Ann. Bangladesh Agric. (2015) 19 : 1-10 *ISSN 1025-482X*

ALTERATION OF HYPHAL MORPHOLOGY AND SUPPRESSION OF SPORANGIAL PRODUCTION OF *Phytophthora capsici* BY ANTAGONISTIC RHIZOBACTERIA

M. Kamruzzaman[1], M. A. Haque[2] and M. T. Islam[2], N. Chandra[2]

Abstract

The study was conducted with six bacterial strains namely, *Delftia* sp. strain BTL001, *Pseudomonas aeruginosa* strain BTL002, *Pseudomonas* sp. strain BTL004, *Staphylococcus arlettae* strain BTL006, *Psseudomonas* sp. strain BTL007 and *Pseudomonas* sp. BTL008, two isolates namely, BTL003 and BTL005 along with two reference strains, *Lysobacter capsici* 10.4.5 and *L. oryzae* Y6269 during 2012-2013. All these bacterial isolates and strains except the reference strains were derived from the rhizosphere of host and non host plants including both crops and weeds of seven different locations of Bangladesh. All of them exhibited antagonistic activity against *P. capsici* on PDA (Potato dextrose agar) medium in dual culture assay. They inhibited the growth of mycelia by producing a clear inhibition zone on PDA medium and exhibited distinct morphological alterations in *P. capsici* hyphae. Light microscopy analyses revealed that hyphal tips of growth inhibited area showed abnormal morphological features (such as curled, excessively branched and swelled) than those of unaffected hyphae in the control plate. Mycelial dry weight and formation of sporangia of *P. capsici* zoospores were significantly reduced by the application of these bacteria. These results indicate that antagonistic bacteria isolated from Bangladesh could be used as an effective agent for biocontrol of late blight pathogen, *P. capsici*.

Keywords: *Phytophthora capsici*, antagonistic bacteria, abnormal morhological features

Introduction

The pathogen *Phytophthora capsici* causes diseases in many vegetables under different families worldwide including Bangladesh (Hossain and Friedt, 2010). It has been reported that, the incidence of disease caused by *P. capsici* on cucurbits causes yield loss as much as 100% (Roberts *et al.,* 2001; Hausbeck and Lamour, 2004). It has a wide range of hosts which includes tomato, brinjal, cucumber, water melon, pumpkin, squash, cocoa, macadamia, and peppers (Aravin *et al*., 2008, Tian and Babadoost, 2003). It can attack all parts of the susceptible host plant at any stage of the growth. The disease caused by them is very difficult to control as they infect host plants through asexually generated characteristic biflagellate motile zoospores from globose shaped sporangia (Islam et al., 2001a). That is why development of effective biologically rational management strategies against the *Phytophthora* is highly needed. Rhizosphere is considered as a unique habitat which harbors a vast majority of microorganisms in a continuous dynamic

[1] Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), BAU Campus, Mymensingh-2202, [2]Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur-1706

state by actions and interactions. Although synthetic antimicrobial compounds have been found to induce morphological alterations in the hyphae of fungal pathogens (Kang *et al*., 2001), antagonistic rhizobacteria could also do the similar functions. In this study we screened eight antagonistic bacteria from sixty isolates on the basis of hyphal growth inhibition and induction of morphological alterations of *P. capsici*. The aim of the study was to observe the induction of morphological alterations of *P. capsici* hyphae with growth inhibition by antagonistic rhizospheric bacteria in dual culture assay.

Materials and Methods

Isolation of bacteria: Soil samples of 200 g were collected from rhizospheric zone of different cultivated crops. One gram of soil sample was taken into conical flask containing 50 ml broth amended with 0.5 g/L ground chitin. Each sample was incubated in a shaker at 150 rpm for 3 days in room temperature. After 3 days of shaking, a loopful of the broth was streaked on a YCA [Yeast cell agar; 18 g Sigma agar, 5 g active dry yeast cell in 1 L doubled distilled water autoclaved for 40 minutes then 50 mg/L penicillin (Sigma Aldrich), 50 mg/L kanamycin (Sigma Aldrich) and 100 mg/L cycloheximide (Sigma Aldrich)] plate as described by Hu Yin (2010). After 5 days, bacterial colonies surrounded by clear zones in which yeast cell were digested were purified on a new YCA plates three times to get single colonies. We isolated 60 isolates both from the host and non-host plants of *P. capsici* and screened their ability to inhibit mycelial growth and alter the morphology of *P. capsici* hyphae on agar plates. The six strains were identified by some physiological and biochemical tests and 16s- rDNA sequencing. Finally, we have the strains and two isolates of bacteria for further observations against *P. capsici*.

Observation of hyphal morphological alterations

Bacterial antagonistic activity against *P. capsici* was observed on Potato Dextrose Agar (PDA) medium with three replications. Dual culture assay was done using streak cultured bacterial colonies and a 6 mm i.e. mycelial plug of the pathogen cut from the edge of an actively growing culture plate with a sterile cork borer, and placed 3 cm apart on the agar plate. Both pathogen and bacteria were inoculated on the agar plate at the same time. Bacteria used were taken from pure streaked plate incubated at 25ºC for 24 h. Inhibition zone was determined after incubation at 25ºC for 5 days measuring the radial growth of pathogen. Percent inhibition of mycelial growth was calculated by the following equation (Sundar *et al.,* 1995).

$$\% \text{ Inhibition of mycelial growth} = \frac{x-y}{x} \times 100$$

Where,

X= mycelia growth of pathogen in absence of antagonists

Y= mycelia growth of pathogen in presence of antagonists

Morphological characters of approaching hyphae were observed under a compound light microscope (400×, XSZ-107 BN, China) and images were recorded with a digital camera (NICON, COOLPLIX P6000, NIKON crop., Japan.4.8/3.7 V=0.5/0.67.4× Optical zoom EDVR, Vietnam).

Time- course observation of mycelial dry weight after treatment with bacterial isolates

A 6 mm diameter block of *P. capsici* mycelia was taken from the pure culture with sterilized borer into 100 ml PD broth and the mixture were kept at room temperature (25˚C). After 3 days, bacterial suspension of 100 µl having 1×10^8 colony forming unit was added. Keeping the same temperature Mycelial dry weight was taken after 2 and 4 days of inoculation and compared with the control weight of *P. capsici* mycelia.

Effect of bacteria on formation of sporangia

In order to observe the effect of bacteria on sporangial development of *P. capsici,* first the bacteria were grown in NB (D+ glucose 1 g/L, peptone 15 g/L, sodium chloride 6 g/L and yeast extract 3 g/L, final pH 7± 0.2) medium for 3 days. A mycelial block of 6 mm in diameter was taken from a pure culture of *P. capsici* culture into a multi-well plate containing 500 µl sterilized distilled water (pH 7.0). Bacterial suspension of 500 µl having 1×10^8 colony forming units was added and the solution was kept at room temperature (25ºC) for 3 days in the dark. After 3 days, the multi-well plate was observed under compound light microscope to investigate the effect of bacterial suspension on sporangia formation in the bacterial treated plates and except bacterial treated control plate. The number of sporangia was counted in 10 microscopic fields for each treatment and also compared with the control.

Results

Antagonistic activity of bacterial isolates against *P. capsici*

Mycelial growth inhibition: Antagonistic bacteria induced characteristic morphological alterations in the *P. capsici* hyphae growing towards bacterial colonies compared to the untreated control along with development of clear inhibition zones on the agar medium (Fig. 1). Bacteria showed antagonistic effects against *P. capsici* on PDA compared to the control plate which were similar to the effect of *L. capsici* 10.4.5 and *L. oryzae* Y6269. All the strains and isolates exerted reduced relative radial growth of hyphae compared to the untreated *P. capsici* hyphae (Table 1). A clear inhibition zone was observed after 5 days of incubation. The highest inhibition of radial growth (74.44%) was found in isolate BTL003 followed by *Staphylococcus* sp. strain

Table 1. Effect of bacterial isolates on radial growth of *P. capsici* mycelium.

Bacterial isolate	Radial Growth(cm)	% Inhibition
Delftia sp. strain BTL001	1.51 ± 0.09b	66.51 ± 2.0cd
Pseudomonas aeruginosa strain BTL002	1.50 ± 0.00b	66.81 ± 0.15cd
BTL003	1.25 ± 0.03e	74.44 ± 2.79a
Staphylococcus sp. strain BTL004	1.38 ± 0.01d	69.38 ± 0.35bc
BTL005	1.50 ± 0.01b	66.66 ± 0.25cd
Staphylococcus sp. strain BTL006	1.29 ± 0.01e	71.34 ± 0.18b
Psseudomonas sp. strain BTL007	1.48 ± 0.01bc	67.18 ± 0.33cd
Pseudomonas sp. strain BTL008	1.56 ± 0.02b	65.41 ± 0.52d
Lysobacter capsici 10.4.5	1.39 ± 0.01cd	68.98 ± 0.18c
Lysobacter oryzae Y6269	1.50 ± 0.00b	66.66 ± 0.00cd
Control	4.57 ± 0.07 a	0.00 ± 0.00e

Data presented here are average value ± SE (Standard error) of at least three replications. Mean values within a common letter do not differ significantly by Duncans Multiple Range Test (DMRT) at $p<0.05$.

BTL006, *Staphylococcus* sp. strain BTL004, *Pseudomonas* sp. strain BTL007 and *Delftia* sp. strain BTL001, respectively (Table 1). *L. capsici* strain 10.4.5 and *L. oryzae* Y6269 showed 68.98% and 66.66% inhibition, respectively (Table 1). The lowest inhibition of radial growth was detected in *Pseudomonas* sp. strain BTL008 (Table 1).

Growth inhibition through induction of characteristics morphological alterations in hyphae

Irregular branching and necrosis along with increased diameter and irregular direction of *P. capsici* hyphae was observed under compound light microscope during interaction with *Delftia* sp. strain BTL001. When the pathogen's hyphae grew towards the colony of *P. aeruginosa* strain BTL002 and *Staphylococcus* sp. strain BTL004 in PDA plates, it induced excessive apical branching and also the swelling while excessive apical branching and bulbous appearance of the hyphae was recorded in *P. capsici* affected by *Pseudomonas* sp. strain BTL008 (Table 1 and Fig. 1). Reduction of radial growth as well as irregular and excessive branching of the hyphae was found during the interaction of *P. capsici* with *L. capsici* 10.4.5 and isolate BTL003. BTL005 exerted abnormal bulbous appearance and curling and *Staphylococcus* sp. strain BTL006 exhibited curling of the hyphae of *P. capsici,* while *Pseudomonas* sp. strain BTL007 and *Lysobacter oryzae* Y6269 caused necrosis, formation of bulbous structure and varying diameter of *P. capsici* hyphae.

Effect of bacteria on formation of *P. capsici* sporangia

To see the effect of antagonistic bacterial isolates on the production of *P. capsici* sporangia, nutrient free bacterial cells were added to multi dish containing mycelia block incubated for sporangia development. The lowest number of sporangia was recorded in the dish containing BTL003 (55%). The highest percentage of sporangia was formed in control treatment (total number of sporangia in control was considered as 100 %) which was followed by bacterial treatments *Staphylococcus* sp. strain BTL006 (80%), BTL005 (78%), *Pseudomonas* sp. BTL008 (71%), *Pseudomonas aeruginosa* strain BTL002 (66%), *Pseudomonas* sp. strain BTL007 (65%), *Staphylococcus* sp. strain BTL004 (61%) and *Delftia* sp. strain BTL001 (60%), respectively (Fig. 2).

Time- course Inhibitory effects on mycelial growth (dry weight) of *P. capsici*

After 3 days growth of *P. capsici* in PDB (Potato Dextrose Broth), the weight of mycelia was 100 mg. However, post incubation mycelial dry weight in control was increased to 120 mg in 5 and 7 days (Fig. 3). However, after treatment with the bacterial isolates mycelial dry weight was initially increased but finally reduced significantly compared to the untreated control. At day 7, bacterial strain *P. aeruginosa* strain BTL002 reduced mycelial dry weight from 110 mg to 100 mg (Fig. 3). Similarly, BTL003, *Staphylococcus* sp. strain BTL004, BTL005, *Staphylococcus* sp. strain BTL006, *Pseudomonas* sp. strain BTL007 and *Pseudomonas* sp. BTL008 reduced mycelial

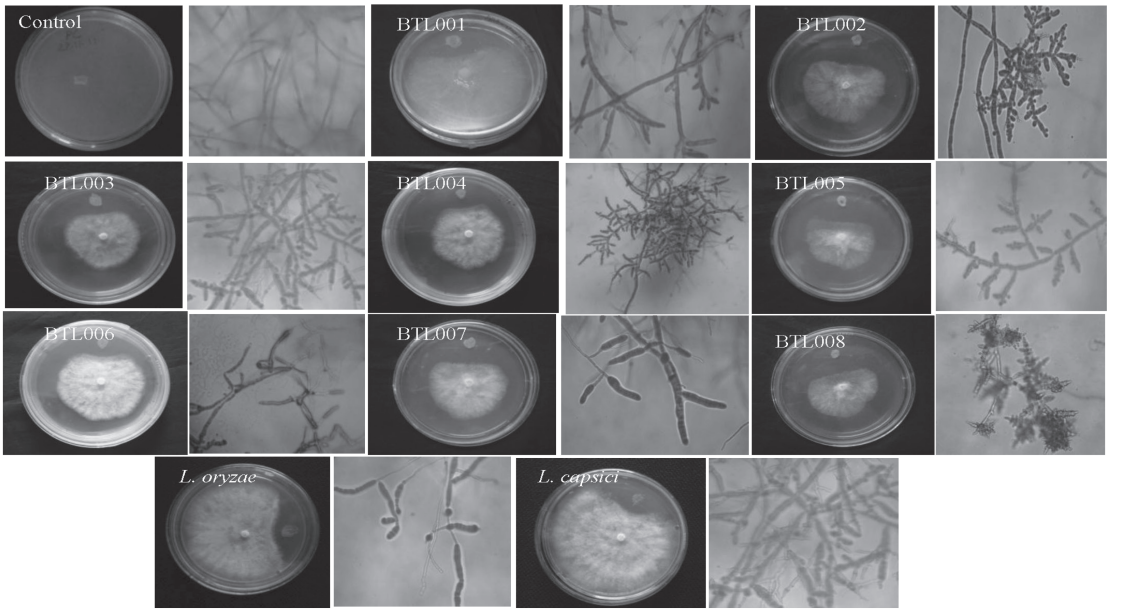

Fig.1. *In vitro* interaction of bacterial isolates, *L. oryzae* Y6269 and *L. capsici* 10.4.5 with *P. capsici* on PDA plate; *Delftia* sp. BTL001 induced irregular branching and necrosis along with increased diameter and irregular direction; *P. aeruginosa* strain BTL002 and *Staphylococcus* sp. strain BTL004 induced excess apical branching; swelling and excessive apical branching and bulbous appearance of the hyphae by *Pseudomonas* sp. BTL008; *L. capsici* 10.4.5 and isolate BTL003 exhibited reduced radial growth as well as irregular and excessive branching of the hyphae; BTL005 exerted abnormal bulbous appearance and curling and *Staphylococcus* sp. strain BTL006 exhibited curling of the hyphae of *P. capsici; Pseudomonas* sp. strain BTL007 and *Lysobacter oryzae* Y6269 caused necrosis, bulbous structure formation and varying diameter of hyphae.

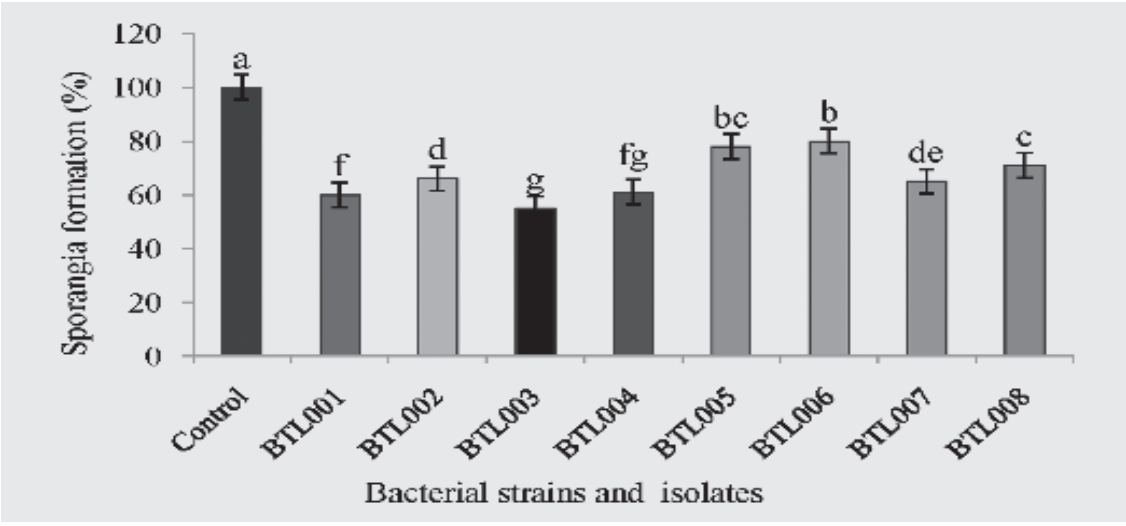

Fig.2. Effect of bacterial isolates *Delftia* sp. strain BTL001, *Pseudomonas aeruginosa* strain BTL002, BTL003, *Staphylococcus* sp. strain BTL004, BTL005, *Staphylococcus* sp. strain BTL006, *Pseudomonas* sp. strain BTL007 and *Pseudomonas* sp. strain BTL008 on sporangia formation of *P. capsici* over control.

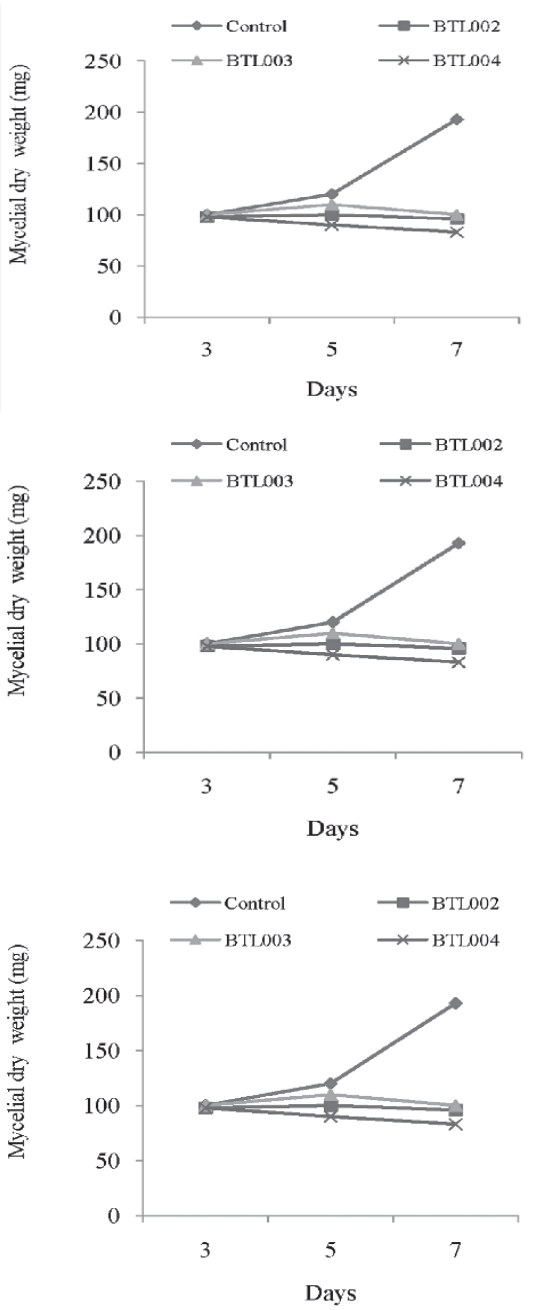

Fig.3. Time- course effects of bacterial treatment (*P. aeruginosa* strain BTL002, BTL003, *Staphylococcus* sp. strain BTL004, BTL005, *Staphylococcus* sp. strain BTL006, *Pseudomonas* sp. strain BTL007 and *Pseudomonas* sp. strain BTL008) on mycelial dry weight of *P. capsici*

dry weight from 100 mg to 96 mg, 90 mg to 83 mg, 160 mg to 150 mg, 97 mg to 93 mg, 85 mg to 76 mg and 90 mg to 73 mg, respectively (Fig. 3). *L. capsici* and *L. oryzae* also reduced mycelial dry weight from 98 mg to 93 mg and 108 mg to 103 mg, respectively (Fig. 3). The highest mycelial dry weight (193 mg) was found in untreated control followed by BTL005 (150.0 mg), *L. oryzae* Y6269 (103.0 mg), BTL003 (100.0 mg), *P. aeruginosa* strain BTL002 (96.0 mg), *L. capsici* 10.4.5 (95.0 mg) and *Staphylococcus* sp. strain BTL006 (93.0 mg). The lowest mycelial dry weight (76 mg) was recorded in *Pseudomonas* sp. BTL008. These results suggested that in broth culture, *Pseudomonas* sp. BTL008 was the most antagonistic to the *P. capsici.*

Discussion

We observed that rhizospheric antagonistic bacteria exerted morphological alterations along with inhibition zone in hyphae of *P. capsici* in dual culture assay. The morphological alterations induced by antagonistic bacteria were comparable to those induced by known antimicrobial chemical compounds (Islam and Tahara, 2001b). Induction of excessive branching in *P. capsici* on encountering *L. capsici* 10.4.6 and isolate BTL003 was apparently similar to those caused by antimicrobial compounds viscosinamide and phenazine-1-carboximide in other Peronosporomycetes and other fungi (Thrane *et al.*, 1999; Bolwerk *et al.,* 2003). Both of these compounds are secondary metabolites produced by *P. fluorescens* strains DR54 and WCS365, respectively (Chin-A-Woeng *et al.,* 1998; Nielsen et al., 1999). We also found that Methanol (MeOH) solubles obtained from culture fluid of *P. aeruginosa*

strain BTL002 and *Staphylococcus* sp. strain BTL004 induces excessive apical branching and curling and *Pseudomonas* sp. strain BTL008 induces excessive apical branching and bulbous appearance in the hyphae of *P. capsici*; this indicates the involvement of secondary metabolites produced by *P. aeruginosa* strain BTL002, *Staphylococcus* sp. strain BTL004 and *Pseudomonas* sp. BTL008 in the induction phenomenon of excessive branching. Similarly, the curly growth of *P. capsici* hyphae in dual culture with *Staphylococcus* sp. strain BTL006 and by MeOH solubles from culture fluid was similar to that observed in *A. cochlioides* AC-5 due to a biocontrol rhizoplane bacterium belonging to *Lysobacter* (Islam *et al.*, 2005) that produces xanthobaccin A (Nakayama *et al.,* 1999). Curly growth of the *Fusarium oxysporum* f. sp. *radicis-lycopersici* hyphae was also found during interaction with *Ps. fluorescens* WCS365 (Bolwerk *et al.,* 2003). The necrosis, bulbous structure formation, extensive vacuolation and varying diameter of *P. capsici* hyphae by BTL007 and *Lysobacter oryzae* Y6269 indicated they secreted antibiotics which might have similar to effects of zarilamide (Bolwerk *et al.,* 2003). It was observed that bacteria travelled about 1–2 cm towards growing hyphae within a week of incubation and densely colonized the hyphoplane, resulting in necrosis and bulbous appearance of the hyphae (Islam *et al*., 2005). The migration of the bacteria to the hyphae and local colonization suggest that chemotactic substances could be involved. However, it is not yet known whether the lethal action of *Pseudomonas* sp. strain BTL007 and *L. oryzae* Y6269 is due to toxin production or competition with the pathogen.

Stanghellini and Miller, (1997) suggested that rhamnolipids produced by *Ps. aeruginosa* can lyse zoospore of *Pythium* aphanidermatum and *P. capsici* at 5 to 30 µg/mL concentrations. Kim *et al.* (2000 a, b) also demonstrated that rhamnolipids have not only zoosporicidal activity, but also inhibit spores germination and hyphal growth of several pathogens. In our study, we found that the bacterial isolates significantly reduced the sporangia of *P. capsici*. One isolate, BTL003 reduced sporangia formation by 45% from the untreated control hyphae of *P. capsici*. Similarly, *Delftia* sp. BTL001 followed by *Staphylococcus* sp. strain BTL004, *Pseudomonas* sp. strain BTL007, *P. aeruginosa* strain BTL002, *Pseudomonas* sp. BTL008, BTL005 and *Staphylococcus* sp. strain BTL006 reduced sporangia formation by 40%, 39%, 35%, 34%, 29%, 22% and 20% respectively from the untreated control hyphae. Moreover, suspension of bacterial isolates reduced the mycelial dry weight of *P. capsici* remarkably. The mechanism of controlling was considered that rhamnolipids can insert into the cell plasma membrane and destruct the membrane structure, ultimately achieving the inhibition of pathogens (Kulkarni *et al.,* 2007). Biofungicide containing rhamnolipids was used to control zoosporic plant pathogenic fungi, including downy mildew, *Pyhtium* and *Phytophthora* sp. (Nielsen *et al.,* 2006) on a variety of crops, including tubers and vegetables, citrus fruits, ornamental plants, trees, shrubs, bedding plants and turf grasses. But till now, few studies investigated the inhibitory effects on *P. capsici*. Previous experiments demonstrated that *Pseudomonas* sp. BS1 identified can produce dirhamnolipids (Liu *et al.,* 2011). In this study, our results clearly indicated that suspension of *Pseudomonas* sp. BTL008 along with other bacterial isolates

and strains had relatively less to strong inhibitory effects on the hyphal growth of *P. capsici*, the formation of zoosporangium and the germination of zoospore, suggesting that these may inhibit the normal growth and development of pathogens by affecting *P. capsici* mycelium growth, zoosporangium formation, zoospore germination, etc. But, whether dirhamnolipids are the substance to inhibit *P. capsici* is required to in-depth study in future. In summary, the experimental results provide a theoretical basis for the use of antagonistic rhizobacteria on the biological control of Phytophthora blights, which points a new research direction for the biological control of Phytophthora blights.

Conclusion

In conclusion, our dual culture observations showed that antagonistic rhizospheric bacteria induced diverse characteristic morphological alterations in the hyphae of phytopathogenic *P. capsici*. Hyphal morphological alterations were associated with growth inhibition and the induction of characteristic morphological changes and indicated antagonistic activity against the *P. capsici*. However, it is necessary to carry out further studies on the isolation of the metabolites responsible for these changes and to understand their mode of action and their usefulness as biocontrol agents.

References

Aravind, R., A. Kumar, S.J. Eapen and K.V. Ramana. 2008. Endophytic bacterial flora in root and stem tissues of black pepper (Piper nigrum L.) genotype: isolation, identification and valuation against Phytophthora capsici. Letters in Appl. Microbiol. 48: 58-64.

Bolwerk, A., A. L. Lagopodi, A. H. M. Wijfjes, G. E. M. Lamers, T. F. C. Chin-A-Woeng, B. J. J. Lugtenberg and G. V. Bloemberg. 2003. Interactions in the tomato rhizosphere of two Pseudomonas biocontrol strains with the phytopathogenic fungus Fusarium oxysporum f. sp. radicis-lycopersici. Molecular Plant–Microbe Interactions 16: 983–993.

Chin-A-Woeng, T. F. C., G. V. Bloemberg, A. J. vander Bij, vander K. M. G. M. Drift, J. Schripsema, B. Kroon, R. J. Scheffer, C. Keel, P. A. H. M. Bakker, H. V. Tichy, F. J. deBruijn, J. E. Thomas-Oates and B. J. J. Lugtenberg. 1998. Biocontrol by phenazine-1- carboximide-producing Pseudomonas chlororaphis PCL1391 of tomato root rot caused by Fusarium oxysporum f. sp. radicis-lycopersici. Molecular Plant–Microbe Interactions 11: 1069–1077.

Hossain, M. A., and W. Friedt. 2010. Isolation and mating type determination of *Phytophthora capsici* and possibilities of its biological and chemical control. A conference on ”World Food System- A contribution from Europe”. Tropentag, September 14-16, Zurich.

Hausbeck, M. K., and H. K. Lamour. 2004. *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Disease 88(12): 1292-1302.

Islam, M. T., T. Ito and S. Tahara. 2001a. Morphological studies on zoospores of *Aphanomyces cochlioides* and changes during interaction with host materials. Journal of General Plant Pathology 67: 255–261.

Islam, M. T. and S. Tahara. 2001b. Repellent activity of estrogenic compounds toward zoospores of the phytopathogenic fungus *Aphanomyces cochlioides*. Zeitschrift fur Naturforschung C 56: 253–261.

Islam, M. T., Y. Hashidoko, A. Deora, T. Ito and S. Tahara. 2005. Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne Peronosporomycetes. Appl. and Environ. Microbiol. 71: 3786-3796.

Kulkarni, M., R. Chaudhari and A Chaudhari. 2007. Novel tension-active microbial compounds for biocontrol applications. Gen. Concepts. Integrated Pest Dis. Manage., 3: 295-304.

Kang, Z., L. Huang, U. Krieg, A. Mauler-Machnik and H. Buchenauer. 2001. Effects of tebunconazole on morphology structure, cell wall components and trichothecene production of Fusarium culmorum in vitro. Pest Management Science, 57:491–500.

Kim, B. S., J. Y. Lee and B. K. Hwang. 2000a. In vivo control and in vitro antifungal activity of rhamnolipid B, a glycolipid antibiotic, against *Phytophthora capsici* and *Colletotrichum orbiculare*. Pest Manage. Sci., 56: 1029–1035.

Kim, S. H., E. J. Lim, S. O. Lee, J. D. Lee and T. H. Lee. 2000b. Purification and characterization of biosurfactants from *Nocardia* sp. L-417. Biotechnol. Appl. Biochem., 31: 249-253.

Liu, T., J. M. Hou, S. Bi, Y. H. Zuo and J. Jing. 2011. Isolation and characterization of a biosurfactant-producing bacterium from Daqing oil-contaminated sites. Afr. J. microbial. Res., 2011, 5(21): 3509-3514.

Nielsen, C. J., D. M. Ferrin and M. E. Stanghellini. 2006. Efficacy of biosurfactants in the management of *Phytophthora capsici* on pepper in recirculating hydroponic systems. Can. J. Plant Pathol., 3: 450-460.

Nakayama, T., Y. Homma, Y. Hashidoko, J. Mizutani and S. Tahara. 1999. Possible role of xanthobaccins produced by Stenotrophomonas sp. strain SB-K88 in suppression of sugar beet damping-off disease. Appl. Environ. Microbiol. 65: 4334–4339.

Nielsen, T. H., C. Christophersen and J. AnthoniUand Sørensen. 1999. Viscosinamide a new cyclic depsipeptide with surfactant and antifungal properties produced by Pseudomonas fluorescens DR54. J. of Appl. Microbiol. 87: 80–90.

Roberts, P. D., J. R. McGovern, A. T. Kucharek and J. D. Mitchell. 2001. 10 December 2007. Vegetable diseases caused by *Phytophthora capsici* in Florida, PP-176.

Sundar, A. R., N. D. Das and D. Krishnaveni. 1995. *In-vitro* antagonism of *Trichoderma* spp. against two fungal pathogens of Castor. Indian J. Plant Protect. 23(2): 152-155.

Stanghellini, M. E. and R. M. Miller. 1997. Biosurfactants, their identity and potential efficacy in the biological control of zoosporic plant pathogens. Plant Dis., 81, 4-12.

Tian, D. and M. Tian and M. Babadoost. 2003. Host Range of *Phytophthora capsici* from Pumpkin and Pathogenicity of Isolates. Plant Disease.88(5): 485-489.

Thrane, C., S. Olsson, T. H. Nielsen and J. Sørensen. 1999. Vital fluorescent stains for detection of stress in *Pythium ultimum* and *Rhizoctonia solani* challenged with viscosinamide from *Pseudomonas fluorescens* DR54. Apple. Environ. Microbiol. 56: 908- 912.

Yin, H. 2010. Detection methods for the genus *Lysobacter* and the species *Lysobacter enzymogenes*. Dissertation and Thesis in Biological Sciences, University of Nebraska, Lincoln, USA.