{-1}$ at 30°C for 16 h.

2.9 Fermentation culture of strain DM-54

After changing a single factor each time, such as carbon source, nitrogen source, and inorganic salt, the fermentation medium was formulated according to experimental needs in a 250 mL triangular flask with 50 mL medium. The strains that were cultured well were inoculated on the fermentation medium using 15% inoculation volume, then were cultivated on shaking tables under the condition 200 $r \cdot min^{-1}$ at 30°C for 48 h. The fermentation liquid was filtered to remove bacterial cells, and the inhibitory activities were measured with the Cylinder-Plate Method. The sizes of the bacteriostatic rings characterized inhibitory activity.

3 Results and analysis

3.1 Isolation of bacterium from soil samples

The results showed that massive bacteria existed in the soil samples. Antagonistic bacteria against *Verticillium dahliae* may be among these bacteria. After isolating and purifying the single colonies with different characteristics, we got 891 strains of bacteria. Those strains mostly had the following morphologic characteristics: gray (a few yellow), opaque colonies, size inequality, thin and flat, wrinkled and arescent surface, neat verge, crateriform shape, with radiation or irregularity.

3.2 The initial screening result of antagonistic bacterium against the *Verticillium dahliae*

Growth dual tests were done between the isolated bacterial strains and *Verticillium dahliae* Kleb separately. Eighty-three strains were obtained from the total of 891 strains after antagonistic activity tests between those strains and *Verticillium dahliae*. Twenty strains showing a relatively higher antagonistic activity were chosen to undergo secondary screening. The results showed that 4 strains had inhibition zones with diameters beyond 16 mm, 10 strains had diameters ranging from 10 mm to 16 mm, and 6 strains had no antagonistic activity. No antagonistic activity among those 6 strains could be detected due to low fermentation broth concentrations. The DM-54 strain was selected because it had the highest antagonistic activity, with an inhibition zone diameter of up to 19 mm.

3.3 Identification of strain DM-54

3.3.1 Colonial morphology

The colonial morphology of the DM-54 strain after cultivation in NA culture for 24 h is shown in Fig. 1. They are nearly round in shape, together with the features of verge irregularity, surface wrinkles and a glossy, crateriform eminence, with a white but opaque color, and no pigment.

3.3.2 Thallus morphology

Strain DM-54 cultured for 24 h was handled with Gram staining, and was examined under an oil-immersed microscope for observation of the morphology and size. The results demonstrated that the strain belonged to the Gram-positive bacteria, having a long rhabditiform shape,

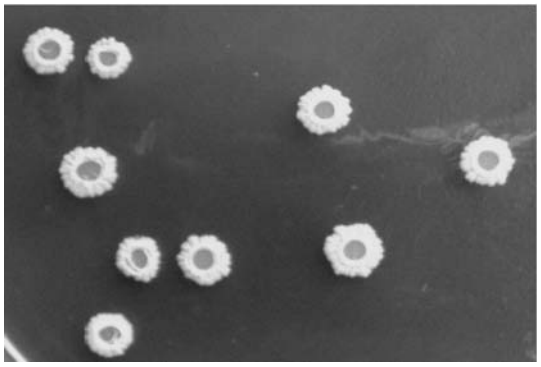

Fig. 1 Colonial morphology of stain DM-54

0.6–0.9 μm×1.8–2.5 μm, an endospore as well as a uniformly stained cell matrix.

3.3.3 Physiological and biochemical features

The physiological and biochemical characteristics of strain DM-54 are shown in Table 1.

3.4 Analysis of 16 S rDNA sequence

The 16 S rDNA isolated from strain DM-54 was 1480 bp long. Resemblance analysis was carried out between this sequence and other 16 S rDNA sequences using the BLAST program. Strain DM-54 and the other nine strains together formed a cladogram according to resemblance. Based on the results shown in Fig. 2 and Table 2, the DM-54 strain was preliminarily identified as a number under *Bacillus*. Furthermore, the resemblance between the DM-54 strain and the strain NBRC 15535s of *Bacillus amyloliquefacien* was 99.43%. According to the resemblance analysis between the 16S rDNA sequences, morphological observations, physio-biochemical characteristics, and further referring of the physio-biochemical characteristics results to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1984) and the Manual of Systematic And Determinative Bacteriology (Dong and Cai, 2001), the DM-54 strain was finally identified as *Bacillus amyloliquefacien*. The resemblance of the physio-biochemical characteristics reached up to 98%.

The strain was qualified as *Bacillus amyloliquefacien* according to analysis of the 16S rDNA sequence similarity, combined with morphological observation and physiological and biochemical reactions. The 16S rDNA sequence similarity reached 99.43%.

This sequence has been received by GenBank (Accession Number: EU826021).

3.5 Determination of antagonistic substance of strain DM-54

Antifungal activities in 0–30%, 30%–60% and 60%–80% protein solution were determined respectively. The results

Table 1 Physiological and biochemical characteristics of strain DM-54

items tested	experimental result	items tested	experimental result
anaerobic growth	–	malonate utilization	–
growth in pH 5.7 nutrition broth	+	saline tolerance	
production of H_2O_2 enzyme	+	2%	+
V-P determination	+	5%	+
the pH of V-P determination	6.12	7%	+
anti-lysozyme	+	10%	+
amylolysis	+	12%	–
nitrate reduction	+	sugar and spirits fermentation	
nitrite reduction	–	glucose	+
citrate utilization	+	manicol	+
gelatine liquefication	+	lactose	+
methyl red	–	growth temperature	
lacmus milk	A	5°C	–
cellulose decomposition	–	10°C	+
tyrosine hydrolysis	–	20°C	+
casein hydrolysis	+	30°C	+
indole	+	40°C	+
phenylalanine deaminase	–	50°C	–
lecithinase	–		

Note: "A" represents litmus milk turning to red.

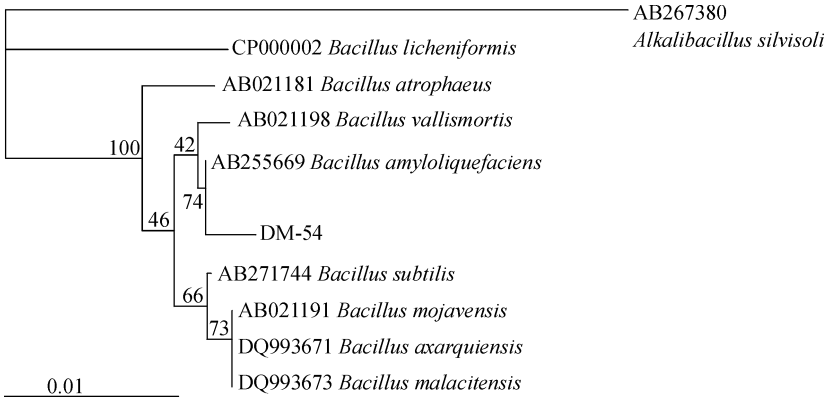

Fig. 2 Phylogenetic tree of the 16 S rDNA sequence of strain DM-54 and related strains

Table 2 Comparison of similarities between several norm stains and strain DM-54

serial-number	specific name	strain-number	similarity/%
DQ993671	*Bacillus axarquiensis*	LMG 22476	98.99
DQ993673	*Bacillus malacitensis*	LMG 22477	98.99
AB021191	*Bacillus mojavensis*	IFO15718	99.14
AB271744	*Bacillus subtilis*	NBRC 13719	99.28
AB255669	*Bacillus amyloliquefaciens*	NBRC 15535	99.43
AB021198	*Bacillus vallismortis*	DSM11031	99.36
AB021181	*Bacillus atrophaeus*	JCM9070	99.21
CP000002	*Bacillus licheniformis*	ATCC 14580	97.16
AB267380	*Alkalibacillus silvisoli*	HN2	93.28

showed that the 30%–60% protein solution had antifungal activity, but the 0–30% and 60%–80% protein solutions had no antifungal effect. Heated at 121°C for 10 min, the 30%–60% protein solution lost its antifungal activity. After an extraction of the fermentation liquid with the same volume of chloroform, the extract also lost its antifungal activity. To sum up, the antagonistic substance of strain DM-54 could be a protein.

3.6 Effects of medium compositions on antagonistic protein production

Each experimental treatment below was repeated three times, and the antibacterial circle diameters were expressed as mean = $x \pm$ standard error.

To the fermentation medium was added different carbon sources of up to 1% and then fermentation was done on shaking tables for 48 h at 30°C at 200 $r \cdot min^{-1}$. The inhibitory activities of the fermentation liquid towards *Verticillium dahliae* Kleb were measured separately. Results showed that the antagonistic protein production from fermentation had the highest inhibitory activity when using dextrin as carbon source, followed by corn flour (Table 3).

When using 1% dextrin as carbon source, and by adding different nitrogen sources of up to 1% into the fermentation media which were fermented on shaking tables for 48 h, we found that the DM-54 strain had better utilization of organic nitrogen sources than inorganic nitrogen sources, and the antagonistic protein production had the highest inhibitory activity when using soya peptone as nitrogen source based on the measured result of the inhibitory activities of *Verticillium dahliae* Kleb in the fermentation liquid (Table 4).

When using 1% dextrin as carbon source and 1% soya peptone as nitrogen source, and adding 0.02% inorganic ions to the fermentation medium to study the effects of different inorganic ions on DM-54 fermented antagonistic protein production, we found that Mg^{2+} and Ca^{2+} were beneficial to antagonistic protein production, and Zn^{2+} had an intensive inhibition of enzymatic activity (Table 5).

According to the single factor experiment results above, an orthogonal experiment with four factors at four different levels was designed by using dextrin, soya peptone, $CaCl_2$ and $MgSO_4$ as materials. Results are shown in Table 6.

The orthogonal experiment indicated that $R_{\text{dextrin}} > R_{MgSO_4} > R_{\text{soya peptone}} > R_{CaCl_2}$. Thus, dextrin was the most important influencing factor, followed by $MgSO_4$, soya peptone and $CaCl_2$.

Table 6 shows that the best combination was $A_4B_4C_1D_2$, which did not exist in the orthogonal experiment. After determination tests, the antibacterial circle diameter was

Table 3 Effect of carbon source on antagonistic protein production of strain DM-54

carbon source	maizena	glucose	sucrose	lactose	starch	dextrin	mannitol	maltose
inhibition zone diameter/mm	16.80 ± 0.29	15.70 ± 0.58	14.80 ± 0.58	16.00	16.00 ± 0.50	17.67 ± 0.76	16.20 ± 1.16	15.33 ± 1.04

Table 4 Effect of nitrogen source on antagonistic protein production of strain DM-54

nitrogen source	peptone	beef extract	yeast extract	tryptone	soy peptone	urea	sodium nitrate	sulfate ammonium
inhibition zone diameter/mm	15.60 ± 1.68	17.80 ± 1.26	18.00 ± 0.50	18.00 ± 1.32	18.67 ± 0.76	0	0	0

Table 5 Effect of ions on antagonistic protein production of strain DM-54

inorganic salt	Mn^{2+}	Fe^{2+}	Na^{+}	Mg^{2+}	K^{+}	Zn^{2+}	Cu^{2+}	Ca^{2+}
inhibition zone diameter/mm	17.80 ± 2.02	11.00 ± 0.00	13.80 ± 0.76	19.30 ± 0.57	12.80 ± 1.26	0	17.67 ± 1.53	19.70 ± 1.16

Table 6 Orthogonal experimental design and results of culture media

number	A (dextrin)	B (soy peptone)	C ($CaCl_2$)	D ($MgSO_4$)	inhibition zone diameter/mm
1	0.5	0.5	0.01	0.01	13.00 ± 0.00
2	0.5	1.0	0.03	0.02	14.00 ± 1.41
3	0.5	2.0	0.05	0.05	10.00 ± 0.00
4	0.5	3.0	0.07	0.10	11.00 ± 0.00
5	1.0	0.5	0.03	0.05	11.75 ± 1.06
6	1.0	1.0	0.01	0.10	13.50 ± 0.71
7	1.0	2.0	0.07	0.01	11.75 ± 1.06
8	1.0	3.0	0.05	0.02	14.75 ± 1.06
9	2.0	0.5	0.05	0.10	14.75 ± 0.35
10	2.0	1.0	0.07	0.05	13.00 ± 0.00
11	2.0	2.0	0.01	0.02	14.50 ± 2.12
12	2.0	3.0	0.03	0.01	15.75 ± 0.35
13	5.0	0.5	0.07	0.02	16.75 ± 0.35
14	5.0	1.0	0.05	0.01	17.50 ± 3.54
15	5.0	2.0	0.03	0.10	13.50 ± 2.12
16	5.0	3.0	0.01	0.05	16.25 ± 1.06
*K*1	12.00	14.06	14.31	14.50	
*K*2	12.94	14.25	13.75	15.00	
*K*3	14.50	12.69	14.25	12.75	
K4	16.00	14.44	13.13	13.19	
R	4.00	1.75	1.18	2.25	

Note: *K* stands for average; *R* stands for range.

found to be 20.2 mm. Thus, the optimal medium constitution was 5% dextrin, 3% soya peptone, 0.02% $MgSO_4$, 0.01% $CaCl_2$.

Fermentation time, inoculation quantity, initial pH and broth content were determined with the orthogonal experiment. An orthogonal experiment with four factors at four levels was designed. Results are shown in Table 7.

According to analysis of the orthogonal experiment, the optimal fermentation time was 48 h, when activity reached the maximum, although less broth content could have induced higher activity that might have something to do with the strain's strict aerobic feature and provided sufficient oxygen that could have been beneficial to the strain's antagonistic protein production. The greater the

Table 7 Orthogonal experimental design and results of culture conditions

number	time/h	bottle filling size/mL	inoculum size/%	pH	inhibition zone diameter /mm
1	24	30	2	6.0	17.25 ± 0.35
2	24	50	4	7.0	16.00 ± 2.83
3	24	75	8	8.0	15.25 ± 0.35
4	24	100	10	9.0	16.75 ± 0.35
5	48	30	4	8.0	19.75 ± 1.06
6	48	50	2	9.0	19.25 ± 1.06
7	48	75	10	6.0	17.25 ± 1.06
8	48	100	8	7.0	16.25 ± 1.77
9	72	30	8	9.0	17.88 ± 0.18
10	72	50	10	8.0	18.00 ± 1.41
11	72	75	2	7.0	16.75 ± 1.06
12	72	100	4	6.0	15.75 ± 1.77
13	96	30	10	7.0	21.00 ± 1.41
14	96	50	8	6.0	18.75 ± 1.06
15	96	75	4	9.0	15.25 ± 0.35
16	96	100	2	8.0	14.75 ± 0.35
K1	16.31	18.97	17.00	17.25	
K2	18.13	18.00	16.69	17.50	
K3	17.09	16.13	17.03	16.94	
K4	17.33	15.88	18.25	17.28	
R	1.82	3.09	1.56	0.56	

Note: *K* stands for average, *R* stands for range.

inoculation quantity, the higher the activity could be, and the highest activity appeared at the initial pH of 7.0.

The optimal fermentation condition was 48 h fermentation time, 30 mL broth in a 250 mL triangle-bottle, 10% inoculation quantity and initial pH 7.0.

Under the same condition, the protein antagonistic activity of the fermentation liquid was detected after fermenting for 48 h at 30°C at the rotating velocity of 150 $r \cdot min^{-1}$, 180 $r \cdot min^{-1}$, 200 $r \cdot min^{-1}$ and 220 $r \cdot min^{-1}$, respectively. Each treatment had 3 replicates, showing that the optimal shaking table velocity was at 200 $r \cdot min^{-1}$ (Fig. 3).

Additionally, under the same condition, the protein antagonistic activity of the fermentation liquid was detected after fermenting at 200 $r \cdot min^{-1}$ for 48 h at the temperatures of 25°C, 32°C, 37°C and 42°C, respectively, with three replicates per treatment, indicating that the optimal fermentation temperature was 32°C, where the highest protein antagonistic activity was seen (Fig. 4).

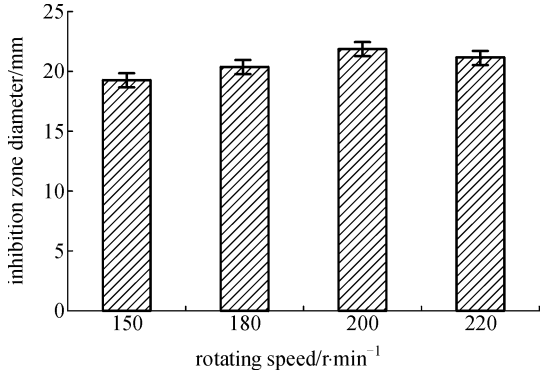

Fig. 3 Effect of rotating speed on antagonistic protein activity of strain DM-54

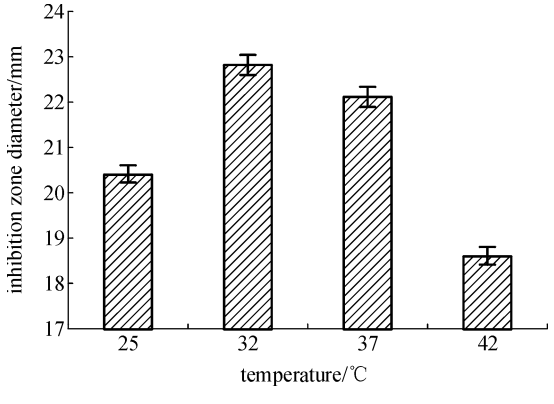

Fig. 4 Effect of temperature on antagonistic protein activity of strain DM-54

4 Discussion

Cotton verticillium wilt is a major disease constraining the production of cotton in China. In order to reduce the harmful effects of chemical fungicides to our environment and health, researchers are searching for alternatives with high security and high effectiveness. Breeding of cotton cultivars against verticillium wilt is not an easy job (Yang et al., 2006). At present, many bacteria or fungi can produce peptide or protein matter with biological activity (Zhou et al., 2007), especially those in the genus *Bacillus*. Some bacteria in the genus *Bacillus*, which are both resistant to adverse environments and usually to bacterial and fungal pathogens as well, are dominant microorganisms in the soil and plant microecologial systems, and many natural isolates with great potency have been screened and applied widely in the bio-control of plant diseases (Zhou et al., 2006).

Therefore, the application of bio-control products has wide prospects. The DM-54 strain that we screened has strong antibacterial activity towards *Verticillium dahliae*. The bio-control efficiency and antimicrobial mechanism of the strain need further research.

In the screening process, most strains with antibacterial activity to *Verticillium dahliae* belong to *Bacillus*. *Bacillus* can produce endospores (Quan et al., 2006) with strong reproductive capacity. So *Bacillus* is feasible for industrial production. It is also a nonpathogenic bacteria widely existing in nature, harmless to people and livestock and does not pollute the environment. As early as 1945, Johnson et al. had reported that *Bacillus* can produce antibacterial substances. Researchers in different countries have great interest in what a biocontrol factor is expected to be. Furthermore, *Bacillus* has a strong anti-reversion force and its colonization in the soil is relatively easy. It has great significance for the practical production of antagonistic bacterium liquid preparations. Therefore, in later screening work, screening *Bacillus* first, then doing further rescreening can greatly reduce the workload.

The DM-54 strain has excellent performance, but only in a reasonable fermentation process can the potential value be brought into full play. In addition to the restricted nutrition factors, the right fermentation condition is another important factor for antibiotic fermentation (Jiang et al., 2007). Fermentation is a complex and dynamic vital process. In this experiment, we only studied some of the major factors affecting the optimization of the fermentation conditions. Further experiments are needed to bring it into industry.

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