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OPTIMIZATION OF FERMENTATION MEDIUM AND FERMENTATION CONDITIONS OF BIOCONTROL STRAIN A192 AGAINST PEPPER ANTHRACNOSE

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Abstract

Chili anthracnose is one of the three most common and serious diseases in chili peppers. In the early stage of this experiment, a fungal strain A192 was screened from the rhizosphere soil of vegetable crops, which had a good antagonistic effect on *Colletotrichum scovillei*. It was identified as *Penicillium restrictum*. To maximize its antifungal effect, the fermentation medium and conditions of strain A192 were optimized. Through single factor experiments, orthogonal experiments, and response surface experiments, the optimal fermentation medium for strain A192 was determined to be dextrin 30.03g/L, diammonium dihydrogen phosphate 3.08g/L, and $ZnSO_4 \cdot 7H_2O$ 1.79g/L. Build a model using Design Expert software and predict an inhibition rate of 85.56% under the above conditions. The actual inhibition rate obtained after verification is 85.53%, which is close to the predicted value and increases by 24.24% compared to the initial inhibition rate. Meanwhile, the optimal fermentation conditions are: liquid volume of 100 mL, temperature of 25°C, and fermentation for 10 days.

Key words: Chili anthracnose; Biocontrol fungi; *Penicillium restrictum*; Response surface methodology.

Introduction

Capsicum annuum L., is also known as Qin chili and sea chili Chili anthracnose is a worldwide plant fungal disease that occurs on pepper plants and is caused by *Colletotrichum* spp. and is considered a serious threat. Chili anthracnose pathogen is one of the most common endophytic fungi among plant pathogens, and due to its economic and scientific importance, it is ranked as the eighth most important plant pathogenic fungus by plant pathologists (Cheng & Sui, 2015). The genus *Colletotrichum* causes economically devastating diseases in a variety of hosts. These include cereals, legumes, vegetables, trees and fruits (Shi, 2020). The plant disease was first reported in the United States in 1790 and has since been reported in many parts of the world. There are more than 20 species of *Colletotrichum* that have been reported to cause chilli anthracnose (Silva *et al*., 2019). *C. scovillei*, a member of the *C. acutatum* species complex, is considered to be the major cause of severe losses in pepper production in tropical and temperate countries such as Brazil, China, Indonesia, Japan, South Korea, Malaysia and Thailand (Khalimi *et al*., 2019). Anthracnose caused huge economic losses, with a loss of approximately 29.5% of crop yields. Anthracnose often occurs after the fruit is harvested, leading to reduced yield and fruit quality, thereby reducing the economic benefits of chilli peppers.

The control measures of pepper anthracnose mainly include agricultural control, biological control and chemical control (Wang *et al*., 2019), in actual production, chemical control is the main measure to control pepper anthracnose. However, the actual production of traditional agents carbendazim, methylthiobacillam and mancozeb and other effects are not ideal, and the long-term use of easy to produce drug resistance (Zhou *et al*., 2020). Currently, most biological control strategies for plant diseases are based on the selection of antagonistic micro-organisms from nature and the inhibition of the growth of pathogen through nutrient competition, re-parasitism, induced resistance and the production of antibiotics, hydrolytic enzymes and other means (Whipps, 1997). Currently reported antagonistic microorganisms of pepper anthracnose are more diverse, including antagonistic bacteria, antagonistic fungi and antagonistic actinomycetes, and some of the research results of the antagonistic microorganisms of the inhibitory components and antagonistic mechanisms have also been explored and studied (Jiang & Song, 2014).

The composition of the fermentation medium influences the supply of nutrients and microbial cell metabolism, and the productivity of inhibitory substances for the fermentation process depends on the medium used. Among the main cultured nutrients, carbon and nitrogen sources usually play a dominant role in fermentation productivity, as these nutrients are directly related to biomass and metabolite formation (López *et al*., 2010). In addition, the properties and concentrations of carbon sources can regulate secondary metabolism through phenomena such as inhibition of catabolism (Hajjaj *et al*., 2001). Therefore, in order to maximize the antifungal effect of the bio-prophylaxis strain fermentation broth, it is necessary to optimize its fermentation medium and fermentation conditions. Orthogonal and response surface design methods have been used in many studies to optimize fermentation media due to the wide range of quantitative and qualitative variables involved in the process (Ren *et al*., 2017). This study used the single factor alternate method and response surface methodology to explore the optimal carbon source, nitrogen source, inorganic salt, pH, fermentation time, fermentation temperature, and liquid volume in flask of the fermentation medium, that in order to obtain the optimal fermentation condition parameters of the antagonistic strain A192. Thereby improving the inhibitory effect on chili anthracnose and providing a theoretical basis for the development of biocontrol agents for this strain and the prevention and control of crop diseases.

Material and Methods

Test materials: Strain A192 (*P. restrictum*), *C. scovillei.* The above two strains were isolated and provided by Plant Disease Institute of Jilin Agricultural University.

Potato dextrose agar (PDA) medium: Potato 200.0g·L^{-1}, Glucose 20.0g·L^{-1}, agar 15.0g·L^{-1};
Potato dextrose broth (PDB) medium: Potato 200g·L^{-1}, Glucose 20g·L^{-1};
Media for carbon source test: Carbon source (Glucose/ Sucrose/Soluble starch/Dextrin/Maltose/Corn protein/ Lactose / Mannitol / Fructose) 20.0 g·L^{-1};
Media for nitrogen source test: KH_2PO_4 3.0 g·L^{-1}, $MgSO_4$ 1.5 g·L^{-1}, Glucose 20 g·L^{-1}, Nitrogen source (Peptone / Yeast extract powder / Beef extract / Tryptone / Urea / Potassium nitrate / Ammonium chloride / Peanut meal / Bran / Ammonium phosphate) 2.0 g·L^{-1};
Media for inorganic salt test: Yeast extract paste 2.0 g·L^{-1}, Peptone 2.0 g·L^{-1}, Glucose 20 g·L^{-1}, Inorganic salt ($CuSO_4·5H_2O$/$ZnSO_4·7H_2O$/$MgSO_4·7H_2O$/$FeSO_4·7H_2O$/$CaCO_3$/$K_2HPO_4·3H_2O$/KH_2PO_4/NaCl) 1.0 g·L^{-1};
Media for pH test: KH_2PO_4 3.0 g·L^{-1}, Yeast extract paste 2.0 g·L^{-1},Peptone 2.0 g·L^{-1}, $MgSO_4$ 1.5 g·L^{-1}, Glucose 20 g·L^{-1}, the pH of the sterilized medium was adjusted to 5.0, 6.0, 7.0, 8.0, and 9.0 with 1.0 mol·L^{-1} HCl and 1.0 mol·L^{-1} NaOH, respectively.

Test methods

Screening of optimal components in fermentation medium: Single-factor experiment: Two 8 mm discs of strain A192 were inoculated into 35 kinds of carbon source, nitrogen source, inorganic salt and pH test medium respectively. Set up 3 replicates, sterile water mixed with PDA medium at a ratio of 1:5 was used as a control group. And shake culture at 25℃ and 175 rpm for 7 days. The inhibition of anthracnose by the fermentation filtrate of strain A192 after fermentation by different components of the medium was determined according to the following formulae and analysed for significance using SPSS software.

$$I = \frac{C - T}{C} \times 100\%$$

where, I = Percent growth inhibition, C = Growth in control, T = Growth in treatment.

Orthogonal experiment: The optimal combination of components was obtained by L_9 (3^4) orthogonal experimental design (Table 1) with 4 factors of carbon source, nitrogen source, inorganic salt, and pH, and 3 levels of the better three components of each factor in the single factor test with intuitive analysis.

Optimization of fermentation medium components additions: Based on the optimal components obtained by orthogonal test, the addition of carbon source, nitrogen source and inorganic salt was optimized by response surface experiment. A group of optimal liquid medium suitable for fermentation of strain A192 was screened out.

Single-factor experiment: The effect of dextrin addition (15 g·L^{-1}、20 g·L^{-1}、25 g·L^{-1}、30 g·L^{-1}、35 g·L^{-1}), Ammonium phosphate addition (1.5 g·L^{-1}、2 g·L^{-1}、2.5 g·L^{-1}、3 g·L^{-1}、3.5 g·L^{-1}) and $ZnSO_4·7H_2O$ addition (1 g·L^{-1}、1.25 g·L^{-1}、1.5 g·L^{-1}、1.75 g·L^{-1}、2 g·L^{-1}) on the inhibition rate was investigated sequentially using one-factor-at-a-time experimentation. The initial fermentation condition was 25℃, 175 rmp, 7 d, and pH 6.0.

Response surface experiment: According to the results of single factor test, the parameter range of response surface test for each component addition was determined (Song *et al*., 2018; Wang *et al*., 2012). A 3-factor, 3-level response surface test was conducted using the Box-Behnken experimental design and analysis method to determine the optimal parameters for the fermentation of strain A192 (Shu *et al*., 2016). Analyze and process the data with Design Expert 8.0.6.

Optimization of fermentation conditions: Set the liquid volume as 50 mL, 75 mL, 100 mL, 125 mL, 150 mL (250 mL conical flask), fermentation time as 4 d, 5 d, 6 d, 7 d, 8 d, fermentation temperature as 15℃, 20℃, 25℃, 30℃, 35℃. The inhibition rate of fermentation broth was determined, and the optimal fermentation conditions were selected.

Results and Analysis

Screening of optimal components in fermentation medium: Strain A192 was fermented by media with different carbon sources, nitrogen sources, inorganic salts and pH, and its inhibition effect varied widely.

Nine carbon sources and no added carbon sources were screened, and the results showed that the three lowest inhibition rates were: maltose (47.49%) < fructose (49.36%) < no added carbon source (61.72%); the three highest inhibition rates were: dextrin (71.05%) > sucrose (70.96%) > mannitol (70.47%). Moreover, after fermentation with medium supplemented with dextrin, sucrose or mannitol. Their inhibition rates were significantly better than those of other carbon sources at the *P=0.05* level, ranging from 9.67% to 9.76% higher than that of the initial carbon source (glucose).

Ten nitrogen sources and not added were screened, and the three lowest inhibition rates were: Bran (55.42%) < No added nitrogen source (55.94%) < Beef paste (57.73%); the three highest inhibition rates were: diammonium hydrogen phosphate (88.77%) > ammonium chloride (74.61%) > urea (71.60%). After fermentation with medium supplemented with diammonium hydrogen phosphate, ammonium chloride or urea, the bacterial inhibition rate was significantly higher than that without additional nitrogen source or with other nitrogen sources, and the bacterial inhibition rate was increased by 10.31% to 27.48% compared with that of the initial nitrogen source (without additional nitrogen source) (Fig. 1).

Table 1. Factors and levels of orthogonal design.

Level	Factor			
	A carbon source single factorource	C Inorganic salt	D pH	
1	Dextrin	Ammonium phosphate	$ZnSO_4·7H_2O$	5
2	Sucrose	Ammonium chloride	$CuSO_4·5H_2O$	6
3	Mannitol	Urea	$FeSO_4·7H_2O$	7

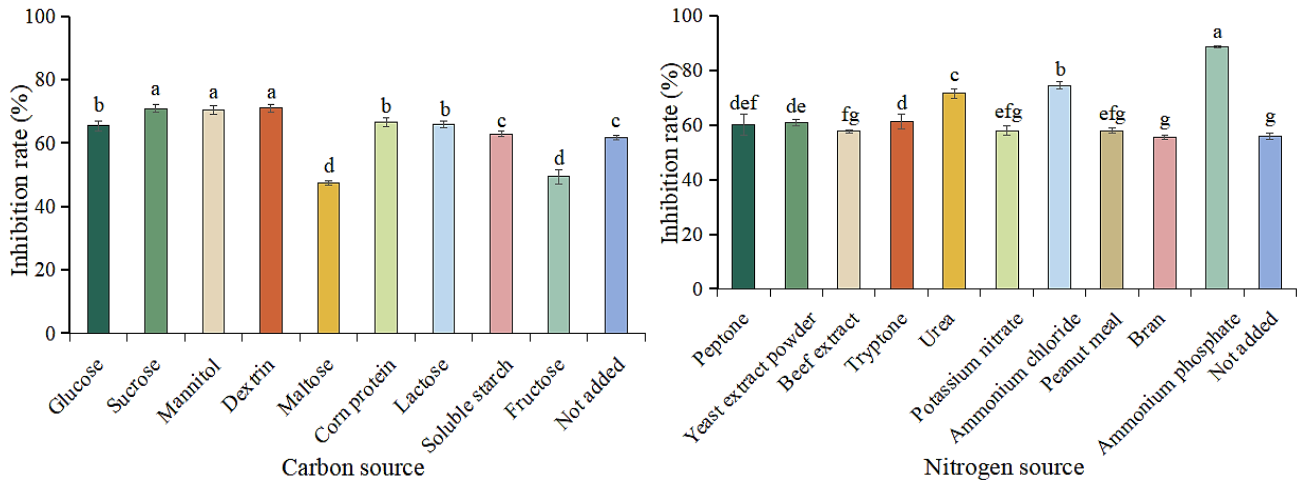

Fig. 1. Inhibition rate of fermentation with different carbon and nitrogen sources different lowercase letters indicate significant differences at the p=0.05 level.

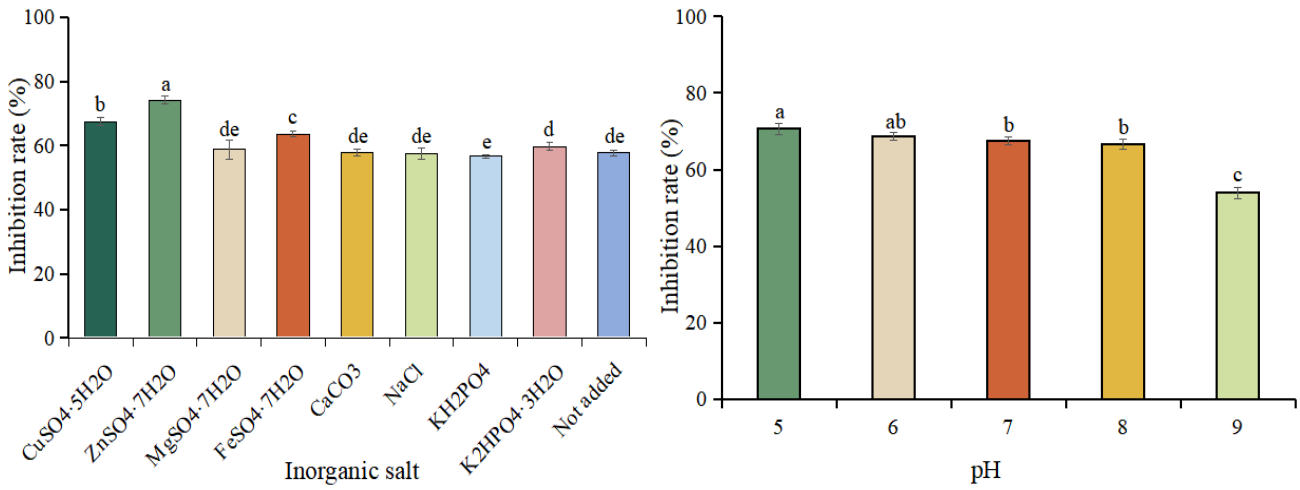

Fig. 2. Inhibition rate of fermentation with different inorganic salt and pH Different lowercase letters indicate significant differences at the p=0.05 level.

After that, 8 kinds of inorganic salts and no added inorganic salts were screened, and it can be seen from Fig. 2 left that the 3 kinds with the lowest inhibition rate are: KH_2PO_4 (56.24%) ＜No added inorganic salts (56.62%) ＜ NaCl (57.51%); and the 3 kinds with the highest inhibition rate are: $ZnSO_4·7H_2O$ (74.15%) ＞$FeSO_4·7H_2O$ (67.48%) > $CuSO_4·5H_2O$ (63.56%). The inhibition rate after fermentation using $CuSO_4·5H_2O$ medium with added $ZnSO_4·7H_2O$, $FeSO_4·7H_2O$ or was significantly higher than that without added inorganic salts, with an increase of 2.27% to 12.86%.

In the pH screening test, the best inhibition of fermentation was shown when the pH was 5, 6 and 7.

Screening of optimal components in fermentation media: The intuitive analysis of the orthogonal experiments can be seen. Factor B has the largest R value of 8.70, which is the primary factor affecting the inhibition rate. The R values of factor A and factor C are 2.84 and 1.04 respectively; The R value of factor A is only 0.83. The order of importance of the influence on the inhibition rate was nitrogen source, carbon source, inorganic salt, and PH. The results showed that the optimal components of fermentation medium were $A_1B_1C_1D_1$, that is, the highest inhibition rate of 79.77% was achieved when the carbon source was dextrin, the nitrogen source was Ammonium phosphate, the inorganic salt was $ZnSO_4$-$7H_2O$, and the pH was 5 (Tables 2 and 3).

Single factor test: As can be seen from Fig. 3, there was a large difference in the inhibitory effect of strain A192 on chili anthracnose pathogen after fermentation in the medium with the addition of 15~35 g·L^{-1} dextrin. When the dextrin was added at 30 g·L^{-1}, the highest inhibition rate (85.11%) was observed for chili anthracnose pathogen, and the excessive addition of dextrin was detrimental to the fermentation of strain A192. The inhibitory effect of Ammonium phosphate at the addition level of 1.5~3.5 g·L^{-1} on the fermentation filtrate also showed significant differences. When Ammonium phosphate was added at 3.0 g·L^{-1}, the highest inhibition rate (86.54%) was observed against *C. scovillei*. There was also a significant difference in the inhibition rate of the fermentation broth when $ZnSO_4·7H_2O$ was added at 1.00~2.00 g·L^{-1}, and the highest inhibition rate against *C. scovillei* (85.81%) was achieved when $ZnSO_4·7H_2O$ was added at 1.75 g·L^{-1}. Therefore, the optimal dextrin, Ammonium phosphate, and $ZnSO_4·7H_2O$ additions to the liquid fermentation medium were 30 g·L^{-1}, 3.0 g·L^{-1}, and 1.75 g·L^{-1}, respectively.

Table 2. The intuitive analysis of the orthogonal experiments.

Test number	Factors				Inhibition rate (%)
	A	B	C	D	
1 ($A_1B_1C_1D_1$)	Dextrin	Ammonium phosphate	$ZnSO_4·7H_2O$	5	79.77 ± 0.8716[a]
2 ($A_1B_2C_3D_2$)	Dextrin	Ammonium chloride	$FeSO_4·7H_2O$	6	71.77 ± 0.8723[d]
3 ($A_1B_3C_2D_3$)	Dextrin	Urea	$CuSO_4·5H_2O$	7	70.78 ± 0.9043[de]
4 ($A_2B_1C_3D_3$)	Sucrose	Ammonium phosphate	$FeSO_4·7H_2O$	7	78.05 ± 0.8379[b]
5 ($A_2B_2C_2D_1$)	Sucrose	Ammonium chloride	$CuSO_4·5H_2O$	5	71.23 ± 0.9554[d]
6 ($A_2B_3C_1D_2$)	Sucrose	Urea	$ZnSO_4·7H_2O$	6	69.56 ± 1.2397[e]
7 ($A_3B_1C_2D_2$)	Mannitol	Ammonium phosphate	$CuSO_4·5H_2O$	6	75.82 ± 0.6912[c]
8 ($A_3B_2C_1D_3$)	Mannitol	Ammonium phosphate	$ZnSO_4·7H_2O$	7	70.80 ± 0.8927[de]
9 ($A_3B_3C_3D_1$)	Mannitol	Urea	$FeSO_4·7H_2O$	5	67.19 ± 0.7422[f]

Note: 0 means not added; different lowercase letters indicate significant differences at the p=0.05 level

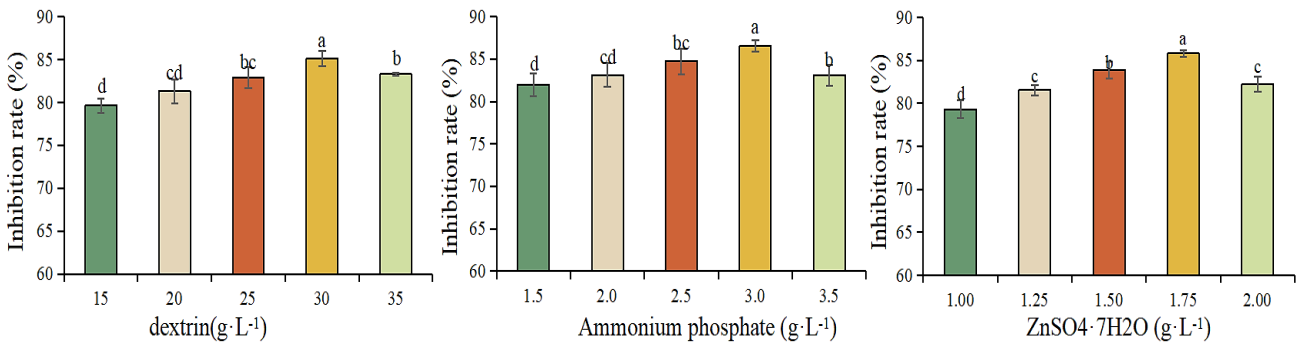

Fig. 3. Effects of dextrin, ammonium phosphate and $ZnSO_4·7H_2O$ addition on the inhibition rate Different lowercase letters indicate significant differences at the *P*=0.05 level.

Table 3. Range analysis results of various factors.

Index	Factor			
	A	B	C	D
K_1	222.32	233.64	220.13	218.19
K_2	218.84	213.80	217.83	217.15
K_3	213.81	207.53	217.01	219.63
k_1	74.11	77.88	73.38	72.73
k_2	72.94	71.27	72.61	72.38
k_3	71.27	69.18	72.34	73.21
R	2.84	8.70	1.04	0.83

Note: K_1, K_2 and K_3 are the sum of all levels of each factor; k_1, k_2 and k_3 are the average values of the level of each factor; *R* value of are the range of k_1, k_2 and k_3

Response surface experiment: The model building and significance examination was done by using medium 1 in the orthogonal experiment. The inhibition rate (Y) was used as the response value, and the amount of dextrin (X_1), ammonium phosphate (X_2), and $ZnSO_4·7H_2O$ (X_3) were used as the 3 factors. And the variable levels are represented by -1, 0, and 1 (Table 4). Design Expert 8.0.6 was used to design a 3-factor, 3-level response surface optimization test to determine the optimal addition combination. The response surface test data are shown in (Table 4).

The regression equation was obtained by fitting the scores in Tab. 3-4 using Design expert V8.0.6.1 software: $Y = 85.53 + 0.073X_1 + 0.34X_2 + 0.066X_3 - 0.39X_1X_2 + 0.040X_1X_3 + 0.028X_2X_3 - 1.40X_1^2 - 1.07X_2^2 - 0.21X_3^2$. From the equation, it can be concluded that in this model, the inhibition rate is most affected by the amount of ammonium phosphate, followed by the amount of dextrin, and least affected by the amount of $ZnSO_4·7H_2O$.

Analysis of variance and significance of the inhibition rate scores (Tables 4-6) shows that the F-value of the model is 20.23 and $p<0.0001$, implying that the model is significant. X_1^2、X_2^2 are extremely significant terms, and X_1、X_2、X_3、X_1X_2、X_1X_3、X_2X_3、X_3^2 are insignificant terms. The F-value of the lack of fit is 2.09 and *P* (=0.24433) > 0.05, implying that the lack of fit is not significant relative to the pure error, indicating that the model has a high reliability. $R^2 = 0.9630$ and $R^2_{Adj} = 0.9154$ indicate that the model has a low error and a strong fit. The difference between $R^2_{Pred} = 0.6158$ and R^2_{Adj} is also within a reasonable range. Adeq Precision = 14.441 > 4 also confirms that the model is ideal.

Table 4. Variables and level of Box-Behnken design.

Level	Factor		
	X_1 Dextrin addition ($g·L^{-1}$)	X_2 Ammonium phosphate addition ($g·L^{-1}$)	X_3 $ZnSO_4·7H_2O$ addition ($g·L^{-1}$)
-1	25	2.5	1.50
0	30	3.0	1.75
1	35	3.5	2.00

Table 5. The results of response surface optimization test for strain A192 fermentation.

Code	Factors and levels			Y Inhibition rate (%)
	X_1 (g·L^{-1})	X_2 (g·L^{-1})	X_3 (g·L^{-1})	
1	-1	-1	0	82.46
2	1	-1	0	83.24
3	-1	1	0	83.67
4	1	1	0	82.89
5	-1	0	-1	83.92
6	1	0	-1	84.13
7	-1	0	1	83.65
8	1	0	1	84.02
9	0	-1	-1	83.59
10	0	1	-1	84.46
11	0	-1	1	83.99
12	0	1	1	84.97
13	0	0	0	85.76
14	0	0	0	85.48
15	0	0	0	85.37
16	0	0	0	85.25
17	0	0	0	85.81

Fig. 4 represents the contour and 3D surface maps of the effect of the interaction of dextrin, ammonium phosphate and $ZnSO_4·7H_2O$ addition on the inhibition rate.

Fig. 4-above shows the interaction between dextrin and ammonium phosphate addition at the central level (1.75 g·L^{-1}) of $ZnSO_4·7H_2O$ addition, indicating that the inhibition rate increases and then decreases with the increase of dextrin and ammonium phosphate addition. Fig. 4-middle shows the interaction between dextrin and $ZnSO_4·7H_2O$ addition at the central level (2.5 g·L^{-1}) of ammonium phosphate addition, indicating that the inhibition rate increases and then decreases with the increase of dextrin and $ZnSO_4·7H_2O$. Fig. 4-below shows the interaction between $ZnSO_4·7H_2O$ and ammonium phosphate addition at the central level (25 g·L^{-1}) of dextrin addition, indicating that the inhibition rate increases and then decreases with the increase of $ZnSO_4·7H_2O$ and ammonium phosphate addition.

The maximum predicted response value (inhibition rate) was 85.56% at the combination of dextrin 30.03 g·L^{-1}, ammonium phosphate 3.08 g·L^{-1} and $ZnSO_4·7H_2O$ 1.79 g·L^{-1} for each factor of the model. Tests were conducted according to the best combination given by the model, and the actual inhibition rate obtained was 81.53%, which was similar to the predicted value, proving the validity of the model. The inhibition rate was 24.24 percentage points higher than that of fermentation with PDB medium (61.29%) before optimization (Fig. 5).

Optimization of fermentation conditions: The highest inhibition was 90.79% at 10 d of fermentation time, which was significantly better than 7 d, 8 d, 9 d and 11 d. The highest inhibition rate was 80.47% at a medium volume of 100 mL (250 mL conical flask), which was not significantly different from 125 mL and 150 mL, but significantly better than 50 mL and 75 mL. At the fermentation temperature of 25°C, the highest inhibition rate was 84.33%, which was significantly better than other temperatures (Fig. 6).

The results showed that the optimal fermentation conditions were at 100 mL medium, 25°C and 10 d.

Table 6. Variance analysis of response surface experiments results.

Source	Sum of Squares	df	Mean Square	F Value	*P*-value Prob > F	Sig.
Model	15.87	9	1.76	20.23	0.0003	**
X_1	0.042	1	0.042	0.48	0.5098	
X_2	0.92	1	0.92	10.53	0.0142	
X_3	0.035	1	0.035	0.40	0.5459	
X_1X_2	0.61	1	0.61	6.98	0.0333	
X_1X_3	0.0064	1	0.0064	0.073	0.7943	
X_2X_3	0.0030	1	0.0030	0.035	0.8575	
X_1^2	8.20	1	8.20	94.08	＜0.0001	**
X_2^2	4.85	1	4.85	55.63	0.0001	**
X_3^2	0.18	1	0.18	2.09	0.1911	
Residual	0.61	7	0.087			
Lack of Fit	0.37	3	0.12	2.09	0.2443	
Pure Error	0.24	4	0.059			
Cor Total	16.48	16				

Note: "*" Indicated significant impact on the results ($0.01<p<0.05$); "**" Indicated that the impact on the results was extremely significant ($p<0.01$)

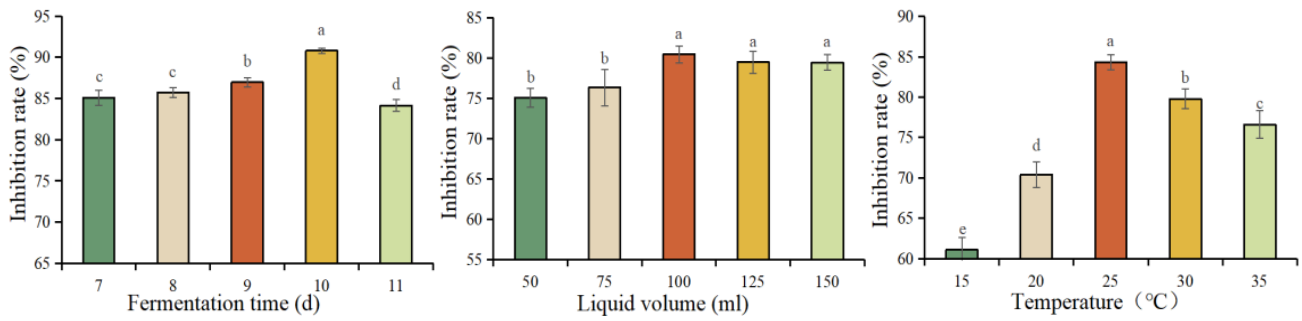

Fig. 6. Inhibition rate of fermentation broth after treatment with different fermentation time, liquid volume and fermentation temperature. Different lowercase letters indicate significant differences at the p=0.05 level.

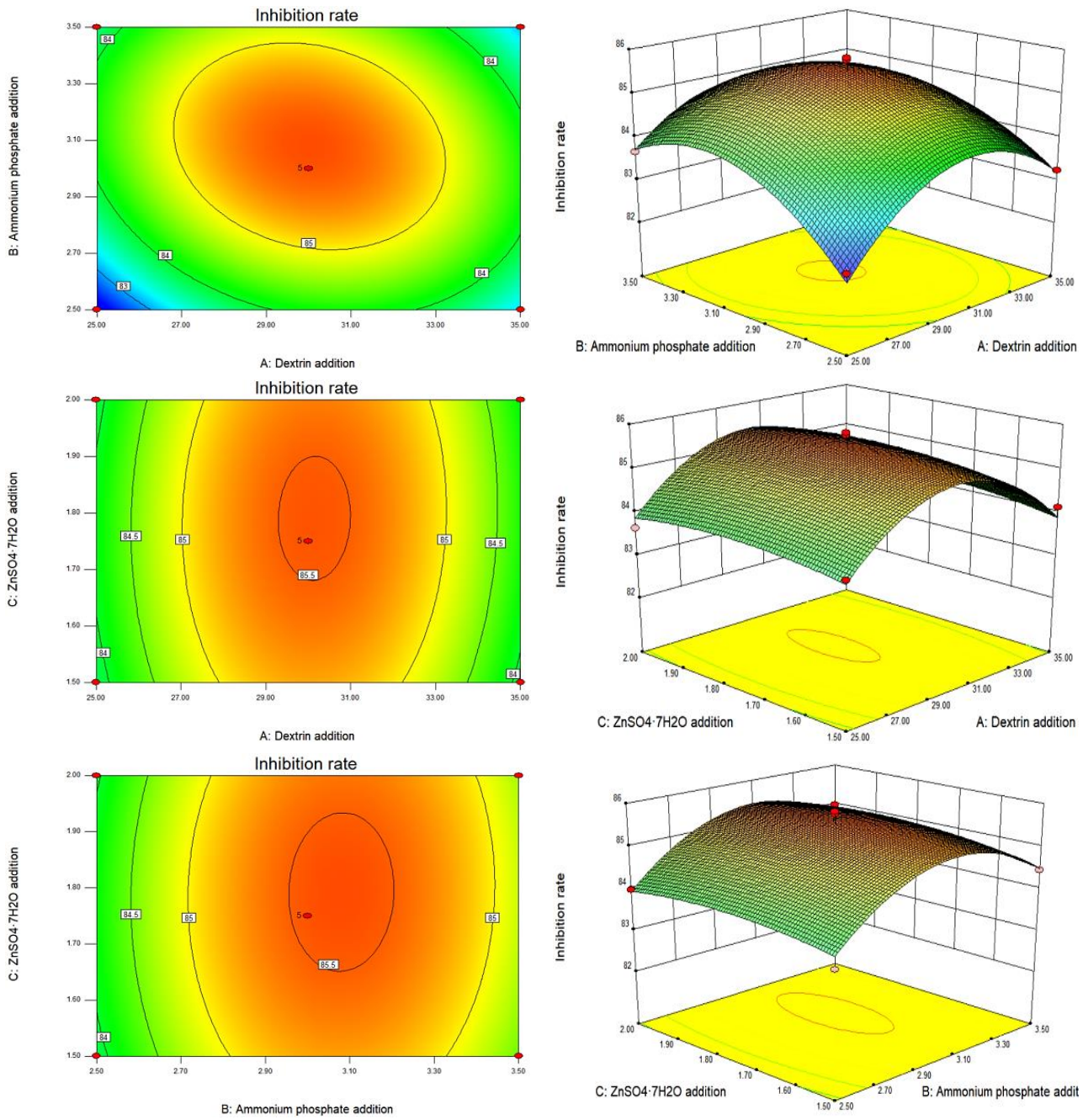

Fig. 4. Contour and 3D surface maps of interactions between factors.

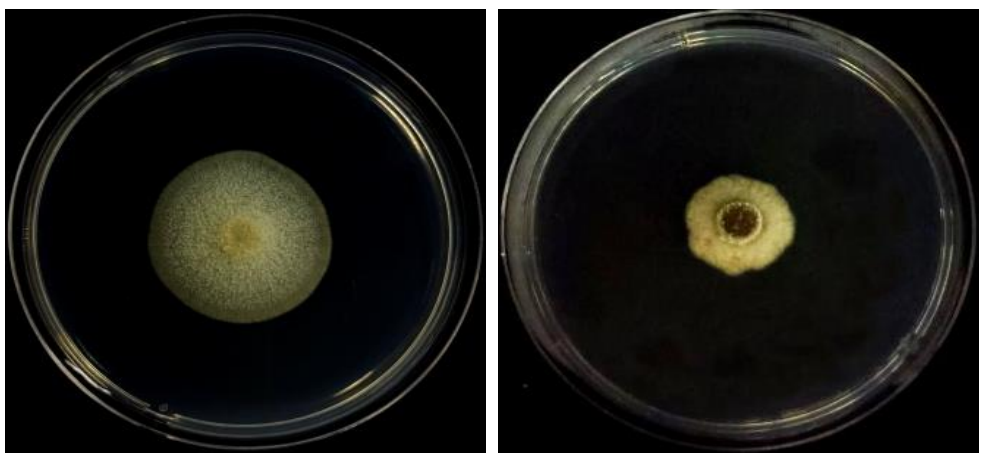

Fig. 5. Comparison of inhibition rate between initial (left) and optimized (right) fermentation.

Discussion

With the growing demand for green agricultural production, biological control as a pollution-free alternative to pesticides has become a hot research topic today (Qiu, 2015). Natural microorganisms and extracts of plant origin are constantly being studied for biocontrol applications. *Penicillium*, as a kind of fungus existing in large quantities in nature, has the advantages of fast reproduction speed, strong spore-producing ability, wide source of medium raw materials and low price, and has great potential in the biological control of plant diseases (Zhang *et al*., 2017). Penicillin was the first antibiotic used in medicine and its discovery was one of the greatest achievements in chemotherapy (Tang, 1957). Subsequently, there have been reports of *Penicillium* in the field of biocontrol. A researcher found that a strain of *P. oxalicum* HB21-1 has a significant antagonistic effect on the root rot pathogen of peppercorns, and can reduce the incidence of the disease, which has potential applications in biological control (Li *et al*., 2023), others screened the antagonistic *P. janthinellum* F1-6 strain from the inter-root soil of healthy plants, which showed 63.64 percent inhibitory, 70.78 percent preventive and 61.03 percent therapeutic efficacy against leaf blight pathogens (Liu *et al*., 2020). Some found that *Purpureocillium lilacinum* 2018-32 was 81.19% and 64.06% effective against root rot and root-knot nematode disease of cucumber, respectively, which were better than similar commercial fungicides (Zhang *et al*., 2021a).

Response surface methodology is often used in multifactor fermentation optimisation, which uses multiple quadratic regression equations to fit the functional relationship between the factors and the response values within a certain range, which can be used to optimise the response values to obtain the optimal process parameters in a quick and easy way. This method is a statistical method for solving multivariate problems with fewer tests, shorter cycle time and high accuracy of the obtained regression equations (Zhang *et al*., 2021b), which is widely used in microbial fermentation and optimisation of culture conditions (Zhou *et al*., 2023; Hassan *et al*., 2020). Application of response surface methodology by some researchers to optimise the fermentation process of the endophytic biocontrol fungus *Paecilomyces lilacinus* WG9 in northern schizothorpe brown spot disease (Zhang *et al*., 2021c). Optimisation of *Streptomyces nosocomialis* fermentation conditions by response surface methodology by some to increase the yield of gougerotin by a factor of 3 (Song *et al*., 2024). Optimisation of culture conditions for liquid fermentation of *Metarhizium rileyi* using response surface methodology to increase biomass per ml of *Metarhizium rileyi* fermentation broth (Xue *et al*., 2023). In this experiment, strain A192 was used as the research object, and the optimal three levels of each of the 35 carbon sources, nitrogen sources, inorganic salts, and pH were screened on the basis of a one-way experiment, respectively, and the optimal medium composition of dextrin, ammonium phosphate, $ZnSO_4 \cdot 7H_2O$, and pH 5 was screened by orthogonal test (Zhang *et al*., 2024; Ma *et al*., 2024). Modelling using Design Expert software predicted that the highest inhibition rate of 85.56% was achieved at the optimal addition levels of dextrin, ammonium phosphate, and $ZnSO_4 \cdot 7H_2O$ of 30.03 $g \cdot L^{-1}$, 3.08 $g \cdot L^{-1}$, and 1.79 $g \cdot L^{-1}$, respectively. The validation test showed that the inhibition rate was 85.53%, which was close to the predicted value and 24.24% higher than that before optimisation. On this basis, the optimal fermentation conditions were derived from a one-way test: 100/250 mL of liquid volume, 25℃, and 10 d of fermentation. This experiment provides a theoretical basis for the future application of biocontrol fungi in actual production, but it needs to be further tested and adjusted in actual production in the future, as it was only carried out in a laboratory shake flask environment.

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