Front. Agric. China 2007, 1(2): 175–178
DOI 10.1007/s11703-007-0029-4

RESEARCH ARTICLE

LIU Xiaoyun, HU Tongle, CAO Keqiang

Biological characteristics of strain F603 of *Epicoccom* sp., an antagonistic fungus for controlling *Phytophthora infestans*

Abstract Factors influencing vegetative growth and spore germination of strain F603 of *Epicoccom* sp., an antagonistic fungus for *Phytophthora infestans* (Mont) de Bary, were studied. Among the different growth media tested, Rye agar was the best medium for its vegetative growth. The range of temperature and pH value for mycelial growth was 5–35°C and 2–12, respectively, with the optimum 25°C and 6–9, respectively. The fungus grew better in Czapek medium with maltose and dextrose as carbon sources and peptone, KNO_3, and $NaNO_3$ as nitrogen sources. The range of temperature for spore germination of strain F603 was 5–35°C, the optimum was 20°C. The range of temperature for sporulation was 10–30°C, and the optimum was 15–18°C.

Keywords *Epicoccom* sp., *Phytophthora infestans*, biological characteristics

1 Introduction

Potato is one of the most important crops in the world. And potato late blight caused by *Phytophthora infestans* (Mont) de Bary is the most serious disease in potatoes. At present, the control of late blight mainly depends on farm chemicals, and the disadvantages of chemical control have been more and more obvious. The biological control of plant diseases, as an effective alternative with less or no pollution to environment, has been assigned more and more importance (Cao et al., 1997; Cao et al., 2001).

Strain F603 of *Epicoccom* sp., a strong antagonistic fungus to *P. infestans*, was selected and purified on the Rye agar (RA) medium in the Laboratory of Plant Disease Epidemiology and Integrated Control of Agricultural University of Hebei, China. The growth inhibition rate of strain F603 for *P. infestans* could reach 73%, and a wide inhibition zone appeared on the RA. In this experiment, we studied the factors influencing vegetative growth and spore germination of strain F603, so as to find out the most suitable conditions for strain F603 to play antagonistic functions against *P. infestans*.

Received September 25, 2006; accepted October 20, 2006

LIU Xiaoyun, HU Tongle, CAO Keqiang (✉)
Biocontrol Center of Plant Diseases and Pests of Hebei, College of Plant Protection, Agricultural University of Hebei, Baoding 071001, China
E-mail: ckq@hebau.edu.cn

2 Materials and methods

2.1 Materials

2.1.1 Fungus

The *P. infestans* isolate was provided by the Laboratory of Plant Disease Epidemiology and Integrated Control mentioned above. Strain F603 was screened to be antagonistic to *P. infestans*. The two fungi were grown in the dark at 18°C on RA.

2.1.2 Growth media

Rye agar (RA) medium, Czapek, potato dextrose agar (PDA), and potato sucrose agar (PSA) were used. The media were poured into 9-cm diameter Petri dishes in hood.

2.2 Methods

2.2.1 Effects of different growth media to vegetative growth of strain F603

The media of PDA, PSA, RA, and Czapek were poured into 9-cm diameter Petri dishes. A 0.6-cm diameter agar disk was cut from the margin of an actively growing culture of strain F603 and then the agar disk was put with the fungal side upward onto the center of each plate. For the purpose of cultivation, the plates were kept under the condition of 18°C for ten days. The diameter of colony was recorded.

2.2.2 Tests of different carbon and nitrogen sources on growth of strain F603

Ten different carbon sources, which were sucrose, maltose, lactose, dissoluble amylum, tri-sodium citrate, D-Galactose,

D-fructose, D-Mannose, dextrose, and control (without any carbon source) were added to Czapek medium at a rate of 20 g/L before autoclaving. The nitrogen source and other ingredients were not changed (Huang, 1994; Wang et al., 1997; Zhang et al., 2003). In another set of tests, 13 different nitrogen sources, which were peptone, beef extract, yeast extract, $(NH_4)_2SO_4$, NH_4NO_3, $(NH_4)_2HPO_4$, $NaNO_3$, KNO_3, NH_4Cl, Ammonium oxalate, L-glycin, L-Asparagine, L-Phenylalanine, and control (without any nitrogen source) at a rate of 2 g/L were tested. After melt at the temperature between 40°C and 50°C, the medium was poured into Petri dishes in hood. For each Petri dish, an agar disk (0.6 mm in diameter), taken from a 10-day strain F603 culture, was placed with the fungal side upward on the center of each plate. All the treatments were incubated at 18°C for ten days.

2.2.3 Tests of different pH value on growth of strain F603

Rye agar (RA) was used for this test. The pH value of RA was adjusted after autoclaving with sterile solutions of 1 mol/L HCl or 1 mol/L NaOH to obtain the following levels: 4, 5, 6, 7, 8, 9, 10, 11, and 12. The medium was poured into 9-cm Petri dishes, a 0.6-cm diameter agar disk, taken from a strain F603 culture, was plated with the fungal side upward on the center of each plate. All the treatments were incubated at 18°C for ten days (Fang, 1998).

2.2.4 Tests of temperature on vegetative growth, sporulation, spore germination of strain F603

The effects of nutrients on mycelial growth and sporulation were assessed in 9-cm Petri dishes. Plates containing RA were used for the tests. Put a 0.6-cm diameter agar disk cutting from the margin of an actively growing culture of strain F603 onto the center of plates. For different temperature on vegetative growth, the plates were put at 5, 10, 15, 20, 23, 25, 28, 30, 35, and 40°C for ten days. The diameters of colonies were measured (Chai et al., 2004; Ji et al., 2004; Zhu, 2003). For sporulation test, the plates were cultivated at 4, 10, 15, 18, 25, 30, 35, and 40°C for ten days. The spores were washed by 10 mL distilled water to form spore suspension. The number of spores was counted by hemocytometer. The effect of temperature on spore germination was assessed on RA. Spore suspension was made by adding distilled water directly into the Petri dishes, which were incubated at 18°C for ten days. A 30-μL drop of strain F603 spore suspension (40 spores in each eyeshot under 10×10) was placed on slides by micropipette. The slides were put into a moisture-saturated plastic chamber with filter paper wetted. The plate was put into incubators of nine different temperature treatments, which were 5, 10, 15, 20, 23, 25, 28, 30, and 35°C.

All the tests above were repeated four times.

2.2.5 Data analysis

The data were analyzed by data processing software (DPS). Statistical analysis was carried out by one-factor variance analysis. Means were compared by Duncan's new multiple range method ($P = 0.05$).

3 Results and analysis

3.1 Effect of different media to the vegetative growth of strain F603

The diameters of the mycelium on different media were measured. The largest diameter (6.51 cm) of strain F603 was on RA followed by those on Czapek (6.51 cm), on PDA (5.98 cm), and on PSA (5.64 cm). The color of strain F603 on RA was black with many spores on the colony. The mycelium on other media was yellow and had no black spores. Figure 1 shows the growing status of strain F603 on different media.

3.2 Effect of different carbon sources on mycelial growth

Comparing to other carbon sources, maltose and dextrose were the best for stimulating strain F603 growth. In contrast, the mycelium did not grow in medium with tri-sodium citrate as carbon source (Fig. 2).

3.3 Effect of nitrogen source on mycelial growth

Figure 3 shows the results of the mycelial growth of strain F603 on Czapek media with different nitrogen sources. It is easy to see that strain F603 can use different kinds of nitrogen sources, in which peptone, $NaNO_3$, and KNO_3 were significantly better ($P = 0.05$) than other nitrogen sources, whereas $(NH_4)_2SO_4$ was the worst with a diameter of 2.95 cm after incubating for 12 days. It is unbelievable that strain F603 that was found on Czapek medium grew quite well even when there was no nitrogen source on the medium.

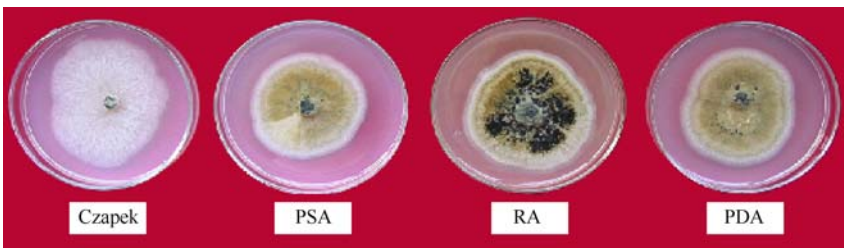
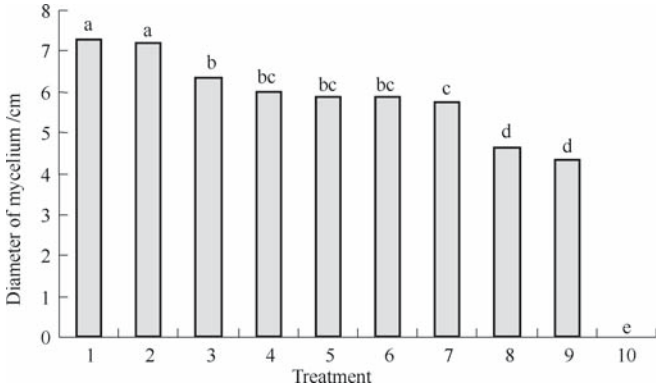


Fig. 1 Modality of mycelium of strain F603 on different media



1: maltose; 2: dextrose; 3: dissoluble amylum; 4: D-mannose; 5: sucrose; 6: D-fructose; 7: control (without any carbon source); 8: lactose; 9: D-galactose; 10: tri-sodium citrate

Fig. 2 The mycelial growth of strain F603 on Czapek media with different carbon sources

3.4 Effect of pH value and different temperature on mycelial growth

The results are shown in Table 1. Growth was significantly ($P = 0.05$) reduced on acidic medium (pH $\leqslant$ 6), the colony was loose and thin. The optimum values were obtained on alkaline medium (pH value 7–9). When the pH value was 7, the diameter of the mycelium reached 7.3 cm after incubating for seven days. So, in general, the mycelium can grow well in neuter and alkaline medium rather than in acidic medium.

The mycelium of strain F603 can grow under temperatures ranged from 5 to 35°C, and the optimum growth temperature was 28°C, the diameter was 6.25 cm after incubating for seven days. The diameter reduced to almost half when the temperature was below 10°C or above 30°C (Table 1).

3.5 Effect of temperature on spore germination and sporulation

The results are shown in Fig. 4. From Fig. 4, we can see that the spores of strain F603 germinated in temperatures ranged from 5 to 35°C. The most germination happened at 20°C with germination rate of 80.23% after 12 h and 93.93% after 24 h of treatment. When the temperature was 5°C or above 35°C, the spores could hardly germinate.

The temperature range for sporulation was between 10°C and 30°C. Temperatures outside this range could significantly inhibit sporulation. The maximum sporulation was at the temperature of 18°C. While at the temperature of 25°C and above, the sporulation was almost stopped.

Table 1 Effect of different pH value and different temperature on mycelial growth

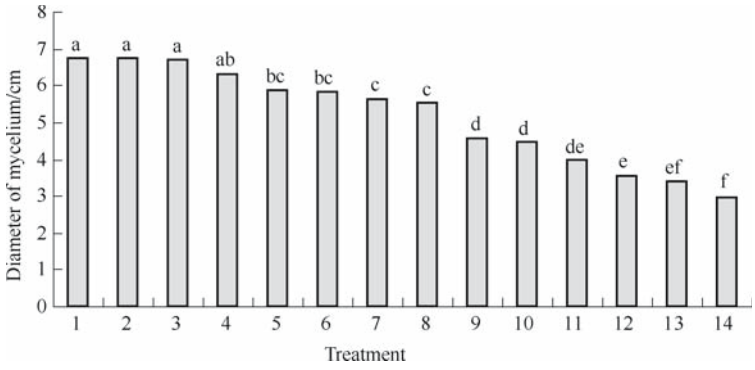
pH	Mycelium diameter/cm	Temperature /°C	Mycelium diameter /cm
7	7.30 a	28	6.25 a
8	7.00 ab	25	6.11 a
9	7.00 ab	23	5.66 ab
6	6.98 b	20	5.18 b
12	6.80 bc	15	4.00 c
10	6.53 cd	30	3.39 c
11	6.43 d	10	2.48 d
5	4.65 e	5	1.35 e
4	3.28 f	35	0.98 e
3	2.43 g	40	0.00 f
2	1.38 h	—	—

Notes: The data followed by the same letter in one column means there was no significant difference by DPS at $P = 0.05$.

4 Discussion

Now more and more people are paying attention to biocontrol of plant diseases, just because of its safety to humans and livestock. However, references of biocontrol on potato late blight were rarely seen.

Zhou Xudong et al. (1999) reported that fungal species in *Epicoccum* was a saprophyte in *Tomicus piniperda* L. Liang and Lu (2002) reported that fungus *Epicoccum nigrum* Link was in rhizosphere of crops in Liaoning province, China. Chen Yong et al. (1999) reported *Epicoccum purpruascens* was one of pathogenic fungi isolated from naturally infected



1: peptone; 2: $NaNO_3$; 3: KNO_3; 4: control (without any nitrogen source); 5: beef extract; 6: L-glycin; 7: yeast extract; 8: L-asparagine; 9: $(NH_4)_2HPO_4$; 10: L-phenylalanine; 11: NH_4NO_3; 12: ammonium oxalate; 13: NH_4Cl; 14: $(NH_4)_2SO_4$

Fig. 3 The mycelial growth of strain F603 on Czapek media with different nitrogen sources

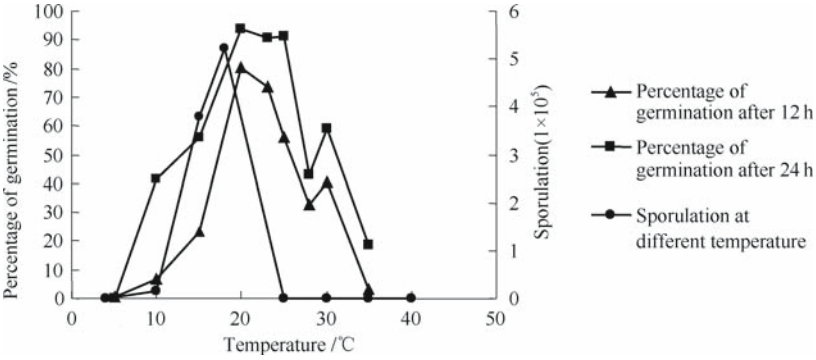


Fig. 4 Percentage of germination and sporulation of strain F603 at different temperatures

Echinochloa crus-galli var. *mitis* (Pursh) Peterm in China. Tong Yunhui et al. (2003) reported that *Epicoccum prupruascens* was a biocontrol agent to *Botrytis cinerea* Pers. *Epicoccom* sp. as a biocontrol agent on potato late blight has not been reported, and neither have its biological characteristics. In this survey, the effects of carbon, nitrogen source, pH value, temperature on the growth of mycelium, and the effect of temperature on the spore germination were tested. Through the tests, the results showed that the mycelium of strain F603 can grow on PDA, PSA, Czapek, and RA, but strain F603 produced black spores only on RA, while there was almost no spore production on other media. The colonies of strain F603 on PDA, PSA, and Czapek were yellow, which means that the strain was sensitive to nutritions. Strain F603 could use not only different kinds of monosaccharide and amylase, but also several nitrogen sources, in which, maltose and dextrose were the optimum carbon source, and peptone, $NaNO_3$, and KNO_3 were the optimum nitrogen sources. In this study, we found that strain F603 could grow on medium without nitrogen or carbon sources, but never grew on medium containing tri-sodium citrate. Maybe the nitrogen sources could not be used, and would restrain the growth of strain F603. The minimum, maximum, and optimum temperature for growth of strain F603 was 5°C, 40°C and 28°C, respectively. In reference to pH values, the range of pH values giving optimal growth of strain F603 was wide (from 7 to 12). The optimum pH value for growth was 7. Strain F603 could grow at a pH value of 12, and the diameter was not significantly smaller than that at pH value 6. However, the mycelium growing on medium of pH 12 was exiguity, and no spores were produced. The optimum temperature for spore germination was at 20°C after incubating for 24 h. Strain F603 could sporulate in the temperature range of 10–30°C, and the optimum was 15–18°C.

Because strain F603 has the potential to grow well in a wide range of environmental conditions, it could be used as an effective biocontrol fungus against *P. infestans*. Results of this study would be served as a theoretical basis for fermentation and preparation of strain F603 in the future. This research was carried out *in vitro*, further information of strain F603 *in vivo* is to be studied in the future.

References

Cao K Q, Forrer H R, Fried P M (1997). General guideline for the control of potato late blight (*Phytophthora infestans*) in Switzerland. Journal of Agricultural University of Hebei, 20(3): 16–20 (in Chinese)

Cao K Q, Forrer H R (2001). Current status and prosperity on biological control of potato late blight (*Phytophthora infestans*). Journal of Agricultural University of Hebei, 24(2): 52–58 (in Chinese)

Chai Z X, Li J H, Xin J F (2004). Biological characteristics of *Fusarium lateritium* Nees. Acta Phytopathologica Sinica, 34(5): 409–413 (in Chinese)

Chen Y, Ni H W (1999). Pathogenicity of indigenous fungi to *Echinochloa crusgalli* and rice. Chinese Journal of Biological Control, 15(2): 73–76 (in Chinese)

Fang Z D, ed (1998). Research Method of Plant Pathology. Beijing: Agricultural Press (in Chinese)

Huang Y (1994). On the hyperparasite of pear gall rust pathogen—Biological characteristics of hyperparasite. Acta Phytopathologica Sinica, 24(4): 329–335 (in Chinese)

Ji M S, Li B Q, Xu Y (2004). Biological characteristics of *Trichoderma viride* strain TR-8. Journal of Shenyang Agricultural University, 35(3): 195–199 (in Chinese)

Liang C, Lu G Z (2002). Fungi on Crop Rhizospheric soil in Liaoning II. Journal of Shenyang Agricultural University, 33(4): 266–269 (in Chinese)

Tong Y H, Ji Z L, Xu J Y, Chen X J (2003). Research progress on biological control of gray mold. Chinese Journal of Biological Control, 19(3): 131–135 (in Chinese)

Wang S, Bai Y J, Zhou Y L (1997). Pathogen of white false smut of rice. Acta Phytopathologica Sinica, 27(4): 321–327 (in Chinese)

Zhang Y X, Lu G Z, Liang J Y (2003). Biological characteristics of *Cercospora zeae-maydis*. Acta Phytopathologica Sinica, 33(4): 292–295 (in Chinese)

Zhou X D, Ye H, Ding H S (1999). On fungal flora inside egg galleries of *Tomicus piniperda* attacking *Pinus yunnanensis*. Forest Research, 12(5): 556–560 (in Chinese)

Zhu Y Z, Qiang S (2003). The biological characteristics of strain QZ-200 of *Curvularia* sp. for controlling *Digitaria sanguinalis*. Acta Phytopathologica Sinica, 33(6): 524–529 (in Chinese)