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Studies on the Growth Conditions for Paecilomyces griseoviride Strain U-2 and Its Ability to Infect Aphids

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Abstract The growth of *Paecilomyces griseoviride* U-2 strain in different cultures and the effects of temperature, pH, humidity on growth and utilization of carbon and nitrogen sources were reported in this paper. The results of bioassay showed that the cinidiosphore of U-2 strain has infectious activity to aphids (vegetable aphids) and its fermentation liquid (from centrifuging) also has a certain aphid-killing activity.

Key words *Paecilomyces griseoviride*; growth; aphids; infectious activity

From 1992-1995, the authors collected different kinds of dead cotton aphids, soil samples and many other kinds of culture media from throughout Shandong province and classified them into 27 kinds and 130 fungal strains with further screening. The *Paecilomyces griseoviride* U-2 strain that was screened from the above strains had relatively strong aphid-killing activity as seen in bioassays [3]. This paper studied the growth conditions of this strain, the infection of conidium on aphids and the aphid-killing activity of its fermentation liquid.

1. Materials and methods

1.1 Materials

1.1.1 Strain: U-2 strain of *Paecilomyces griseoviride* (the authors separated and preserved it).

1.1.2 Released aphids: vegetable aphids, which were artificially fed and reproduced using cabbage seedling and radish seedling in the greenhouse.

1.2 Methods

1.2.1 Flat bed cultivation: followed standard methods.

1.2.2 Slide glass cultivation and observation method: as per Deqing Zhou's method [5].

1.2.3 Observation of growth temperature: The conidium were placed on glass slides with 0.5% glucose solution end to end, then transferred, moistened and cultivated under different temperature regimes. 500 spores were observed after 24 hours and the germination rate was recorded.

1.2.4 pH: Triangular flasks were filled with pH liquor to a volume of 500ml; 100ml for each flask, repeated in three flasks. 2% HCl and 2 N NaOH was used to adjust pH. A 0.5ml spore suspension was filled in; they were cultivated under 24±1° C for 14 days. After centrifugation, they were dried and weighed at 60° C.

1.2.5 Relative humidity (RH): Different kinds of saturated salt were used for controlling RH [2, 4] ; e.g. H_2O for 100%; KH_2PO_4 for 95%; $ZnSO_4.7H_2O$ for 90%; KCl for 87%; $(NH_4)_2SO_4$ for 81% and $NaC_2H_3O_2.3H_2O$ for 76%. The spore suspension that contained 1% glucose was deposited onto glass slides, and rapidly air-dried. They were then transferred to different kinds of well-secured containers of saturated salts, with temperatures maintained at 20°C. The rate of germination was observed over 24-96 hours.

1.2.6 Utilization of carbon and nitrogen sources: Refer to the Campbell method. Different kinds of sugar solution were made with sterile filtration. The nitrogen source utilized for the basic culture medium employed Duggar liquor. The total gross content was determined using 2% KNO_3. After inoculation, they were transferred and cultivated at 24±1° C for 14 days, the process repeated 3 times.

1.2.7 Study on conidial infection of aphid: A minitype hand sprayer was used to spray the conidium suspension (about $1.5*10^8$/ml) uniformly on aphid populations on cabbage seedlings (cabbage seedlings were planted in small plastic bowls). They were put into an aphid-feeding container with controlled temperature and humidity, and the infection

status was observed and recorded for 8 days. The experiment included a water rinsing treatment that was replicated three times.

1.2.8 Determination of aphid-killing activity of fermentation liquid: The *Paecilomyces griseoviride* U-2 strain spore suspension was inoculated on a potato dextrose agar (PDA) culture medium. It was cultured at 26° C and agitated at 200 rpm for six days. The fermentation liquid was centrifuged at 3000rpm for 20min. The minitype hand sprayer was used to spray aphids on the cabbage seedlings, with the environmental temperature kept at 20-23° C and relative humidity at 75-88%; aphid mortality was counted within 48 hours. The experiment included a water rinsing treatment and was replicated three times.

2. Results and discussion

2.1 The growth status of colony on different media

The colony diameter was 28-42mm; it was grayish-green in color; the fringe was white and uniform; the center was ridged with indistinct circular veins and 4-6 distinct radiation lines; the back was tan and the extravasate was colorless or buff-colored at 25° C for 14 days on Czapek's agar. On PDA the growth was more rapid than that on Czapek's agar: the colony diameter was 35-46mm; it was grayish-green with the back side brown. Grown on the peptone sucrose agar for 14 days, the diameter of the colony was 24-35mm; it was relatively flat or the partial center was a little ridged; the back was yellow and the extravasate was colorless.

2.2 Spore germination

The spore absorbed the water, and became swelled and distorted when the conidium was at 24° C and moisture-saturated ; it started to sprout within 5-7 hours, and bud within 10 hours. The bud tube could protrude from the first stage, second stage or transverse direction of the spore. When the bud tube prolonged to a certain degree, it would generate ramification.

2.3 Hypha fragmentation reproduction

The glass slide culture method was used. It was observed that the reproductive part of hypha ruptured into conidium both on Czapek's agar and on PDA. The ruptured conidium was in the shape of a pole at 1.4-1.9*3.0-4.1μm, usually existing as conglobation or cluster.

2.4 Growth and temperature

The starting growth temperature of U-2 strain was 8° C. The best growth temperature was 24-26° C from the point of spore germination and the speed of colony growth.

2.5 The influence of humidity on germination of conidium

The germination speed of conidium is relatively rapid and uniform at saturated humidity. Not only the germination rate decreased but also the germination time was prolonging with a decrease in relative humidity. For instance, when RH was 100%, the germination rate was 76.2% at 24h and 96.6% at 96h; when RH was 90%, the germination rate was 9.5% at 24h and 74.5% at 96h; when RH was 76% the germination rate was 0 at 24h and 24.4% at 96h (Table 1).

2.6 The influence of pH on the growth of mycelium

The bioassay results (Table 2) indicated that U-2 strains could all grow within the range of pH 4-10, but the yield of mycelium was relatively high within the range of pH 6.0-6.5. The strain could barely grow when pH was greater than 11.0.

Table 1. The influence of relative humidity on the germination of spore

Relative humidity, RH (%)	Medium	Rate of germination (%)		
		24h	72h	96h
100	H_2O	76.2	89.3	96.6
95	KH_2PO_4	25.5	49.2	82.8
90	$ZnSO_4.7H_2O$	9.5	50.1	74.5
87	KCl	2.4	28.8	40.6
81	$(NH_4)_2SO_4$	0	15.3	28.8
76	$NaC_2H_3O_2.3H_2O$	0	0	24.4

Table 2. The influence of pH on the growth of mycelium

PH	Dry weight of mycelium (g)
4.0	0.5564
5.0	0.8012
6.0	0.8825
6.5	0.9015
7.0	0.8024
8.0	0.7010
9.0	0.4234
10.0	0.3552
11.0	0.088

2.7 The utilization of carbon and nitrogen sources

The utilization of carbon sources: 11 carbon sources such as glucose, sucrose and raffinose etc. were bioassayed (Table 3). All the carbon sources could be utilized except inulin, sorbose, rhamnose and Lactose. But the yield of mycelium was the highest if sucrose was the carbon source, and was the second highest if glucose was the carbon source.

The utilization of nitrogen sources: 9 nitrogen sources such as nitryl, amido and nitroso etc. were bioassayed (Table 4). KNO_3, $NaNO_3$ and $(NH_4)_2HPO_3$ was relatively beneficial to the growth of mycelium. This mycelium was not able to utilize $NaNO_2$ and H_2NCSNH_2.

Table 3. The utilization of *Paecilomyces griseoviride* on carbon source

Carbon source	Dry weight of mycelium (g)
Glucose	0.3645
Raffinose	0.2122
Raffinose	0.3042
Maltose	0.3012
Mannose	0.2894
Inulin	0
Sucrose	0.3948
Lactose	0
Sorbose	0
Rhamnose	0
Glycerol	0.1842
Contrast	0

Table 4. The utilization of *Paecilomyces griseoviride* on nitrogen source

Nitrogen source	Dry weight of mycelium (g)
KNO_3	0.6246
$NaNO_3$	0.6056
$(NH_4)_2SO_4$	0.5212
$(NH_4)_2HPO_3$	0.6124
NH_4NO_3	0.4123
$(NH_4)_2CO_3$	0.2789
NH_4Cl	0.3012
$NaNO_2$	0
H_2NCSNH_2	0
Contrast	0

2.8 The infection of conidium on aphid

The bioassay results (Table 5) indicated that *Paecilomyces griseoviride* U-2 conidium has infectious activity to vegetable aphids,

but the infection rate was apparently influenced by ambient temperature and humidity. The infection rate of vegetable aphids could reach 70.2% at the ambient temperature of 20±2° C and humidity of 90%.

2.9 The aphid-killing activity of fermentation liquid

After spraying fermentation liquid and centrifugation liquid, the reduction rates of aphid populations were 42.4% and 80.6% respectively within 24h and 48h. The difference was remarkable contrasting with -3.8% and -5.6% for rinsing. This showed that the *Paecilomyces griseoviride* U-2 strain fermentation liquid had certain aphid-killing activity, which is worthy of further research.

Table 5. The infection of *Paecilomyces griseoviride* conidium on vegetable aphid

Temperature (° C)	Relative humidity (%)	Infection rate (%)
16±2	60	10.2
	70	31.6
	80	52.4
	Above 90	61.2
22±2	60	13.2
	70	40.1
	80	66.3
	Above 90	70.2

Table 6. The aphid-killing activity of *Paecilomyces griseoviride* U-2 strain fermentation liquid

Group Name	Aphid population reduction rate	
	24h	48h
Fermentation liquid	42.4	80.6
CK	-3.8	-5.6

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