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Scanning Electron Microscopy of Strawberry Root Rot (*Pestalotiops photiniae* C16-4) Inhibited by Antagonistic Bacteria

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To better study the inhibition effect of three strains of antagonistic bacteria against Pestalotiops photiniae C16-4 ,the using of scanning electron microscopy (SEM) is a good way. This paper studies three kinds of methods (cake sample, insert sample and not fixed sample) for preparation of specimen observing fungus. Through the inhibition of fungus cultivation, we observed the ultrastructure of fungus. Insert sample without fixed was a short, simple, rapid method. Insert sample was more practical for conventional observation and identification of fungi and provided a reference for SEM of other microorganisms. This preparation procedure could be most useful for the routine examination and identification of the fungus. The results show that the changes of hyphae structure after treatment of different antagonistic bacteria inhibition can be seen, which mainly showing adhesion between mycelium, uneven thickness, partly sunken, or fracture, which giving full play to its bacteriostatic action.

Key words: Strawberry Root Rot, Antagonistic bacteria, SEM, Samples, Inhibited.

Strawberries are popular and rewarding plants to grow any where in the world.Its roots may fall victim to rot, which is a widespread disease of annual. As well as perennial matted-row strawberries, root rot pathogens lead to decreasing productivity and longevity of the crop. Yield losses of 30 to 50 percent are common and the productive life of strawberry fields is reduced (Schilde 2011). Strawberry root rot is a general name that caused by various fungus and adverse environmental disease, the main producing areas in the world strawberry root rot pathogens have been reported up to 20 kinds, that are soil-borne disease for more difficult prevention, (Zhang, 2003). To prevent root-rotting, strawberries should be used of chemical pesticides.For a long time, the repeated and extensive use of chemical pesticides can cause soil, water and air pollution, and increase pesticide residues of strawberry fruit, while also killing the beneficial microorganisms, resulting in a resurgence of the disease, and the production of the vicious cycle.

In recent years, the using of antagonistic microorganisms for biological control of plant diseases is subject of concern, its prevention and treatment of some diseases is very obvious (Chen, 2003). The pathogens most frequently isolated from

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affected roots are fungi such as Rhizoctonia Fragariae, Cylindrocarpon, Pythium and Fusarium species.Pestalotiops photiniae C16-4 is an obligately biotrophic fungus it occurs in all parts of the world where strawberries are grown.

Thangavelu (2003, 2008) isolated Bacillus subtitles from rhizosphere soil , it has resistance to Fusarium wilt at 4l%. The antagonistic bacteria N1729 was screened from rhizosphere soil of cotton(Chen et al. 2007), It turns out that can effectively inhibit the growth of pathogenic bacteria. In addition, Wang (1990) isolated R38, R28 strains. It turns out that they have good control the disease of Rhizoctonia solani; C.P. You (2001) isolated Bacillus pilus which has a significant inhibitory effect for the rice blast fungus; abroad prevention by the use of *B. subtilis*, *Rhizoctonia solanie*, *Pythium* sp, *Fusarium* sp and *Botryadiplodia solanituberosi* cause disease, and achieved good results(2000). Many studies show that the inhibitory effect of antagonistic bacteria in the growth caused hyphae the collapse of the cell contents to leak outand also have some germination inhibition.destroy the cell walls of bacteria (chen liang, 2003),

SEM is an important tool for microscopic observation in many fields. The successful using of scanning electron microscope in studies of actinomycetes was made by Williams and Davies (1967). Since the initial work on actinomycetes by Williams and Davies (1967), a large number of strains have been examined and further information on the range of forms of the members of this group has been obtained. In addition, improved specimen preparation and instrument operation have resulted in better resolution at higher magnifications. Investigations of the structure of organisms belonging to different genera of the microorganism with scanning electron microscopes have provided much useful information. (Humphery E, 2006). For the degree of inhibition of antagonistic bacteria against fungus mainly depends on the electron microscope down further observation. So,select the best approach is the key to simple scanning electron microscope, the paper compares the sample preparation method for Pestalotiops photiniae C16-4 ultrastructure of the observed effect. This report is a consequence of a concerted effort to understand the relation of antagonistic bacteria and strawberry root rot.

MATERIALS AND METHODS

The cultures of strawberry root rot C16-4 (Fusarium oxysporium) and Antagonistic bacteria (T4-4, S-30, S-11)were all obtained from the Laboratory of Plant Pathology ,Beijing University of Agriculture.

Media and reagents

0.2 mol / L potassium phosphate,(pH 7.2),0.1 mol / L glutaraldehyde buffer; a graded ethanol series,acetone; PDA media (H2O 1000ml, potato 200g/l,agar,17 g/l,Glucose 20 g/l).

Strain culture

Grown on the different treatment and control of Fusarium oxysporum fungus tablets access PDA medium mixed with antagonistic bacteria, *incubated* at 27°C for 7d.

Media and reagents

The sterilized cover-glass were inserted with the help of sterile forceps at an angle of about 45°C into agar medium plates, until about half cover-glass was dipped in the medium..cutting the cover glass; Perforating cake samples with punch in a solid that grow dense (4mm of diameter).All were fixed for 24 h in 2.5% (w/v) glutaraldehyde in 25 mM sodium phosphate buffer, pH 7.2.

Sample preparation and electron microscopy

The next day they were washed in six 15 min changes of PBS buffer ,followed by a 1.5 h post-fix in the osmium tetroxide, washed with water 6 times for 15 min, and dehydrated through a graded ethanol series (30%, 50%, 70%, 90%,95%, 100% [v/v] ethanol) for 15 min each step. Three 15 min changes in 100% ethanol, followed by three 15 min changes in acetone.The dehydration process was slowly done to discourage the processes of hyphal shriveling that may occur during rapid dehydration. Ultimately, for SEM, the microbial material was critically point dried. The upper surface of each sample was then coated under vacuum with a film of gold (Hitachi E-1010).The gold coating process was completed in 15–20 min and images were recorded with SEM (TESCAN 5136), accelerating voltage of 15 kV.The cultures were directly grown on cover-glass and coated with a film of gold without using any fixative and dehydrating procedures.

RESULTS AND DISCUSSION

The effects results of different sources by SEM

The strain samples observed by SEM can be obtained by solid and liquid medium. The cover-glass of insert sample only can be obliquely inserted into the a solid medium. In this study, the figure of fungal samples obtained by inserted *process* has a simple background, good visible, clear structure and distinct level. Besides it is seldom interfered by another substance. We can observe that the mycelium and the spores of normal morphology are arranged in rows, and in uniform size. Their appearances are smooth and they have a good dispersion degree. The shape of spore is cylindrical, which surface with a small amount of concave points (Fig.1-a1-a2). Compared with the cake sample,which it was significantly less than the inserted sample, mainly due to in the processing of samples dehydration, more easily leading to the loss of sample. In the cake samples (Fig.1-b1), the mycelium is piled up together, crossing and overlapping each other. The site meant to be seen was not fully exposed, but still a few not segmental aerial mycelium and germinating spore can be seen. Without any fixed processing insert sample can truly show the morphological structure of the natural growth of mycelium, but part hypha and spore shrinkage deformation; charge accumulation is more obvious; containing more impurities; structure level is not clear, and the mycelium surface has a quantity of attachments. Part of hypha and spore were covered by culture radical or secretions (Fig.1-c1).

The inhibitory effect of different antagonistic bacteria on Pestalotiopsis photiniae C16-4

After the Fusarium axysporum C16-4 were cultured on the PDA plate culture media mixed with different antagonistic bacteria filtrate T4-4, S-30, S-11, the results showed that under the action of antagonistic bacteria T4-4, the mycelium rupture, and spores are acerbate depression, and take on petaloid under the metabolic action of antagonistic bacteria S-11, mycelium was destroyed obviously. It shrinkages, takes on concave point, and distort into deformity. Hypha and spore surface become rough, part spores have different degrees of swelling (Fig. 2-b1-b2).

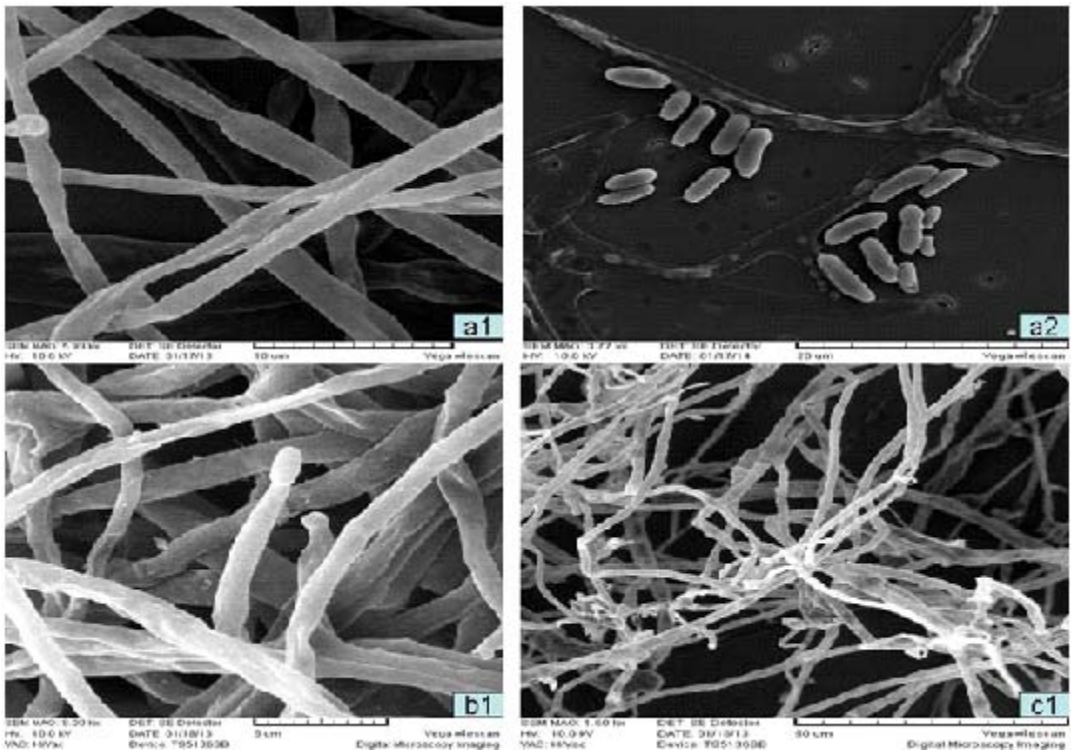
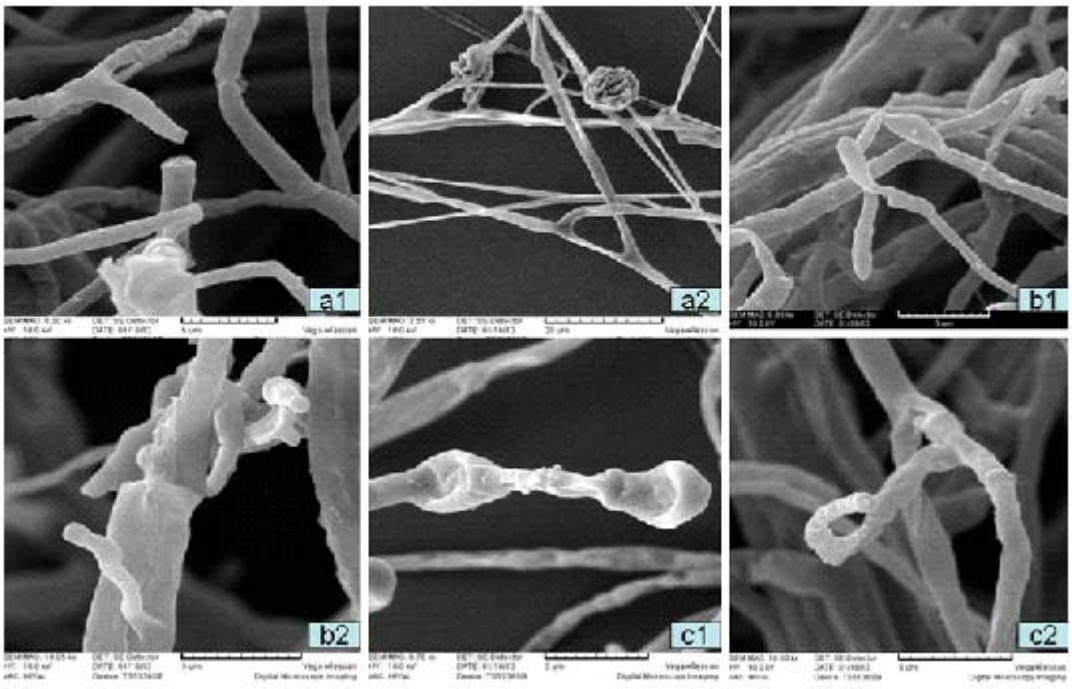


Fig. 1. SEM observation of the morphological characterisitcs of fungus under different culture conditions a1 and a2 inserts samples b1: bacteria cake samples, c1: not fixed sample



a1 and a2 : The effect of C16-4 inhibited by Antagonistic bacteria T4-4
b1 and b2 : The effect of C16-4 inhibited by Antagonistic bacteria S-11
c1 and c2 : The effect of C16-4 inhibited by Antagonistic bacteria S-30

Fig. 2. The inhibitory effect of different antagonistic bacteria on pestalotiopsis photiniae C16-4 by SEM

Germinating spores are sunken with the inhibition effect of S-30 antagonistic bacteria. Hypha swell and its separated coarse increase significantly (Fig. 2-c1-c2).

Morphology had played a major role in distinguishing Fusarium oxysporum from other sporing and in the characterization of microorganism species. The tools of the microorganism morphologists are bright field microscope. These permit to observe undisturbed cultures grown on Petri dishes. *However* for observation of surface features of spores, use of scanning electron microscope is essential (Williams *et al.*, 1989). Tresner et al. used electron microscopy to differentiate Streptomyces species on the basis of the fine structure of spore chain morphology, spore surface, shape of sporangia, formation of single spores, spore surface textures like smooth, warty, rugose, spiny etc. These characteristics merit importance in recent days microorganisms taxonomy also (Castillo et al., 2006; Dastager et al., 2008). As the preparation procedure is so simple and rapid, this *method* could be a very suitable and useful method for the routine examination and identification of specimens.

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