ANTAGONISTIC INTERACTIONS BETWEEN THE BACTERIAL ANTAGONIST Pseudomonas fluorescens Q2-87 AND PATHOGENIC Pythium spp. IN RELATION TO HYPHAL ULTRASTRUCTURAL ANALYSIS

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ABSTRACT

Effects of the biocontrol agent Pseudomonas fluorescens Q2-87 that is known to produce the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) on phytopathogenic Pythium spp. in relation to electron microscopic analysis of the hyphal cell walls were investigated. The outcome of the antagonistic interactions between this strain and Pythium spp. by means of light and scanning electron microscope, when co-cultivated on potato dextrose agar was described. Light micrographs are presented that displayed hyphal distortion, swollen shapes and unusual apical branching. In addition, scanning electron micrographs are also presented that displayed degradation of the cell wall layers and revealed the cellulosic wall microfibrillar skeleton. A similar cell wall degradation was observed when PDA medium was amended with the synthetic antibiotic 2,4-DAPG. Thus, it might be concluded that the natural antibiotic 2,4-DAPG is primarily responsible for antagonistic process of the Omycete P. ultimum induced by P. fluorescens Q2-87. Our results based on ultrastructural observations provide evidence that the modes of this strain action in inhibition, preventing colonization by Pythium are mainly associated with major cell wall degradation, and eventually death of Pythium cells, as a result of extensive cell wall breakdown.

Keywords: 2, 4-diacetylphloroglucinol, Pseudomonas fluorescens Q2-87, Pythium ultimum, ultrastructural analysis

INTRODUCTION

A number of Pseudomonas strains have been intensively studied as possible biocontrol agents for soil-borne fungal pathogens (Dunne et al. 1996; Weller and Thomashow 1999; Thomashow et al. 2000; Landa et al. 2002). Among them are P. fluorescens strains F113 (Fenton et al. 1992), CHAO (Schnider et al. 1995; Duffy and Defago 1997) and Q2-87 (Harrison et al. 1993; Mazzola et al. 1995). The biocontrol effect of all three strains is related, in part, to their ability to produce the antibiotic 2, 4-DAPG (Vincent et al. 1991; Shanahan et al. 1992). However, strain CHAO also produces a second antibiotic, pyoluteorin (Natsch et al. 1998). Pseudomonads that produce the phenolic antibiotic 2,4-DAPG inhibit a broad spectrum of phytopathogenic fungi (Nowak-Thompson et al. 1994, Sharifi-Tehrani et al. 1998) and control a number of root and seedling diseases (Sharifi-Tehrani et al. 1998). There are three lines of evidence support the importance of 2, 4-DAPG production in biological control. First, mutations in the biosynthetic pathway resulted in reduced biocontrol activity (Vincent et al. 1991; Nowak-
Thompson et al. (1994). Second, the population magnitude of 2, 4-DAPG producers correlated with disease suppressiveness of the soil and in situ antibiotic production (Raaijmakers et al. 1999). Third, diverse 2, 4-DAPG-producing *Pseudomonas* spp. have been isolated from the rhizosphere of many different crop plants (McSpadden-Gardener et al. 2000; Picard et al. 2000).

Inhibition of a plant pathogen by producing antimicrobial compounds via biosynthesis, degradation of pathogenicity factors of the pathogen and biosynthesis of cell wall-degrading enzymes have been considered as a major mode of action in terms of fluorescent pseudomonads (Keel and Défago 1997; Whipp 2000). 2,4-DAPG has been shown to be the only antibiotic compound produced by *Pseudomonas fluorescens* Q2-87 (Harrison et al. 1993; Bonsall et al. 1997). In addition, it has been confirmed that mutant-negative strains of this bacteria lost the ability to inhibit plant pathogens (Vincent et al. 1991). The mechanism by which this antibiotic exerts its mode(s) of action to antagonize a phytopathogen in relation to ultrastructural analysis by means of scanning electron microscopy is not yet studied. Interaction between organisms may involve cellular communication mediated by diffusible substances.

To improve our understanding of the interaction between a genus of Oomycete, *Pythium*, as a model system of a phytopathogen, and *P. fluorescens* Q2-87 producing 2, 4-DAPG, as a biocontrol agent, we investigated the outcome of antagonistic process between these two organisms in relation to ultrastructural changes of hyphal cell walls. To clarify the specific role of the phenolic antibiotic 2, 4-DAPG produced by this strain in this process against *Pythium*, a synthetic 2, 4-DAPG was also applied.

**MATERIALS AND METHODS**

*Pythium isolates and culture conditions*

*Pythium* species used in this study were *P. ultimum*, *P. aphanidermatum* and *P. intermedium*. *P. ultimum* was isolated from cowpea in Georgia, USA and kindly supplied by Dr. Jeffret Hoy (Agricultural Experiment Station, LSU). However, *P. aphanidermatum* and *P. intermedium* were isolated from turfgrass in Michigan State, USA. All cultures were maintained on a solid V8 medium which consisted of 200 ml V8 juice, 3.0 g CaCO₃, 15.0 g agar and made up to one liter with distilled water.

**Bacterial culture and growth media**

Lyophilized *P. fluorescens* strain Q2-87 (NRRL B-23374) was obtained from the Agricultural Research Service Patent Culture Collection (National Center for Agricultural Research, USDA, Peoria, Illinois). Cultures were grown on 100 ml tryptone soy broth medium (TSB, 30 g/L Difco, Detroit, MI). They were maintained at 24°C on tryptone glucose yeast medium (TGY) which consisted of 5.0 g tryptone, 5.0 g yeast extract, 1.0 g glucose, 1.0 g KH₂PO₄, 15.0 g agar and made up to one liter with distilled water, pH 7.0, prior to autoclaving.
In vitro antagonistic interactions

*P. fluorescens* cultures were grown on potato dextrose agar (PDA, Difco, Detroit, Michigan), pH 6.6, at 24°C for 24 h while *Pythium* species cultures were grown for 3-4 days on PDA medium. Hyphal tips from the actively growing mycelia were then used in dual cultures. Interactions between these two living organisms were studied on PDA media as described by Schmidli-Sacherer et al. (1997). A PDA medium was chosen in this study, because the ability of the biocontrol *P. fluorescens* to antagonize the growth of *Pythium* spp. depends on characteristics of the growth medium (Maurhofer et al. 1995). Sterilized glass slides (50 x 75 mm) were placed in Petri-dishes and 2 mm depth of PDA was added. After solidification, bacteria were streaked directly onto the glass slides on one side and 5-mm-diameter of *Pythium* plugs were simultaneously co-cultivated at 1 cm along the bacteria on the opposite side. Control slides were cultivated with *Pythium* only. Treated and untreated slides were incubated at 24°C. The interaction, i.e. inhibition, areas began to form after 24 h and were observed in situ by light microscope as evidence that the bacterium prevented colonization of the slide by *Pythium* by producing the antibiotic 2, 4-DAPG under these cultural conditions. Light observations were made without use of cover glasses as this in itself could be traumatic *Pythium* cells and affect their dynamic responses, e.g. cytoplasmic streaming, or cause cell lysis. Strips of agar from the intermediate area having *P. ultimum*, *P. aphanidermatum* or *P. intermedium* were taken, at specified times unless otherwise stated, after bacterial application and were processed for scanning electron microscopy.

Antagonistic activity of synthetic 2,4-DAPG

Preliminary experiments with synthetic 2,4-DAPG (purchased from Toronto Research Chemicals Inc., Toronto, Canada) concentrations ranging from 0 to 100 μg/ml indicated that the growth of *Pythium* spp. was completely inhibited by 50-20 μg/ml. 2, 4-DAPG was dissolved in 95% ethanol (EtOH) and added to the media. The highest concentration of EtOH in the 2, 4-DAPG media was 0.1% and thus 0.1% of EtOH was used as a control medium. Antagonistic interactions between 2, 4-DAPG and *P. ultimum* representing *Pythium* spp. was performed by the following assay.

Preparation of the glass slides containing media was done as described above. 5-mm-diameter plugs of *Pythium* were excised from the youngest growing margin of a 4-day-old culture and cultivated on one side of the glass slides. 24 h later, aliquots of 80μg/ml synthetic 2,4-DAPG were applied at 0.5 cm along the bacteria on the opposite side of the plugs. Control slides were cultivated with *Pythium* only and applied with aliquots contained 0.1% EtOH. Treated and untreated cultures were incubated at 24°C. Inhibition zones were observed in situ by light microscope after 24 h of 2,4-DAPG application. Strips of agar, from the intermediate area having *P. ultimum* that interacted with diffusible of the synthetic antibiotic, were taken at specified times after application and were processed for scanning electron microscopy.

Scanning Electron Microscopy (SEM)

Mycelial samples from the interaction regions were cut into 5 mm² pieces (two pieces from each slide) and fixed by immersion in 4%
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glutaraldehyde (Sigma Co.) in 0.1 M phosphate buffer (pH 7.0) for 2 h at 25°C. Dehydration was conducted through a graded ethanol series in deionised water (Glaeser 1974). When in 100% ethanol, specimens were critical-point dried in an Balzers critical point dryer with CO₂ as the transitional fluid, then mounted on aluminum stubs using adhesive tabs and sputter coated with approximately 24.5 nm of gold for 3.5 min in an Emscope SC 500 unit and purged with argon gas. Specimens were examined at various magnifications in situ with a JEOL 6300 scanning electron microscope, manufactures by Japan Electron Optics Limited.

There were 5 replicates for each antagonist-pathogen combination and control. The experiment was repeated twice with similar results. P. ultimum induced by synthetic 2, 4-DAPG showed identical results to that induced by natural 2, 4-DAPG produced by P. fluorescens. Subsequently, 2, 4-DAPG micrograph at 96 h was just presented.

RESULTS

In vitro antagonistic experiment

In the glass slide single cultures, P. ultimum grew actively and colonized the entire agar surface within 4 days following cultivation. However, in dual cultures, contact between P. fluorescens producing 2,4-DAPG and P. ultimum did not occur by 4 days after co-cultivation. Antagonistic interactions between these two living organisms were clearly discerned by very limited growth and the complete absence of mycelial hyphae in the intermedium area along bacterial colonies (Fig. 1, A and B). Similar result was also noted in terms of the synthetic compound 2, 4-DAPG (Fig. 1, C and D). In addition, when agar samples from the interaction region between P. fluorescens and P. ultimum were deposited at the margin of an actively growing Pythium colony, mycelial growth was halted. By contrast, the Pythium easily overgrew agar disks taken from un-inoculated PDA medium (not shown).

Morphological changes under light and scanning electron microscope

At the microscopic level, the control hyphae of Pythium have a regular diameter, normal growth and relatively linear shape during the time-course of investigation. Pythium hyphae grown in dual culture exhibited morphological changes mainly characterized by hyphal distortions (Fig. 1, E and F). Swollen shapes were clearly observed in P. intermedium compared to P. ultimum (Fig. 1, F and H). In addition, the surface of the cell wall of treated Pythium became smooth instead of rough in all Pythium spp. under study compared to their controls (Figs. 1, E-H, 2, and 3).

Time-course investigation of the cell wall events of P. ultimum and P. aphanidermatum

Fig. 4 shows that sequential degradation of the cell walls of P. ultimum after inducing by P. fluorescens Q2-87 was observed in the intermediate area where diffusible bacterial metabolite interacting with Pythium cells (Fig. 2, B-H).
Fig. 1. Light micrographs of *P. ultimum* inhibited by either *P. fluorescens* Q2-87 or synthetic 2,4-DAPG for 96 h. *P. ultimum* grown in single culture (A and C), *P. ultimum* grown in dual culture and PDA media applied with 80 μg/ml 2,4-DAPG (B and D, respectively). Note: the white area depicts growth of *P. ultimum* and the black area depicts the zone of inhibition. Light micrograph of *P. ultimum* inhibited by *P. fluorescens* Q2-87 for 96 h, in the intermediate area where diffusible bacterial antibiotic and *Pythium* are interacting, mycelial hyphae of *Pythium* grown in a single culture showing normal growth (E), mycelial hyphae of *Pythium* grown in dual culture showing irregular in shape and expanded in size (F). Scanning electron micrographs of *P. intermedium* inhibited by *P. fluorescens* Q2-87 for 96 h, in the intermediate area where diffusible bacterial antibiotic and *Pythium* are interacting, *Pythium* grown in single culture showing normal hyphae having rough outer surface, *Pythium* grown in dual culture showing swollen shapes and smooth outer surface (H).
However, control mycelial hyphae grown in single culture showed no degradation and a rougher outer surface of the cell walls over the time course of study (Fig. 2, A-G). However, *P. ultimum* induced by natural diffusible antibiotic 2, 4-DAPG lost its uniform folding patterns during the progress of degradation process (Fig. 2, B and D). After 72 h, looseness and disconnection of the outer layer of the cell wall was observed (Fig. 2F) followed by complete degradation of this layer and appearance of wall microfibril skeleton by 96 h (Fig. 2H). Identical results were also seen after synthetic 2, 4-DAPG application (Fig. 3D) in comparison with the induction by living biocontrol agent *P. fluorescens* (Fig. 2H). Similar ultrastructural analyses were also noted during the time course study of *P. aphanidermatum* induced by the same strain (Fig. 4) with considering that the folding patterns vary between these two species (Figs. 2A, and 4A). However, microfibril skeleton were not markedly seen in *P. aphanidermatum* by 96 h (Fig. 4H).

**DISCUSSION**

Phenolic antibiotic-produced by fluorescent pseudomonads have been used effectively in biological control of many soilborne phytopathogens (Pierson 1997). Among the modes of biocontrol action mediated by fluorescent pseudomonads are production of the secondary metabolites (Howell and Stipanovic 1980; Howie and Sustow 1991; Thomashow and Weller 1995) and/or hydrolytic enzymes (Jones et al. 1986; Défago et al. 1996). According to Haas et al. (1991), Vincent et al. (1991), Shanahan et al. (1993), Harrison et al. (1993), Keel et al. (1996), and Bonsall et al. (1997), *P. fluorescens* Q2-87 has the ability to antagonize a wide range of plant pathogens via producing the secondary metabolite 2,4-DAPG. Indeed, mutant strains of *P. fluorescens* lacked the ability to produce 2,4-DAPG, also lost the ability to inhibit plant pathogens (Fenton et al. 1992; Carrol et al. 1995). In agreement with the former authors, we isolated and identified 2,4-DAPG from a chemically-defined broth culture of the strain Q2-87 by chromatographic means (data will be reported elsewhere). In the present study, *in vitro* antagonistic process between *Pythium* and the Q2-87 or synthetic 2,4-DAPG was observed (Fig. 1, A-D). These data are in agreement with previously reported inhibitory activities of *P. fluorescens* on various plant pathogens, including *Pythium* spp. (Fenton et al. 1992; Levy et al. 1992; Shanahan et al. 1992; Harrison et al. 1993; Hultberg et al. 2000). At microscopic level, *Pythium* spp. cultivated in dual culture relative to that cultivated in single culture revealed that mycelial hyphae were mainly characterized by irregular in shapes and expanded in size (Fig. 1, E and F). Our results are in line with Paulitz et al. (2000) who reported that alterations of *Pythium* cells were occurred after application with *P. aurofaciens* producing the antibiotic 2,4-DAPG. They described the nature of these morphological changes as swollen and abnormal appearance of the hyphae. In the present study, scanning electron micrographs of *Pythium* in dual culture relative to that in control documented the sequential cell wall degradation, over the time course study, induced by the Q2-87 strain (Figs. 2, 8424
and 4). The outer cell wall layer, i.e. amorphous layer, at least, was completely degraded and revealed the underlying microfibrillar β-1, 4-glucan skeleton (Fig. 2H). However, the cell wall microfibrils were not seen in P. aphanidermatum that induced by the same strain (Fig. 6) indicating that degradation process did not reach to the level that cell wall skeleton might be seen. Extending the time-course of analysis may reveal this ultrastructure. Similar results were also observed after synthetic 2,4-DAPG application (Fig. 3), confirming that Q2-87 strain may exert its biocontrol action via producing the antibiotic 2,4-DAPG. In terms of revealing cell wall microfibrils presented here, similar results were obtained by Bartnicki-Garcia and Wang (1983) via scanning electron microscopic examination after exo-β-1,3-glucanase application. These authors reported that amorphous glucan layer that completely covers the microfibrils of Phytophthora palmivora could be selectively removed by hydrolyzing with exo-β-1,3-glucanase activity to reveal the underlying microfibrillar β-1, 4 glucan skeleton. In addition, Cherif et al. (1992) found that application of exoglucanase, a β-1, 4 glucan celluliohydrolase with specific affinity for β-1,4-linked glucans, to ultrathin sections of Pythium ultimum showed that gold particles were preferentially associated with the internal wall portion closely appressed against the plasma membrane whereas the outer portion was devoid of labeling. Consequently, it may be concluded that constituents of microfibrils are considered to be cellulosic (β-1,4 glucan) and they disappeared from the mycelial walls upon hydrolyzing with cellulase, following a previous hydrolyze with β-1,3-glucanase as also reported by Bartnicki-Garcia and Wang 1983. However, constituents of amorphous material are considered to be noncellulosic glucans (β-1,3- and β-1,6 linked glucan) as suggested by Bartnicki-Garcia and Wang (1983).

Based on the foregoing results, the scanning electron micrographs (Figs. 2, and 3) indicate that noncellulosic β-glucans existing in the outer layer of Pythium cells was completely degraded by β-1,3 glucanase to reveal the internal wall layers, cellulosic β-1,4 glucan microfibrils (Figs. 2 and 3D). Further, comparing figures 2H and 3D with the correspondence control (Figs. 2G, and 3A), the present results suggest that there are two different structural elements in Pythium cells: long microfibril and nonfibrillar or amorphous substance. This architectural design is similar to that of other fungal walls as reported by Bartnicki-Garcia and Wang (1983), and Wesselis (1990). Additionally, microscopic investigations reveal that growth inhibition of both P. ultimum and P. intermedium induced by either P. fluorescens Q2-87 or the synthetic compound 2,4-DAPG correlated with both hyphal degradation observed via SEM (Figs 4 and 5) and marked structural changes including vacuolation, condensation and aggregation of the cytoplasm, as well as cytoplasmic organelles breakdown (Figs. 5, A-D). Because such cell reactions were, in fact, induced in the absence of intimate contact between Pythium cells and P. fluorescens, the possibility that diffusible 2,4-DAPG metabolite (s) may be Responsible for the observed disturbances appears realistic.
Fig. 2. Scanning electron micrographs of *P. ultimum* induced by *P. fluorescens* Q2-87 during the time-course indicated of cell wall hyphal degradation, in the intermediate area where diffusable bacterial antibiotic and *Pythium* are interacting. Mycelial hypha was grown in single culture for 24 h having normal growth and much rougher outer surface (A) compared to that grown in dual culture showing less rougher outer surface (B). Mycelial hypha was grown in single culture (C) for 48 h compared to that grown in dual culture (D) showing relatively smooth cell wall relative to the control. Mycelial hypha was grown in single culture (E) for 72 h compared to that grown in dual culture (F) showing looseness and disconnection of the outer layer of the cell wall. Mycelial hypha was grown in single culture (G) for 96 h compared to that grown in dual culture (H) showing complete degradation of the outer layer, at least, of the cell wall and revealing wall microfibril skeleton. Magnifications were indicated within Figs.
Fig. 3. Scanning electron micrographs of *P. ultimum* induced by the synthetic 2,4-DAPG at 80 μg/ml for 96 h. *Pythium* grown in single culture (A) compared to that grown in dual culture showing complete degradation of the outer layer, at least, of the cell wall and revealing wall microfibril skeleton (B). The same as in B at higher magnifications indicated (C and D).
Fig. 4. Scanning electron micrographs of *P. aphanidermatum* induced by *P. fluorescens* Q2-87 producing 2,4-DAPG, during the time-course of hyphal degradation, in the intermediate area where diffusible bacterial antibiotic and *Pythium* are interacting. Mycelial hypha was grown in single culture for 24 h showing normal hypha with a much rougher outer surface (A) compared to that grown in a dual culture (B). Mycelial hypha was grown in single culture (C) for 48 h compared to that grown dual culture (D) showing a relatively smooth cell wall relative to that in control. Mycelial hypha was grown single culture (E) for 72 h compared to that grown in dual culture (F) which show a smooth outer layer of the cell wall. Mycelial hypha was grown in single culture (G) for 96 h compared to that grown in dual culture (H) which show looseness and disconnection of the outer layer of the cell wall. Magnifications were indicated within the Figs.
Fig. 5. Transmission electron micrographs of *P. ultimum* induced by *P. fluorescens* for 48 h, in the intermediate area where diffusible bacterial antibiotic interacting with hyphae of *P. ultimum*. Cross section of *P. ultimum* grown in single culture having normal structural components (A). Angle of section through a hypha of *P. ultimum* grown in dual culture showing structural changes mainly characterized by an increase in the number of vacuoles (B). Induced *P. ultimum* for 96 h showing disorganization of cytoplasmic organelles and a cell was devoid of all living components (C and D, respectively). Bars indicated in within figures.
Similar disturbances in the overall fungal organization have been reported in the case of fungal cells exposed to either antibiotics (Klecan et al. 1990; Hajlaoui and Bélanger 1993, Benhamou et al. 1996), or Sterol-binding fungicides (Fuller et al. 1990). Because of plasma membranes have been reported to be a potential target for antibiotics (Lewis and Papavizas 1987), changes in the plasma membrane permeability may have promoted internal osmotic imbalances, leading to alterations such as cytoplasm disorganization and aggregation. Since P. fluorescens Q2-87 is well known to produce the secondary metabolite 2,4-DAPG during its stationary phase, strong support for the concept of antibiotic as the main mechanism by which Q2-87 operates against Pythium cells is provided by the finding that agar disks collected from the bacterial-free intermediate region between Pythium cells and Q2-87 could trigger the same sequence of events seen by SEM and transmission electron microscopy (not shown). These biocontrol effects led ultimately to cell wall degradation and cell death (Figs. 2, B-H, 3, B-D, and 4, B-H). So far, little is known about pathogen cell (wall) degradation mediated by Pseudomonas-produced antibiotics. In fact, most studies have been concentrated on the role of hydrolytic enzymes in the antagonistic processes against some diseases (Saksirirat and Hoppe 1990) and not on the possible involvement of antibiotics. Askary et al. (1997) were the first to suggest that antibiotics, causing cytoplasm disorganization, preceded parasitism and subsequent internal colonization of the plant pathogen S. fuliginea. Antifungal synergisms between enzymes and either toxic or antibiotic metabolites have just been reported over the past few decades (Köller et al. 1992; Yang et al. 1993; Shimböck et al. 1994). One of the examples being the fungal antagonist, Gibberella viridae, while gliotoxin, a fungitoxic metabolite, has long been thought to be the main mechanism of biocontrol by this fungus, recent studies have shown that cell wall degrading enzymes were also involved in this process (Di Pietro et al. 1993). These authors suggest that synergistic effect of the enzymes was likely due to partial cell wall hydrolysis which facilitated rapid diffusion of the toxin. Similar results by us were also found during the interaction between Pythium cells and Pseudomonas strain Q2-87, since higher levels of β-1,3 glucanase and cellulase (β-1,4, glucanase), and protease were found in Pythium cells after 2,4-DAPG application (data will be reported elsewhere). Thus, antibiotics and hydrolytic enzymes may be involved in the antagonistic process mediated by Pseudomonas strain Q2-87.

Overall, marked ultrastructural changes induced in Pythium cells by Q2-87 (Figs. 2H, and 4H) were found to be associated with hyphal distortion, swollen shapes, abnormality, and inhibited growth that seen by light and scanning electron microscopy (Figs. 1, A-H). These significant changes may be explained by the fact that two cell wall textures described above including the outer layer might be required for Pythium growth and development and may be related to the need for controlled plasticity of the apical zone where expansion takes place, as also suggested by Bartnicki-Garcia and Wang (1983). Thus, one might conclude that cell wall degradation would affect hyphal growth and development because of the importance of amorphous layer in hyphal wall morphogenesis. In line with our results, Meyer et al.
(1976) found in a related study that germination of the Oomycete Phytophthora palmivora cysts after application with exo-β,1,3-glucanase, the outer wall layer is eroded and the germ tubes were distorted, swollen in shapes and unusual in apical branching. It might be concluded that degradation of Pythium cell walls, which contain, β-1,3 glucan and β-1,4 glucan, require enzymes that can hydrolyze polymers of glucose with various glycosidic linkages (Bartnicki-Garcia and Wang 1983). β-1,3-glucanase and cellulase (β-1,3-glucanase) are recognized as important hydrolytic enzymes because they attack the most common cell wall-forming polymers in Oomycete hyphae. As a result, partial or complete degradation of these structural polymers would have adverse effects upon the growth and differentiation of fungi (Bartnicki-Garcia and Wang 1983; Pouloue 1992; Fridlender et al. 1993; Schirmböck et al. 1994).

CONCLUSION

The results of the present investigation demonstrate that antibiotic of Pythium spp. triggered by P. fluorescens Q2-87 induces cell wall degradation that adversely affects Pythium growth and development and shed more light on the mechanism by which antibiotic is expressed at ultrastructural level. Our results suggest that there are, of course, hydrolytic enzymes participated in the cell wall degradation of Pythium. However, the source and nature of these enzymes are required further investigation. Could these have been produced by the bacteria? Could the mechanism of biocontrol involve enzyme production as well as antibiotic production. These questions led us to further study of the mechanism of action of biocontrol P. fluorescens Q2-87 of a major plant pathogen, Pythium.

REFERENCES

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التفاعلات المضادة بين المضاد البكتيري سينديوموناس فلوريسنس Pseudomonas fluorescens Q2-87 والمضاد المرضي اثناع Pythium فيما يتعلق بالتركيب الدقيق للهيفات

سرح على جمال الدين يوسف١- كامل أحمد حسين ظرورة١- جوزيف فارجس١
قسم أنثاث الزراعي- جامعة قناة السويس بالإسماعيلية- مصر
و قسم امراض النبات- جامعة ميشيغان- الولايات المتحدة الأمريكية١

تم دراسة تأثيرات عامل المقاومة الجيوبية سلالة 2,4-diacetylphloroglucinol على المضاد المرضي فيضال فيما يتعلق بالتفاعل الدقيق للهيفات. تحت تلك الدراسة، تم استخدام كل من الميوكروسكوب الضوئي والإلكتروني للدراسة. كل منها معروف ببيئة البيطاطس- داكروز- أجري. وفقًا للدراسة، يمكن أن يحد الميوكروسكوب الضوئي الهيفات الناتجة عن الميكونوموناس في بيئة البيطاطس. بالإضافة إلى ذلك، فإن الميوكروسكوب الضوئي يحسن تحلل التفاعلات الجينية الخلوية بينما يظل البكتيري في بيئة البيطاطس. في النهاية، يمكن أن تكون هذه النتائج مفيدة في تطوير عامل المقاومة الجيوبية 2,4-diacetylphloroglucinol.