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Screening based on Ultraviolet Mutagenesis of Beauveria Bassiana Strains with Stronger Spores Yielding and Quality, Stress Tolerance and Virulence

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Abstract

Beauveria bassiana is a typical insect pathogenic fungus and has a strong killing effect on a variety of arthropod insects. Herein, we used the method of UV mutagenesis to carry out undirected mutation on the original strains of Beauveria bassiana and selected 5 mutant strains. After phenotypic and virulence determination of the mutant strains, we believe that B2, B3 and B5 are excellent strains and have been deposited.

Keywords

Beauveria Bassiana; Ultraviolet Mutagenesis; Virulence.

1. Introduction

As a typical filamentous entomopathogenic fungus, *Beauveria bassiana* can effectively kill most field arthropod insects, and has extremely high biocontrol potential, meanwhile, it has also been the study model for the gene mutation in fungus. In the natural environment, *B. bassiana* exist mainly in the form of aerial conidia [1]. Firstly, under suitable conditions, conidia germinate across the insect surface, afterwards, conidia will produce germinal structures such as germ tube and appressorium and secrete corresponding hydrolase which penetrates the host-wall and enters the insect hemocoel. Then, the differentiated hyphae form mycelium and multiply in the form of unicellular blastospore in the hemocoel of the host, which consumes host nutrition, secretes toxins, fight host immune system and destroys the body's tissue structure, eventually leading to the death of the host. Upon the host death, blastospores turn back to hyphae, which must penetrate again through the cuticle for outgrowth and conidiophore development so as to produce conidia on cadaver surface for survival, dispersion or new infection cycle [2-4]. During these growth or infection processes, entomopathogenic fungi encounter adverse environmental stress and the host-insect immune defense. Therefore, stress tolerance ability, cell size and maintenance of cell wall integrity are pivotal for fungal growth, conidiation, and environment adaption and host insect, as well as virulence [5-6].

In the process of fermentation production technology, researchers use a variety of mutagenesis methods to improve microbial strains to increase the yielding of target products [7]. In the rice production process, mutagenesis induction can produce genetically stable mutants that help to increase yield [8]. At present, the mutagenesis methods commonly used in laboratories are ARTP mutagenesis and ultraviolet mutagenesis (UV-mutagenesis). Among them, UV-mutagenesis is more common, and it has the advantages of high safety performance, simple experimental conditions and easy operation.

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2. Materials and Methods

2.1. Strains and Culture Conditions

The wild-type (WT) strain of *B. bassiana* ARSEF 2860 and its mutants were routinely grown on SDAY plates (4% glucose, 1% peptone, 1% yeast extract, and 1.5% agar) at 25°C under light/dark cycles of 12:12 h for conidia. Conidial suspensions at a concentration of 10^7 conidia/ml (the same below unless specified) were incubated in nitrogen-limited broth (NLB: 4% glucose, 0.4% NH_4NO_3, 0.3% KH_2PO_4 and 0.4% $MgSO_4$) at 25°C with shaking (110 rpm) for blastospores and in germination broth (GB: 2% sucrose and 0.5% peptone) for germination assessment.

2.2. UV-mutagenesis of the WT Strains

UV- mutagenesis system (Jinan Jiekang Purification Equipment Factory) was used for mutation. 200 μl conidial suspensions were spread on sterilized stainless steel plates and exposed to UV. The working power of the system was set to 15W, the wavelength was 250nm, and the irradiation time was 1min. After UV treatment, the steel plates were eluted by 0.02% Tween-80. The eluate was inoculated in SDAY plates. After 3 days of incubation at 25°C, pick the larger colonies to make a suspension and continue to inoculate it in SDAY medium for cultivation for the subsequent experiments.

2.3. Calculation of Spores Yielding

Conidial suspensions of 5 candidates were spread evenly on cellophane-overlaid SDAY plates. Three colony plugs (5 mm diameter) were bored daily from each plate using a cork borer and suspended in 1ml of 0.02% Tween 80 and numbered one day intervals from 5 days to calculate the conidiation.

To estimate the blastospore yield, 100μl of conidial suspension of each candidate mutant and WT were incubated in 50 ml of NLB. The yielding of blastospores was counted with a microscope at 12h intervals from 2 days onwards.

2.4. Determination of Conidial Germination and Stress Resistance

Aliquots of 100 μl conidial suspensions of testing strains were transferred into 900μl GB. Germination rates were examined at 2 h intervals and the half germination time (GT_{50}) was required to measure the conidial germination capacity.

The aliquots of 1μl conidial suspensions of WT and candidate were spotted on the plates of SDAY and CZA medium respectively supplemented with a gradient of NaCl (30mg/ml), H_2O_2 (2mM), Congo red (CR: 30μg/ml), Carbendazim (CAR: 0.2μg/ml) for cell chemical stress. Colonies diameter were cross-measured from 5th day to 9th day and calculated to the relative inhibition rate.

Conidial thermotolerance was assayed by exposing conidial samples to 45°C heat stress for up to 90 min, followed by modeling analysis of relative germination rates over the intensities of the stresses for half lethal time (LT_{50}) estimates of each strain.

2.5. Assay of Virulence

Galleria mellonella larvae (3 instar each) was used to perform the virulence bioassays. Three teams of 30 larvae were soaked in 30 ml conidial suspensions for 10 s for the infection, then, immediately transferred them into breathable boxes at 25℃ for normal growth. The death records were observed every 12h, and as an indicator for assessing larvae mortality, LT_{50} is a death trend index obtained by probabilistic analysis.

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3. Results and Analysis

3.1. Generation of Mutant Strains

Five candidate strains with larger colonies and stronger hyphae compared to WT were selected from the strains produced by UV mutagenesis and named as B1, B2, B3, B4, B5. Conidia scraped from each strain were made into a 10^7 conidia/ml suspension for subsequent determination.

3.2. Analysis of Spores Yielding

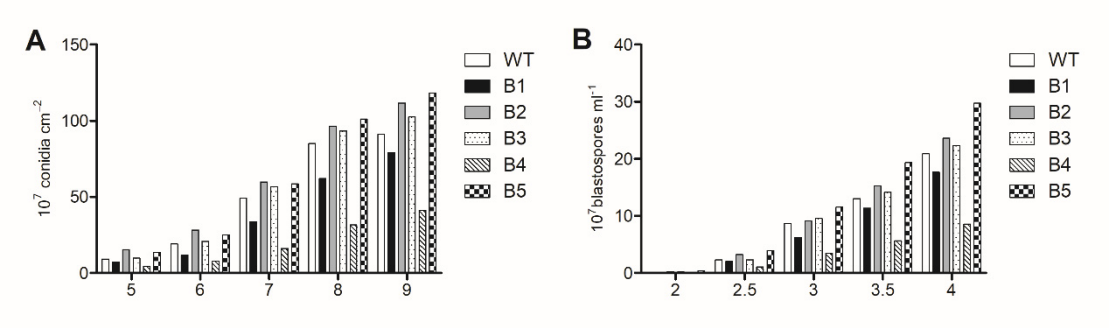

Fig 1. Spore Yield Analysis Chart

Spore production is positively correlated with fungal virulence. For conidiation capacity, B2, B3 and B5 are significantly better than WT, while B1 and B4 are weaker than WT (A) which is a crucial indicator for the adsorption of conidia to the host. For blastospores, the proliferation of blastospores consumes nutrients in the host, and the hyphae produced cause mechanical damage to the host and lead to death. In the process of simulating the production of blastospores in the hemocoel of insects, the yielding of blastospores was the best with B5 and the worst with B4 (B).

3.3. Analysis of Germination and Multiple Stress Tolerance

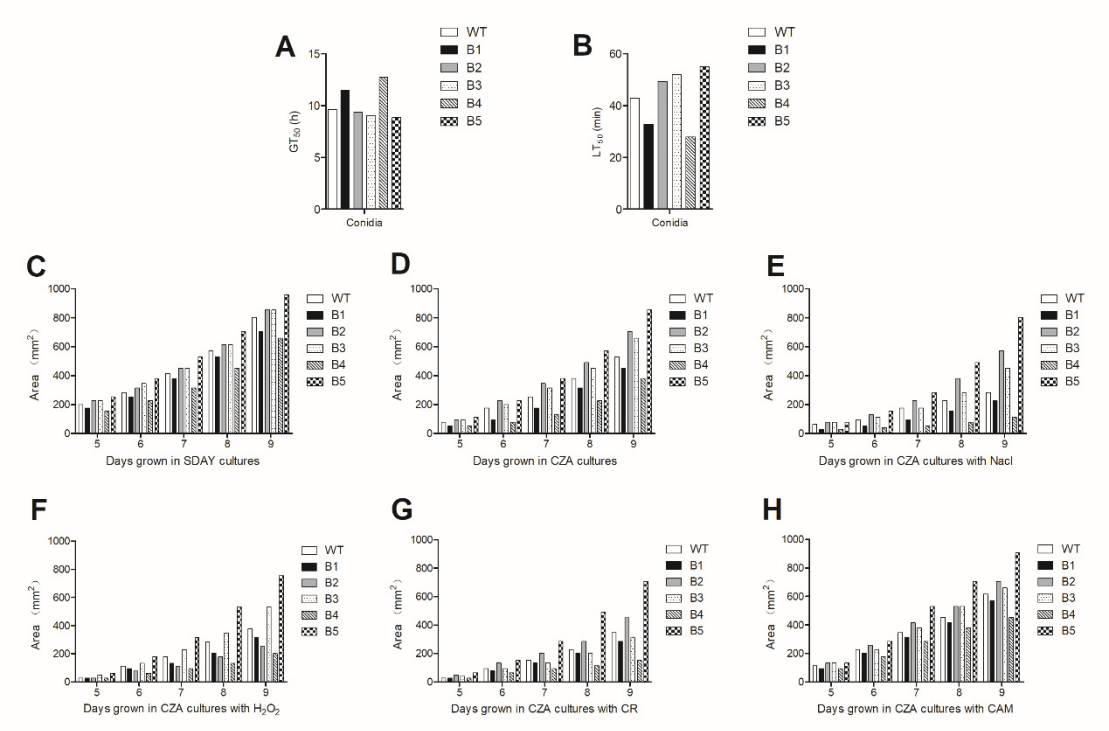

Fig 2. Analysis of Germination and Multiple Stress Tolerance

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We can see from the figures that the GT_{50} of strains B2, B3 and B5 was lower than that of WT, which indicated that the germination speed was significantly accelerated. For heat shock, B2, B3 and B5 strains have a longer LT_{50}, demonstrating that they have stronger heat resistance than other strains. For chemical stress, we found that different strains have different sensitivity to chemical stress. Our results suggested that B1 and B4 are sensitive to NaCl, H_2O_2 and CR, B2 is sensitive to H_2O_2 and B3 is sensitive to CR, but only B4 is sensitive to carbendazim. Among these strains, B5 is the most resistant and does not show sensitivity to any chemical stress.

3.4. Analysis of Strains Virulence

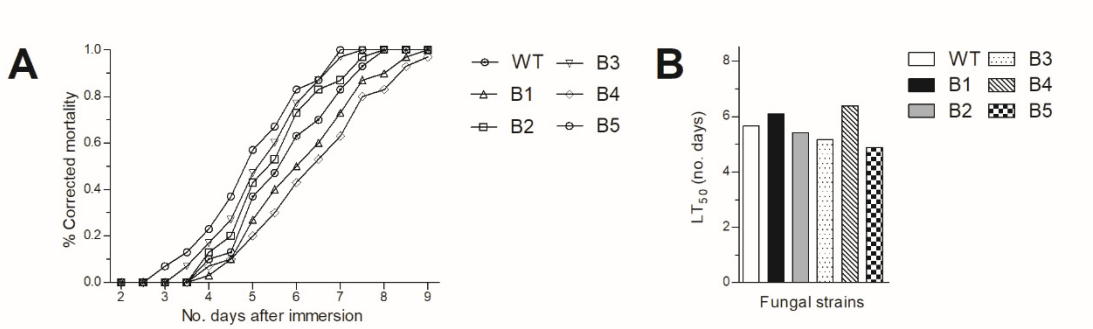

Fig 3. Analysis of Strains Virulence

Here, we used corrected mortality and LT_{50} of larvae to measure the virulence of six strains. The pathogenicity of strain B5 is stronger than other strains obviously, however, the strain B4 is the most weaken that its mortality does not arrive 100% in 9th day. The LT_{50} of B5 is 19.7% lower than WT, meaning that its virulence is increased.

4. Conclusion

Today's society is committed to the development of green and organic agriculture, and the replacement of chemical pesticides by microbial pesticides will surely become a major development trend. As a typical biocontrol fungus, *B. bassiana* is widely used in agricultural production, which also makes the screening of excellent fungal strains a major focus for laboratories to overcome. Using the screening method of ultraviolet mutagenesis, we selected 5 mutant strains from the original strains of *B. bassiana*, and identified the yield, quality and virulence of the conidia and blastospores of the mutant strains. Our results show that B2, B3 and B5 are improved strains, while B1 and B4 are weaker than WT. This experiment not only screened out three excellent strains, but also provided a method and vision for the improvement and identification of fungal strains.

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