Effect of Aluminium oxide on inhibition activity of a deep-sea Penicillium sp. against Aspergillus parasiticus

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Abstract. In this study, the effects of aluminium oxide content, micro particle diameter and rotational speed on the activity of fermentation broth of deep-sea *Penicillium* MHE1 in inhibiting the growth of *Aspergillus parasiticus* NFRI-95 hypha and aflatoxin production were studied by uniform design. The optimal combination is obtained by regression analysis and experimental verification. The optimal combination is 1.015 g / L and 101.5 mesh in diameter for aluminium oxide, and 108.6 r / min for rotation incubation. Under these conditions, the mycelium inhibition rate of fermentation broth of strain MHE1 to Aspergillus parasiti NFRI-95 reached 85.88%, and the aflatoxin inhibition rate reached 100%, which increased by 7.1% and 9.2% respectively compared with that before optimization.

1. Introduction

The ocean is a huge, untapped resource, especially a deep sea with high salinity, high pressure, low temperature, little nutrition, and no light[1]. As an important group of marine microorganisms, the characteristics of unique metabolic pathway and genetic background, as well as the rich and diverse structure and activity of their metabolites, the deep-sea fungi have become a hot spot in the research of secondary metabolites among marine microbes[2]. In the study of the metabolites of deep-sea fungi, they play a significant role in antifungal activities, but there is no report on the inhibition of aflatoxin up to date[3]. Aflatoxin is a derivative of difuranoxano-naphtho-ketone, which contains a difuran ring and a coumarin ring in its molecular structure. A total of 18 kinds of aflatoxins, such as BI, B2, G1, G2, B2α, G2α, M1, M2, P1 have been isolated, among which aflatoxin B1 (AFB1) has the strongest toxicity and carcinogenicity[4]. Aflatoxin B1 (AFB1) is a secondary metabolite produced mainly by *Aspergillus flavusus*, *Aspergillus parasitic sus* and *Aspergillus Niger*. It is naturally found in crops such as peanut, cottonseed, corn, wheat and rice[5]. The strong protein secretion ability of filamentous fungi is adapted to the way they grow under natural conditions[6]. It shows that the filamentous fungi have an obvious characteristic, that is, the variable mycelium morphology in fungus liquid fermentation. Due to the morphological characteristics of filamentous fungi, a serious problem caused by the increase of biomass in submerged culture of filamentous fungi is high viscosity[7]. Generally speaking, high viscosity also means that the mass transfer and mixing conditions become worse during fermentation, thus exacerbating the inconsistency of the fermentation region[8]. Even in laboratory-scale fermentation experiments, the homogeneity of fermentation broth is still difficult to guarantee.

Considering the significant effect of fungal mycelium morphology on fermentation products, the control of mycelium morphology has always been the focus of process control, and it is also the difficulty of the production of filamentous fungi at present [9]. Therefore, it is necessary to obtain the optimal fermentation products by controlling the fermentation form of the filamentous fungi.

In this paper, the effects of different amount and sizes in diameter of aluminium oxide microparticles on the anti-mycelium and antiaflatoxigenic activities of a deep-sea *Penicillium* strain MHE1 fermented under different rotational speeds were studied by uniform design, aiming at increasing the yield of active substances and obtaining the best active product which against *Aspergillus parasitic us* through stable fermentation by adding aluminium oxide microparticles to the fermentation system.

2. Materials and methods

2.1. Medium and aluminium oxide

PDA (Potato Dextrose Agar Medium): Potato 200g, glucose 15g, water 1L, pH6.5; GY (Glucose Yeast): glucose 20g, yeast 5g, deionized water1L.

Aluminium oxide with different mesh of 100, 200, 400, 600, 800, 1000, 1200 were purchased from Chemical Company.

2.2. Fungal strains

The deep-sea *Penicillium* strain MHE1 and *Aspergillus parasitica* NFRI-95 used in this study are from the Institute of Applied and Marine Microbiology, Harbin Institute of Technology (Weihai). Because *Aspergillus parasitic* NFRI-95 can produce an orange-red norsolorinic acid (NA) which is the first stable intermediate product in the process of aflatoxin synthesis, and the strain has no nor-1 gene function[10], so it is relatively safe and reliable in application. Therefore, it can be visually screened out high activity strains with anti-mycelium and antiaflatoxin.[11] The deep-sea *Penicillium* MHE1 has the activity of inhibiting NFIR-95 mycelium growth and NA production by tip-culture assay.

2.3. Uniform Design of fermentation conditions for Aluminium oxide microparticles

The DPS data processing system is used to design the mixing level of three factors: the diameter of aluminium oxide (100, 200, 400, 600, 800, 1000 and 1200 mesh), the amount of aluminium oxide (1, 2.5, 5, 7.5, 10, 15, 20 g/L), and the rotational speed (100, 200, and 300 r/m). Accordingly, a total of 13 groups of fermentation schemes were obtained, and the MHE1 was fermented according to the scheme.

2.4. Determination of antifungal and anti-aflatoxin activity

The strain MHE1 was cultured in PDA medium in shaking flask for 6 days. After fermentation, supernatant was collected by centrifugation at 6000 r/m for 10 min, then GY was added to the supernatant for supplement of nutrients and pH was adjusted to 6.5 in order to support the growth and aflatoxin production by *Aspergillus parasiticus*, which was used to determine the antifungal and anti-aflatoxin activity of MHE1 supernatant by tip culture methods[12]. The inhibition rate to mycelium growth and aflatoxin production were calculated according to the following formula: The inhibition rate of mycelium (%) = (fresh weight of mycelium in control group - fresh weight of mycelium in experimental group) / fresh weight of mycelium in control group * 100%; Inhibition rate of toxin = (OD of control group - OD of experimental group) / OD of control group * 100%.

3. Results

3.1. Effect of addition of aluminium oxide micro particles on antifungal and anti-aflatoxin activity of fermentation broth.

From Table 1, we can see that the addition of aluminium oxide micro particles into the fermentation medium has a significant effect on the anti-mycelium and anti-aflatoxin activities of fermentation cell-

free supernatants. The highest inhibition rate of mycelium appears in the N7 fermentation scheme which is 91.37±3.99%. The minimum inhibition rate of mycelium appears in the N11 fermentation scheme which is 25.16±16.58%. For inhibiting the production of orange norsolorinic acid that is inhibiting aflatoxin production, the highest inhibition rate was found in the N12 fermentation scheme which is 98.67±0.9%. The lowest inhibition rate was found in the N11 fermentation scheme which is 38.18±24.05%. In order to obtain the optimal fermentation scheme, the anti-mycelium rate and the anti-aflatoxin rate were respectively taken as the goal, and regression analysis of quadratic polynomial stepwise, regression analysis of multiple factor and interaction items, multiple factor and square regression analysis and stepwise regression analysis of partial least squares quadratic polynomial were used to optimize the fermentation scheme by DPS uniform design software.

Table 1. Effect of addition of aluminium oxide microparticles on antifungal and anti-aflatoxin activity of fermentation cell-free supernatants

scheme	AVG ±STDEV anti-mycelium rate	AVG ±STDEV anti-aflatoxin rate
N1	65.56±13.23%	87.92±9.75%
N2	57.75±4.33%	97.54±1.48%
N3	31.59±9.21%	38.38±6.61%
N4	65.31±16.63%	90.12±10.02%
N5	59.37±9.55%	90.24±3.89%
N6	52.29±3.34%	97.67±0.56%
N7	91.37±3.99%	97.27±3.51%
N8	49.33±33.18%	83.11±15.42%
N9	62.28±18.00%	68.24±31.31%
N10	54.20±18.67%	91.12±2.11%
N11	25.16±16.58%	38.18±24.05%
N12	49.39±13.12%	98.67±0.90%
N13	54.44±15.66%	79.86±6.41%

3.2. Optimization and Experimental Verification of the fermentation Scheme

Based on the data in Table 1**,** regression analysis of quadratic polynomial stepwise, regression analysis of multiple factor and interaction items, multiple factor and square regression analysis and stepwise regression analysis of partial least squares quadratic polynomial were optimized in DPS software. Among them, the partial least square considering the interaction term simulates the optimization operation and obtains the operation regression equation is more ideal, as follows:

Regression equation of anti-mycelium rate:

$y1=0.7904601-0.009145a-0.000284b-0.001243c-0.000025a\times b+0.000092a\times c+0.00000b\times c$

Regression equation of anti-aflatoxin rate:

$y2=1.1040671-0.01107a-0.000344b-0.001504c-0.00003a\times b+0.000111a\times c+0.00000b\times c$

Where, a is amount of aluminium oxide (g/L), b is aluminium oxide diameter (mesh), c is rotational speed (r/min).

Under the optimal predictive inhibition rate to mycelium growth (y1=0.676) and aflatoxin production (y2=0.9821), the optimized various factors is as follows: a=1.015; b=101.5002; c=108.6

According to the optimized scheme, fermentation was carried out and the antifungal and anti-aflatoxin activity of fermentation cell-free supernatants was tested. The results show that the anti-mycelium rate and anti-aflatoxin rate of cell-free supernatants obtained under optimized fermentation conditions were 85.88% and 100% respectively (Figure 1), which were in good agreement with the predicted anti-mycelium rate (67.6%) and the predicted anti-aflatoxin rate (98.21%). The inhibition rate to mycelium growth and aflatoxin production under optimized fermentation conditions were higher than those before optimization, which were 80.17% and 91.57% respectively. Therefore, the

addition of aluminium oxide microparticles could increase the bioactive metabolite production against mycelium growth and aflatoxin production by Aspergillus parasiticus.

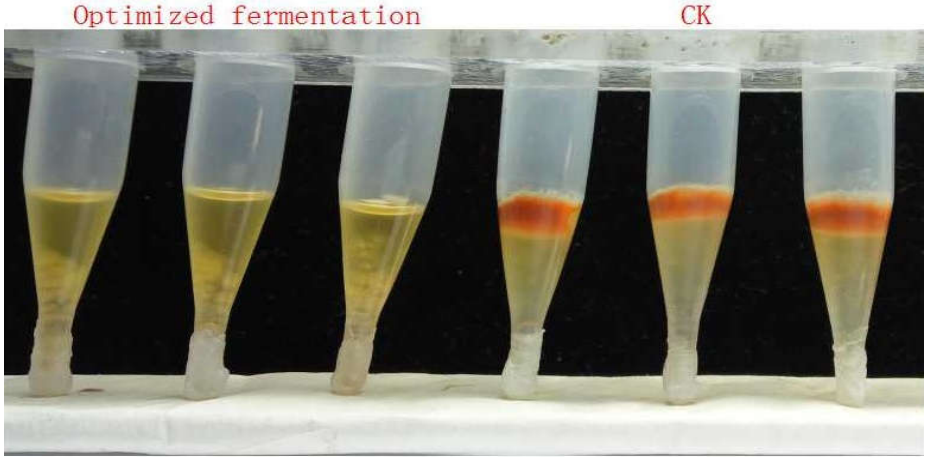

Fig. 1 Antifungal effect of strain MHE1 after fermentation optimization.

4. Conclusion

The work of inhibiting aflatoxin has been paid more and more attention, and it is a serious hazard to food and human health. In recent years, some scientific researchers have made further progress on the mechanism of aflatoxin and the mechanism of toxin synthesis, however, the study of biological control methods that can completely inhibit fungal growth and aflatoxin synthesis is still not optimistic [13]. The aim of this study was to investigate the effect of aluminium oxide microparticles on the inhibition of *Aspergillus parasiticus* NFRI-95 by secondary metabolites of deep-sea *Penicillium* strain MHE1. It was found that the addition of aluminium oxide microparticles to fermentation broth could increase the inhibitory effect of MHE1 metabolites on the growth of *Aspergillus parasiticus* NFRI-95 hyphae and the production of orange substance norsolorinic acid. Norsolorinic acid, the orange substance produced by NFRI-95, is the first stable intermediate product in the process of aflatoxin synthesis. Therefore, if norsolorinic acid was inhibited, the aflatoxin biosynthesis was inhibited too. This study has a certain application potential for the prevention and control of aflatoxin contaminated food and feed.

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