Liquid Culture of *Ampelomyces quisqualis*, a Mycoparasite for Biological Control of Powdery Mildews

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Abstract

Ampelomyces quisqualis produced pycnidia but not conidia on malt-yeast extract (MY) agar, while in MY broth the fungus formed both pycnidia and conidia in 7 days. Shaking the culture greatly increased the amount of conidia produced in MY broth. Among the vegetables tested, carrot broth was best for conidial formation under shaking conditions, followed by tomato broth and cucumber broth. The fungus failed to grow in potato broth. When *A. quisqualis* conidia produced in carrot broth were incubated together with *Oidium euonymi-japonicae* conidia on glass slides under moist conditions, parasitism of this mycoparasite on its powdery host was observed for the first time outside the host of the powdery mildew.

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Key words : conidial formation, biological control, mycoparasite, *Ampelomyces quisqualis*, powdery mildew.

INTRODUCTION

Ampelomyces quisqualis Ces. is a mycoparasite capable of infecting both asexual and sexual structures of many powdery mildew species in nature. More than 60 years ago, Yarwood¹¹⁾ reported partial control of red clover powdery mildew with this fungus. Due to its wide host range, the mycoparasite is a highly promising candidate for biological control²⁾. It also has potential for integrated disease management because of its tolerance to a number of fungicides used in the control of powdery mildews¹⁰⁾. The use of mycoparasites in biological control requires mass production of infective propagules. *A. quisqualis* is a slow-growing fungus and production of large amount of spores is usually difficult to obtain. The successful development of a method for mass production of conidia by this fungus with inexpensive and readily available material is reported here.

MATERIALS AND METHODS

Microorganisms *A. quisqualis* was isolated from *Podosphaera leucotricha* (Ell. & Ev.) Salm. on the leaf of *Malus pumila* Mill.³⁾, and cultured on M2 agar⁶⁾ to produce inoculum. Conidia of *Oidium euonymi-japonicae* (Arc.) Sacc. from *Euonymus japonicus* Thunb. were used to test ability of mycoparasite conidia, produced with the method described in this report, to parasitize the powdery mildew host. All the microorganisms used were

collected from Lanzhou, China.

Conditions for conidial formation To compare the difference, in ability to support conidial formation in medium, between solid and liquid states the malt-yeast extract (MY) broth (7.5% malt extract and 0.1 yeast extract) and MY agar (MY broth plus 2% agar) were used. Fifty ml of MY broth was placed in each 250 ml flask before autoclaving, while 25 ml MY agar was added to each 9-cm petri plate after autoclaving. To inoculate the medium, six pycnidia of *A. quisqualis* were added to each flask or evenly distributed on the surface of each plate. For determining the effect of shaking the culture on conidial formation, flasks were incubated on a shaker adjusted to the speed of 100 rpm. Mycelial growth and pycnidial formation were recorded after 2, 4, 6 and 7 days at 25°C. The numbers of conidia were determined on the 7th day with a Pipetman microliter pipette (P-20, West Scientific, Oakland, California, USA)⁹⁾.

For comparing the ability of different vegetable extracts to support conidial formation, 25 g of peeled potato, tomato, carrot or cucumber was chopped into small pieces and boiled for 10 min with 50 ml distilled water in a 250 ml flask. The supernatant was filtered through four layers of cheesecloth and the filtrate was adjusted to 50 ml with distilled water. Flasks were inoculated as described and data were recorded after incubation at 25°C for 7 days on a shaker.

Parasitism of cultured conidia Powdery mildew conidia of *O. euonymi-japonicae* were sprayed on

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Table 1. Effect of culture conditions on mycelial growth and pycnidial formation by *Ampelomyces quisqualis*

Incubation time (day)	Solid medium		Liquid medium			
	Mycelia	Pycnidia	Static		Shaking	
			Mycelia	Pycnidia	Mycelia	Pycnidia
2	+	*	—	—	+	—
4	+	—	—	—	++	+
6	+	+	+	+	+++	++
7	++	+++	+	+++	+++	+++

* Relative mycelial growth and pycnidial formation: —, 0; + < ++ < +++.

** Without conidia.

*** With conidia.

Table 2. Mycelial growth and conidial production of *A. quisqualis* in different vegetable broth under shaking conditions

Medium	Mycelial weight (g/50 ml)	Conidial concentration (No./ml)
Carrot broth	2.7 A*	2.4×10^7 A
Tomato broth	2.6 A	6.0×10^6 B
Cucumber broth	0.1 B	3.2×10^5 C
Potato broth	0	0

* Data followed by the same letter in each column are not significantly different ($p=0.01$) according to Duncan's multiple range test.

the glass slides by blowing air using an atomizer bulb⁴⁾. Fifty μ l conidial suspension of *A. quisqualis* obtained from carrot broth and washed twice with sterile distilled water by centrifugation⁸⁾ was then added onto each glass slide. The glass slide was placed on a V-shaped glass rod in a petri plate containing 10 ml of sterile distilled water³⁾. Plates were sealed with parafilm and incubated at 25°C. The interaction between powdery mildew host and the mycoparasite on the slides were observed once every 6 hr for 3 days.

Data analysis Three replicates were used and each experiment was repeated at least once. Data were analyzed with Duncan's multiple range test.

RESULTS

Mycelial growth of *A. quisqualis* was detectable on the 2nd day on MY agar, but on the 6th day in MY broth without shaking (Table 1). The fungus formed pycnidia after 6 days under both conditions. However, pycnidia produced on MY agar did not contain conidia after 7 days, while those produced in MY broth released 1.3×10^4 conidia per ml of the medium. Shaking the culture greatly improved mycelial growth and pycnidial formation of *A. quisqualis* in MY broth (Table 1). The number of conidia released from pycnidia reached as high as 1.2×10^7 per ml of the medium after 7 days. Liquid and

Table 3. Effect of concentration of carrot broth on mycelial growth and conidial production of *Ampelomyces quisqualis* under shaking conditions

Concentration of carrot broth (%)*	Mycelial weight (g/50 ml)	Conidial concentration (No./ml)
100	2.4 A**	5.6×10^6 B
50	2.2 A	1.9×10^7 A
25	1.3 B	1.5×10^7 A
12	0.6 C	6.0×10^6 B
6	0.6 C	5.2×10^6 B
3	0.2 D	2.8×10^6 B

* Stock broth (100%), prepared by boiling 50 g of chopped carrot in 50 ml of distilled water, was adjusted with distilled water to make various concentrations of broth.

** Data followed by the same letter in each column are not significantly different ($p=0.01$) according to Duncan's multiple range test.

shaking conditions were, therefore, selected for subsequent tests to find an inexpensive material for mass production of pycnidium by this fungus.

Among the materials tested, carrot broth was best for conidial formation of *A. quisqualis*, followed by tomato broth and cucumber broth (Table 2). The fungus grew poorly in cucumber broth and failed to grow in potato broth. Although 100% of carrot broth increased mycelial growth, it greatly decreased the amount of conidia produced (Table 3). Decreasing the stock carrot broth two fold did not significantly affected conidial formation, but substantially reduced the mycelial growth. Further dilution of carrot broth was not good for both conidial formation and mycelial growth.

On the glass slides, *A. quisqualis* conidia around *O. euonymi-japonicae* germinated at higher rate and produced longer germ tube than those away from the powdery mildew conidia. After 24 hr, germ tubes near the host conidia were about 5–10 times longer than the conidia, while those far from the host conidia were only about 0.5–5 times longer. Germ tubes tend to grow toward host conidia. After entering the host, the mycoparasite may emerge from the same or opposite side of the host conidium (Plate I-A, B). Those germ tubes penetrating through host conidia grew 10–20 times longer than those not in contact with the host conidia in 60 hr after inoculation (Plate I-C). The host conidia penetrated by the mycoparasite showed rougher surface than those not be penetrated in 72 hr after inoculation (Plate I-D).

DISCUSSION

The method presented in this report for production of *A. quisqualis* conidia has the advantages of being simple and inexpensive. The concentration of conidia produced in culture medium with this method was about 2.5 times higher than that reported by Szejnberg *et al.*⁹⁾ using

Plate I

Plate I Parasitism of *Ampelomyces quisqualis* on powdery mildew *Oidium euonymi-japonicae* conidia.

- (A) Emergence of the mycoparasite from the same side of the host conidium in 36 hr after inoculation (m, mycoparasite conidium ; pm, powdery mildew conidium).
 (B) Emergence of the mycoparasite from the opposite side of the host conidium in 36 hr after inoculation.
 (C) Penetration of the host conidium by the mycoparasite in 60 hr after inoculation.
 (D) Parasitism of the mycoparasite on the host conidia in 72 hr after inoculation.

potato broth under shaking conditions. Moreover, these conidia were capable of infecting powdery mildew conidia. It is, therefore, suitable for mass production of infective propagules of this mycoparasite for biological control.

Sztejnberg *et al.*⁹⁾ reported growth and conidial formation by *A. quisqualis* in potato broth and potato dextrose broth. However, the fungus failed to grow in potato broth in this study and Chen & Yang¹⁾ was not able to culture it in potato dextrose broth. The difference in geographic origin might account for such discrepancy. Moreover, the Israeli isolate used by Sztejnberg *et al.*⁹⁾ was isolated from *Oidium* sp. parasitizing *Catha edulis*, while the Chinese isolate used in this study was obtained from *P. leucotricha* on *M. pumila*.

Since powdery mildews are obligate parasites, all the previous studies on interaction of mycoparasite and its powdery mildew host were carried out on the host of the powdery mildew^{2,7,10)}. By incubating *A. quisqualis* conidia together with powdery mildew conidia on glass

slides under moist conditions, parasitism of the mycoparasite was shown for the first time outside the host. This may be useful for studying factors affecting parasitism of *A. quisqualis* on its powdery mildew hosts.

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和 文 摘 要

Yu-Huan GU: うどんこ病の生物防除に利用できる菌間寄生菌, *Ampelomyces quisqualis* の液体培養

Ampelomyces quisqualis は麦芽, 酵母エキスを寒天培地上では柄子殻のみを形成し, 分生子を形成しなかったが, 麦芽, 酵母エキスを液体培地で培養すると 7 日間で柄子殻および分生子を形成した。振とう培養によって分生子形成量は増大した。各種植物の煎汁で液体培地を調製して本菌の分生子形成量に及ぼす影響を調べたところ, ニンジン, トマト, キュウリの順に形成量を増大させたが, ジャガイモ培地中では菌の育成が見られなかった。ニンジン液体培地で形成させた本菌の分生子と *Oidium euonymi-japonicae* の分生子とを湿室内のスライドガラス上で培養したところ, 前者が後者の表面に寄生している様相が確認された。