Effect of oligochitosan on development of *Colletotrichum musae in vitro* and *in situ* and its role in protection of banana fruits

Meng Xiangchun[1], Tang Yanxia[1], Zhang Aiyu[1], Huang Xuemei[2], Zhang Zhaoqi[2]*

[1] Inst. Fruit Tree Res., Guangdong Acad. Agric. Sci., Guangzhou, P.R. China

[2] Coll. Hortic., S. China Agric. Univ., Guangzhou, P.R. China, zqzhang@scau.edu.cn

* *Correspondence and reprints*

Received 14 July 2011
Accepted 25 September 2011

Fruits, 2012, vol. 67, p. 147–155

DOI: 10.1051/fruits/2012008
www.fruits-journal.org

Resumen Español, *p. 155*

Effect of oligochitosan on development of *Colletotrichum musae in vitro* and *in situ* and its role in protection of banana fruits.

Abstract — Introduction. Concerns about the potentially harmful effects of fungicides on human health and the environment encourage the search for alternative treatments for perishable fruit postharvest disease control. To this end, the potential use of oligochitosan as a natural antifungal compound to control postharvest anthracnose caused by *Colletotrichum musae* was investigated in banana fruits from the Cavendish group (genome AAA). **Materials and methods**. The influence of oligochitosan on the growth of *C. musae* was determined *in vitro* by micrographic analysis, while its *in situ* antifungal activity was monitored in banana fruits that were artificially injury-inoculated with *C. musae*; the activities of several defense-related enzymes were measured. **Results and discussion**. Oligochitosan at (4 and 8) $g{\cdot}L^{-1}$ markedly inhibited radial mycelial growth of *C. musae in vitro*. The scanning electron micrograph of *C. musae* treated with oligochitosan at inhibitory concentrations showed distortion and thinning of the hyphal wall and reduction in fungus colony diameter. Dipping banana fruits in oligochitosan solution at (5 to 20) $g{\cdot}L^{-1}$ significantly reduced the diameter of the anthracnose lesion, and 20 g oligochitosan$\cdot L^{-1}$ almost reached the same inhibitory effect as 0.5 $g{\cdot}L^{-1}$ of Sportak®, a synthetic fungicide. Activities of defense-related enzymes such as phenylalanine ammonia-lyase (PAL), β-1, 3-glucanase (GLU) and chitinase (CHT), but not polyphenol oxidase (PPO), increased in banana fruits treated with 0.5 g oligochitosan$\cdot L^{-1}$. **Conclusion**. The inhibitory effect of oligochitosan on anthracnose development is due to the combination of a direct antifungal effect on the pathogen and an indirect effect, whereby the activities of defense-related enzymes in the banana fruit were enhanced. To control anthracnose in harvested bananas, treatment with oligochitosan above 20 $g{\cdot}L^{-1}$ may substitute the use of synthetic fungicide.

China / *Musa* (bananas) / postharvest losses / disease control / biological control / anthracnosis / oligochitosan / antifungal properties / induced resistance

Effet de l'oligochitosane sur le développement de *Colletotrichum musae in vitro* et *in situ* et son rôle pour la protection des bananes.

Résumé — Introduction. Les préoccupations causées par les effets nocifs potentiels des fongicides sur la santé humaine et l'environnement encouragent la recherche de traitements alternatifs pour le contrôle de maladies après-récoltes des fruits périssables. À cette fin, l'utilisation potentielle d'oligochitosane en tant que composé antifongique naturel contre l'anthracnose après-récolte causée par *Colletotrichum musae* a été étudiée pour des bananes du groupe Cavendish (génome AAA). **Matériel et méthodes**. L'influence de l'oligochitosane sur la croissance de *C. musae* a été déterminée *in vitro* par analyse micrographique, tandis que son activité antifongique *in situ* a été suivie sur des bananes qui ont été artificiellement inoculées par blessures avec *C. musae* ; les activités de plusieurs enzymes de défense ont été mesurées. **Résultats et discussion**. L'oligochitosane à (4 et 8) $g{\cdot}L^{-1}$ a nettement inhibé la croissance mycélienne radiale de *C. musae in vitro*. La micrographie électronique de *C. musae* traité avec de l'oligochitosane à des concentrations inhibitrices a montré une distorsion et un amincissement de la paroi des hyphes et la réduction de diamètre des colonies du champignon. Le trempage des bananes dans une solution d'oligochitosane (de 5 à 20) $g{\cdot}L^{-1}$ a significativement réduit le diamètre de la lésion de l'anthracnose, et un trempage à 20 g oligochitosane$\cdot L^{-1}$ a presque donné le même effet inhibiteur que 0.5 $g{\cdot}L^{-1}$ de Sportak®, fongicide de synthèse. Les activités des enzymes liés à la défense telles que la phénylalanine ammonia-lyase, la β-1, 3-glucanase et la chitinase ont augmenté dans les bananes traitées avec 0.5 g oligochitosane$\cdot L^{-1}$, mais pas celle de la polyphénol oxydase. **Conclusion**. L'effet inhibiteur de l'oligochitosane sur le développement de l'anthracnose est dû à la combinaison d'effets antifongiques directs et indirects sur le pathogène, de sorte que les activités des enzymes de défense de la banane ont été améliorées. Pour contrôler l'anthracnose dans les bananes récoltées, leur traitement par une concentration d'oligochitosane supérieure à 20 $g{\cdot}L^{-1}$ pourrait remplacer l'utilisation de fongicides de synthèse.

Chine / *Musa* (bananes) / perte après récolte / contrôle de maladies / lutte biologique / anthracnose / oligochitosane / propriété antifongique / résistance induite

1. Introduction

Being a highly perishable fruit, the banana has a short shelf life and suffers severe postharvest losses both in terms of quality and quantity. Anthracnose caused by the fungus *Colletotrichum musae* is a major cause of postharvest losses [1, 2]. The most common control method for this rot is postharvest treatment of banana fruits with synthetic fungicides, such as benomyl and thiabendazole (TBZ) [3]. However, with the development of resistance of *C. musae* to these fungicides, this strategy is losing its efficacy in the postharvest control of banana anthracnose. Besides this, concern regarding fungicide toxicity and potentially harmful effects on human health and the environment is growing, and consumers are demanding alternative treatments to reduce the use of synthetic fungicides for postharvest disease control in perishable fruits such as bananas [4].

Chitosan derived from the outer shell of crustaceans was reported as a promising alternative treatment due to its natural antifungal activity and elicitation of natural defensive responses in plant tissues [5–7]. Chitosan effectively controlled tomato gray and blue mold rot and anthracnose in artificially post-inoculated papaya and mango fruits [5, 8]. Reduced percentages of infection and index severity, caused by *R. stolonifer* inoculation, were also found on peach, papaya and tomato fruit treated with chitosan [9]. Serving as a fruit coating, it can maintain the quality and prolong the storage life of many kinds of fruits, such as banana, strawberry, grape, etc. [10–11].

However, the high viscosity and insolubility of chitosan in a neutral aqueous solution restricts its uses in practice. Oligochitosan, which was prepared by enzymatic hydrolysis of chitosan polymer, is not only water-soluble, nontoxic and biocompatible, but also has versatile functional properties [12, 13]. The fungicidal activity of oligochitosan has been proved for 12 plant pathogens, with the highest inhibition found on *Phomopsis asparagi* (sacc.a) Bubak, *Fusarium oxysporum* (Schl.) f. sp. *cucumerinum* Owen and *Rhizoctonia solani* Kuhn [14]. *In situ* studies showed that oligochitosan inhibited spore germination and mycelial growth of two fungal pathogens in pear fruit and induced activities of chitinase and β-1,3-glucanase of the fruit, indicating its elicitation of plant resistance against fungal disease other than fungicidal activity [15, 16].

Although oligochitosan is regarded as a versatile biopolymer in its applications to agriculture, its potential use as an antimicrobial or preservative compound deserves to be further explored. No information is known about the effects of oligochitosan on postharvest pathogens of banana. In this study, we evaluated the effect of oligochitosan on the development *in vitro* and *in situ* of *Colletotrichum musae*, and its elicitation of defense-related enzymes such as phenylalanine-ammonia-lyase (PAL), polyphenoloxidase (PPO), β-1,3-glucanase (GLU) and chitinase (CHT) in banana fruit.

2. Materials and methods

2.1. Inoculum preparation

Colletotrichum musae, kindly supplied by Prof. Liu, Aiyuan, South China Agricultural University, was maintained in potato dextrose agar (PDA) at 28 °C. The spores were removed from 7-day-old PDA cultures and suspended in sterile distilled water. The suspensions were filtered through three layers of muslin cloth to remove mycelial fragments and adjusted to 1×10^6 conidia·mL^{-1} with a hemacytometer according to Liu *et al.* [8].

2.2. Determination of effective concentration of oligochitosan for controlling fungal growth

Water-soluble oligochitosan with 95% deacetylation, average molecular weight of 5 kDa, low viscosity and pH value of 7.0, was kindly provided by Hainan Zhengye Zhongnong High Technology Co., Ltd. The effects of oligochitosan on mycelial growth were assayed using the method of Yao and Tian [17] with some modifications. Oligochitosan solution was mixed with molten PDA

to reach final concentrations of (1, 2, 4 and 8) $g \cdot L^{-1}$ in a total volume of 10 mL per petri plate. After the agar had solidified, 5-mm disks of *C. musae* were placed in the center of each petri plate and were incubated at 28 °C. Colony diameter was determined 7 days after treatment. Each treatment was replicated three times, ten petri plates were used in each treatment for measuring the colony diameter, and the experiment was repeated twice with similar results.

2.3. Microscopy studies

Seven-day-old fungal cultures of *C. musae* on PDA were used for scanning electron microscopy (SEM) to check the morphological changes caused by oligochitosan, according to the methods described by Alvindia and Natsuaki [18]. Segments (5 × 10) mm in size were cut from the periphery of the colony cultures and immediately placed in a vial containing 3% glutaraldehyde in 0.05 $mol \cdot L^{-1}$ phosphate buffer (pH 6.8) at 4 °C. The segments were fixed for 48 h, then dehydrated for 20 min each in an ethanol series [(30, 50, 70 and 95)%] and, finally, in absolute ethanol for 45 min. The samples were then critical-point dried in liquid carbon dioxide and then mounted on SEM stubs and electroplated with gold-palladium. The samples were viewed in a Philips FEI-XL300 SEM operated at 20 kV at 2,000× magnification.

2.4. Fruit and postharvest treatment

Fresh banana fruits (genome AAA, Cavendish group, cv. BaXi), at the green maturity stage according to the criteria of Jullien *et al.* [19], with fruit firmness of around 50 N, were harvested from a commercial orchard located in Zhongshan, Guangdong province. Banana hands in positions 2–3 from the top of each bunch were cut and divided into individual fingers. Fingers without any visual defects and of uniform size and color were randomly selected and used for this experiment.

Banana fingers were surfaced-sterilized with sodium hypochlorite (2%) for 3 min, rinsed with distilled water and air-dried at ambient temperature (25–28 °C). Air-dried fruits were then dipped for 2–3 min in different concentrations of oligochitosan [(2.5, 5, 10, 20) $g \cdot L^{-1}$] solution and again kept at ambient temperature for drying. Water and 0.5 $g \cdot L^{-1}$ of Sportak® (Bayer CropScience, Germany) dipping were used, respectively, as negative and positive controls. One day after the oligochitosan treatment, three holes of 2 mm deep and 2 mm in diameter were made on one side of each fruit, and spores of *C. musae* were inoculated by placing 10 µL of a conidial suspension (10^6 conidia$\cdot mL^{-1}$) in each of the holes. For each treatment, twenty fruits were inoculated, namely 60 inoculation replicates per treatment. After inoculation, the fruits were incubated at 22 °C and 95% relative humidity in containers wrapped by polyethylene bags with wet paper towels in the bottom of the container. Peel tissues surrounding the wounds of three fingers were sampled after (0, 3, 6, 9 and 12) days for enzyme activity determination. Ten days after inoculation, the decay diameter was measured with a precision ruler Pad. The experiment was repeated three times.

2.5. Measurement of defense-related enzyme activity

One gram of peel tissue was homogenized with 10 mL cold borate buffer (50 $mmol \cdot L^{-1}$, pH 8.8) and sodium phosphate buffer (50 $mmol \cdot L^{-1}$, pH 6.0) containing 1% (w/v) polyvinyl-polypyrrolidone (PVP) for phenylalanine-ammonia-lyase (PAL) and polyphenoloxidase (PPO) crude enzyme extraction, respectively. For β-1,3-glucanase (GLU) and chitinase (CHT) enzyme extraction, ten mL of 50 $mmol \cdot L^{-1}$ sodium acetate buffer (pH 5.0) were used. The homogenate was centrifuged at 4 °C for 15 min at 13,000 × *g* and the supernatant was used for enzyme assay.

The phenylalanine-ammonia-lyase activity was assayed according to the method by Lisker *et al.* [20] with slight modifications. The enzyme extract (1 mL) was incubated with 2 mL of borate buffer (50 $mmol \cdot L^{-1}$, pH 8.8) and 1 mL of L-phenylalanine (20 $mmol \cdot L^{-1}$) for 60 min at 37 °C. The reaction was stopped with 1 mL HCl (1 $mol \cdot L^{-1}$).

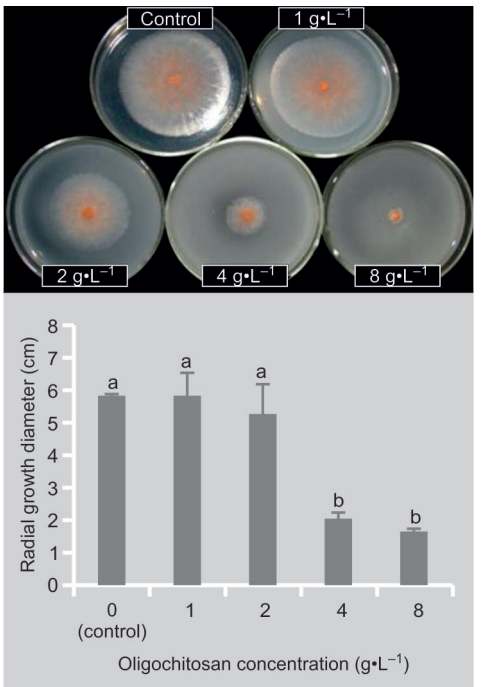

Figure 1.
In vitro culture of *Colletotrichum musae* on PDA media supplemented with different concentrations of oligochitosan and radial growth of *C. musae* mycelia. Colony diameter was measured 7 d after incubation. Bars with different letters are significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

PAL activity was determined by the production of cinnamic acid measured in a spectrophotometer at 290 nm. The blank was the crude enzyme preparation mixed with L-phenylalanine and without incubation. The enzyme activity was expressed as nmol cinnamic acid·h^{-1}·mg^{-1} protein.

The determination of the polyphenoloxidase activity was carried out by adding 0.5 mL of enzyme preparation to 3.0 mL of catechol substrate (500 mmol·L^{-1} in 50 mmol·L^{-1} sodium phosphate buffer, pH 6.0) and the increase in absorbance at 420 nm was measured immediately. The activity of PPO was expressed as U·mg^{-1} protein, where one unit corresponds to an increase of 0.001 in absorbance per min.

The β-1,3-glucanase activity was determined using 0.4% laminarin in 0.05 mol·L^{-1} sodium acetate buffer (pH 5.0) as substrate. The amount of glucose released was calculated based on a glucose standard curve. Reducing sugars were measured at 540 nm using dinitrosalicylic acid reagent. Enzyme activity was expressed as mmol glucose·min^{-1}·mg^{-1} protein.

The chitinase activity was assayed following the method of Deepak *et al.* [21] with N-acetyl glucosamine as the standard. Colloidal chitin in 0.05 M sodium acetate buffer (pH 5.0) was used as substrate. The concentration of N-acetyl glucosamine released after incubation was measured spectrophotometrically at 585 nm using dimethylaminobenzaldehyde reagent. Enzyme activity was expressed as nmol N-acetyl glucosamine·h^{-1}·mg^{-1} protein.

2.6. Data collection and analysis

The experiments were laid out in a completely randomized design with three replications per treatment. Analysis of variance was done using the DPS software and differences between means were determined using Duncan's Multiple Range Test (DMRT) at $P = 0.05$.

3. Results

3.1. Effect of oligochitosan on mycelial growth of *C. musae in vitro*

The radial mycelial growth of *C. musae* on potato dextrose agar (PDA) medium supplemented with oligochitosan was evaluated 7 days after inoculation. As compared with the control, obvious inhibition in the growth of *C. musae* was observed in the plates with oligochitosan at (4 and 8) g·L^{-1}, in which the colony diameters were markedly reduced by 65.4% and 71.6%, respectively, when compared with the diameter of the control colony (*figure 1*). No significant inhibition of mycelial growth was obtained at oligochitosan concentrations of (1 and 2) g·L^{-1}.

The effect of oligochitosan on the morphological changes of *C. musae* was examined by scanning electron micrograph (SEM) (*figure 2*). In the control, normal growth of *C. musae* mycelia on PDA was observed. The hyphae were homogeneous with smooth cell walls and clear development of conidiophore. In the treatment at 2 g oligochitosan·L^{-1}, a slightly distorted mycelium was observed. At the highest oligochitosan concentration (4 g·L^{-1}),

distortion, indentation, rupture, thinning of the hyphal cell wall and reduction of the hyphal diameter of *C. musae* were observed.

3.2. Effect of oligochitosan dipping on banana anthracnose development

Oligochitosan at 5 g·L^{-1} and above considerably reduced banana fruit lesion diameter caused by inoculation of *C. musae* (*figure 3*). The higher the concentration of oligochitosan, the smaller the lesions observed.

Oligochitosan at 20 g·L^{-1} reduced the lesion diameter on the fruit by 67.2% compared with the water control. No significant difference was obtained between 2.5 g·L^{-1} oligochitosan dipping and the control (*figure 3*). Oligochitosan at 20 g·L^{-1} showed almost the same inhibiting effect on anthracnose lesion development as Sportak®, a synthetic fungicide, at a concentration of 0.5 g·L^{-1}. Other than inhibiting the development of anthracnose, oligochitosan appeared to delay ripening, as indicated by inhibition of the fruit degreening (*figure 3*), respiration and pulp softening (data not shown).

3.3. Effect of oligochitosan on defense-related enzymes

The effect of oligochitosan treatment in delaying the anthracnose development was further investigated with respect to the activities of several defense-related enzymes in the banana peel tissue. The activity of phenylalanine-ammonia-lyase (PAL) increased dramatically during the first 3 days then decreased in both control and oligochitosan-treated fruits. Higher PAL activities were recorded in the treated fruits from the 3rd up to the 12th day of storage than in the control fruit, where up to two-fold higher activity was observed on the 9th and 12th days (*figure 4*).

No significant difference in the polyphenoloxidase (PPO) activity was observed between the treated and control fruits during the whole incubating time, except that, on day 3, lower activity was detected in the treated fruits. An increase in PPO activity from 20 U·mg^{-1} protein at 0 d to almost 40 U·mg^{-1} protein on day 6 was recorded in both treated and control fruits (*figure 4*).

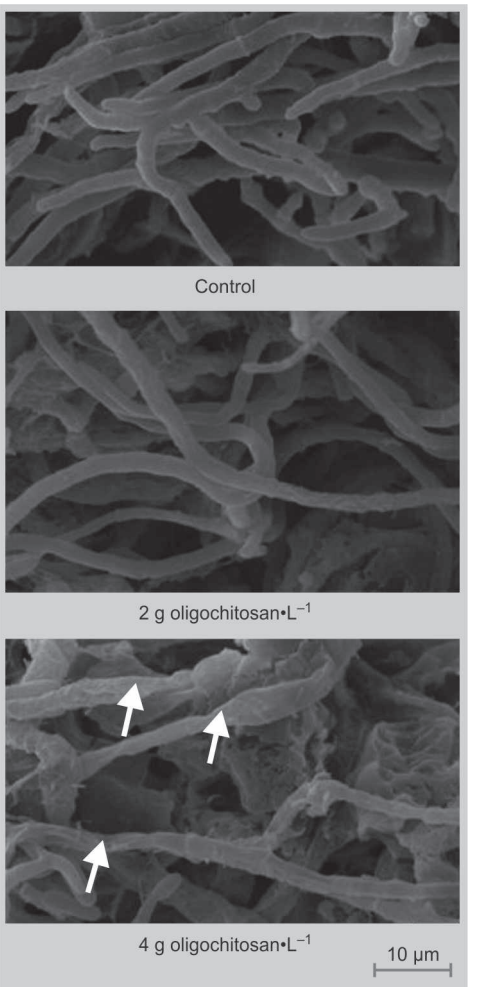

Figure 2.
Scanning electron micrograph (SEM) of *Colletotrichum musae* hyphae exposed to water control and (2 and 4) g oligochitosan·L^{-1} for 6 d at 28 °C. The samples were viewed in a Philips FEI-XL300 SEM operated at 20 kV at 2,000 × magnification. The white arrows show distortion, indentation and thinning of the hyphal wall.

A constant increase in the β-1, 3-glucanase (GLU) activity was observed in both treated and control fruits. However, the treated fruits displayed more marked enhancement in the activity, with (37, 49 and 39)% higher activity on the 6th, 9th and 12th days, respectively, than in untreated fruits. In all evaluations, a higher chitinase (CHT) activity was observed in the treated fruits, when compared with the control (*figure 4*).

4. Discussion

The growing concern regarding the potentially harmful effect of synthetic fungicides on the environment and human health

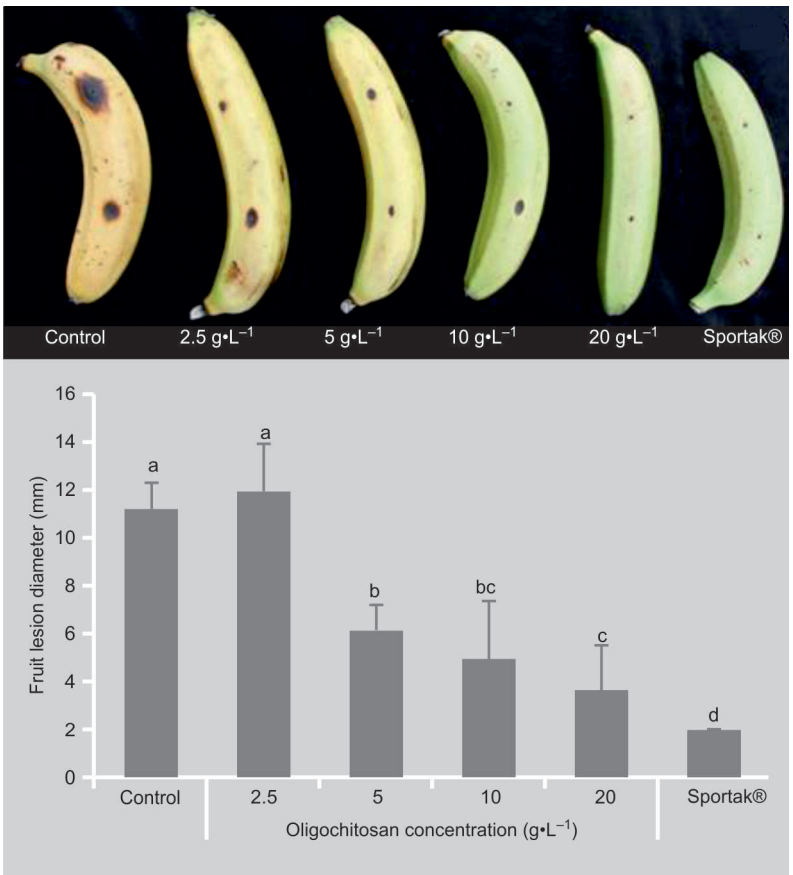

Figure 3.
Fruit lesion diameters on banana fruit treated with different concentrations of oligochitosan or with Sportak® (0.5 g·L^{-1}) and inoculated with *Colletotrichum musae* (bottom), and anthracnose symptoms on the fruit (top). Bars with different letters are significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

requires efforts to explore alternative strategies to reduce postharvest rotting of vegetables and fruits. Among the natural compounds, oligochitosan offers a great potential as a water-soluble and biodegradable substance that has antifungal activity [10, 15, 22]. Previous studies have shown that oligochitosan could induce resistance in plants against fungal diseases on vegetable crops and pear fruit [14, 22]. We studied the potential postharvest use of oligochitosan as an antifungal agent and elicitor of defense reactions to reduce anthracnose caused by *C. musae* in banana fruit.

Our results showed that oligochitosan inhibited mycelial growth of *C. musae in vitro*, and delayed anthracnose development caused by *C. musae* in bananas, validating its antifungal activity [10, 15, 22]. Meng *et al.* reported that 5 g·L^{-1} of oligochitosan or chitosan presented 100% inhibition in mycelial growth of two fungi pathogens from pear fruit, *Alternaria kikuchiana* and *Physalospora piricola in vitro*. Higher concentration was required for *in situ* control of black spot disease in pears caused by *A. kikuchiana*, and around 50% inhibition was obtained by 10 g·L^{-1} of oligochitosan treatment 96 h after inoculation [15]. In our study, a concentration of 8 g oligochitosan·L^{-1} reduced the *in vitro* mycelial growth by 71.6%, and (10 and 20) g oligochitosan·L^{-1} induced more than 50% inhibition of anthracnose caused by *C. musae in situ* 10 days after inoculation. Moreover, we observed that the oligochitosan treatment resulted in cell wall distortion and fragmentation of *C. musae*, which was expressed as reduced mycelial growth *in vitro*. These results proved the direct inhibitory effect of oligochitosan on the pathogen.

Besides its antifungal effect, the chitosan or oligochitosan treatment was also found to elicit defense responses in postharvest fruits. In tomato fruit, postharvest chitosan treatment induced a significant increase in the activities of polyphenoloxidase (PPO) and peroxidase (POD), and enhanced the content of phenolic compounds [8]. Increase in the activities of chitinase (CHI), β-1,3-glucanase (GLU) and peroxidase (POD) was reported in oligochitosan-treated pear fruit [15]. In our study, we found that the activities of phenylalanine-ammonia-lyase (PAL), β-1,3-glucanase (GLU) and chitinase (CHT) in oligochitosan-treated banana fruit increased significantly, indicating that oligochitosan is also an effective agent for inducing defense responses. PAL is involved in the biosynthesis of phytoalexins and lignins, thus contributing to the production of physical and chemical barriers against infection. The observed increase in PAL activity could have triggered the phenylpropanoid pathway, resulting in the release of toxic phytoalexins at the site of *C. musae* penetration.

Higher expression levels of hydrolases such as β-1,3-glucanase and chitinase have been shown to provide enhanced resistance to fungal pathogens. The direct effect of these enzymes on the pathogen is degradation of fungal cell wall components. The indirect effect is the release of some elicitors

from the decaying fungal cell wall that might stimulate other plant defense mechanisms such as phytoalexin accumulation in the host plants [23]. Fruits treated with oligochitosan resulted in increased levels of β-1,3-glucanase and chitinase activity following the pattern of induction observed in chitosan- or harpin-treated melon, mango and apple fruits [15, 24]. The observation in this study that oligochitosan treatment did not result in significant change in PPO activity needs further investigation.

In conclusion, the current study showed that the control of anthracnose in banana fruit with oligochitosan treatment was due to the direct inhibition of growth of *C. musae* and indirectly through enhanced activity of enzymes involved in the biosynthesis of phenolic compounds that impart disease resistance. The results suggest that oligochitosan, which is nontoxic and biocompatible, might be a promising substitute for banana anthracnose control, and might have potential in enhancing the activity of chemical fungicides currently used to control *C. musae* on bananas, allowing lower concentrations of fungicide to be used. There is a need for commercial application trials of oligochitosan, singly or integrated with fungicides or other postharvest disease control methods, to be carried out on banana anthracnose control. According to the *in situ* results presented here, above 20 $g \cdot L^{-1}$ is recommended as the commercial trial concentration of oligochitosan, because a more complicated pathogen and fruit quality or maturity background may occur in commercial use.

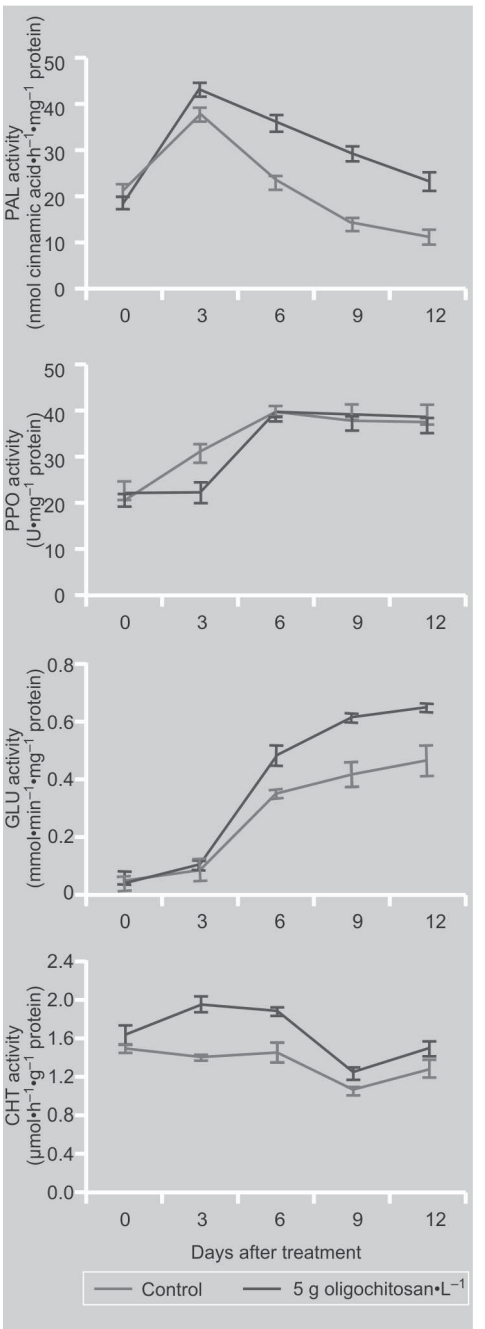

Figure 4.
Activities of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), β-1,3-glucanase (GLU) and chitinase (CHT) in banana pericarp treated with water (control) and 5 g oligochitosan·L^{-1}. Bars represent standard deviations of the means.

Acknowledgements

This research was supported by the research and demonstration of key technologies for promoting the banana industry development program (No. 2009B020201011) and the introduction and application demonstration of postharvest preservation technologies in tropical fruits program (No. 2009B050300006) of Guangdong Science and Technology Department, and the National Natural Science Foundation of China (30771515). We thank Dr. Elda B. Esguerra, from the Postharvest Horticulture Training & Research Center in the University of the Philippines Los Baños, for her help with the language modification of this manuscript.

References

[1] De Costa D.M., Erabadupitiya H.R.U.T., An integrated method to control postharvest diseases of banana using a member of the *Burkholderia cepacia* complex, Postharvest Biol. Technol. 36 (2005) 31–39.

[2] Finlay A.R., Brown A.E., The relative importance of *Colletotrichum musae* as a crown rot pathogen on Windward Island bananas, Plant Pathol. 42 (1993) 67–74.

[3] Khan S.H., Aked J., Magan N., Control of the anthracnose pathogen of banana (*Colletotrichum musae)* using antioxidants alone and in combination with thiabendazole or imazalil, Plant. Pathol. 50 (2001) 601–608.

[4] Maqbool M., Ali A., Alderson P.G., Effect of cinnamon oil on incidence of anthracnose disease and postharvest quality of bananas during storage, Int. J. Agric. Biol. 12 (2010) 516–520.

[5] Eryani A.A., Mahmud T.M.M., Syed S.R., Mohamed A.R., Eryani A.R., Effects of calcium and chitosan treatments on controlling anthracnose and postharvest quality of papaya (*Carica papaya* L.), Int. J. Agric. Res. 4 (2009) 53–68.

[6] Jitareerat P., Paumchai S., Kanlayanarat S., Sangchote S., Effect of chitosan on ripening, enzymatic activity, and disease development in mango (*Mangifera indica*) fruit, N. Z. J. Crop Hortic. Sci. 35 (2007) 211–218.

[7] Win N.K.K., Jitareerat P., Kanlayanarat S., Sangchote S., Effects of cinnamon extract, chitosan coating, hot water treatment and their combinations on crown rot disease and quality of banana fruit, Postharvest Biol. Technol. 45 (2007) 333–340.

[8] Liu J., Tian S.P., Meng X.H., Xu Y., Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit, Postharvest Biol. Technol. 4 (2007) 300–306.

[9] Han C., Zhao Y., Leonard S.W., Traber M.G., Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria × ananassa) and raspberries (*Rubus idaeus*), Postharvest Bio. Technol. 33 (2004) 67–78.

[10] Bautista-Baños S., Hernandez A.N., Valle M.G., Hernandez M., Barka E.A., Bosquez E., Wilson C.L., Chitosan as a potential natural compound to control pre and post harvest diseases of horticultural commodities, Crop Prot. 25 (2006) 108–118.

[11] Meng X., Li B., Liu J., Tian S., Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage, Food Chem. 106 (2008) 501–508.

[12] Zhao X.M., She X.P., Du Y.G., Liang X.M., Induction of antiviral resistance and stimulary effect by oligochitosan in tobacco, Pestic. Biochem. Physiol. 87 (2007) 78–84.

[13] Liu H.M., Cheng S.H., Liu J., Du Y.G., Bai Z.H., Du Y.G., Synthesis of pentasaccharide and heptasaccharide derivatives and their effects on plant growth, J. Agric. Food Chem. 56 (2008) 5634–5638.

[14] Zhang M., Tan T., Yuan H., Rui C., Insecticidal and fungicidal activities of chitosan and oligo-chitosan, J. Bioact. Compat. Polym. 18 (2003) 391–400.

[15] Meng X., Yang L., Kennedy J.F., Tian S., Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit, Carbohydr. Polym. 81 (2010) 70–75.

[16] Kim S.K., Rajapakse N., Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review, Carbohydr. Polym. 62 (2005) 357–368.

[17] Yao H.J., Tian S.P., Effects of pre- and postharvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage, Postharvest Biol. Technol. 35 (2005) 253–262.

[18] Alvindia D.G., Natsuaki K.T., Biocontrol activities of *Bacillus amyloliquefaciens* DGA14 isolated from banana fruit surface against banana crown rot-causing pathogens, Crop Prot. 28 (2009) 236–242.

[19] Jullien A., Chillet M., Malezieux E., Pre-harvest growth and development, measured as accumulated degree days, determine the post-harvest green life of banana fruit, J. Hortic. Sci. Biotechnol. 83 (2008) 506–512.

[20] Lisker N., Cohen L., Chalutz E., Fuchs Y., Fungal infections suppress ethylene-induced phenylalanine ammonia lyase activity in grapefruits, Physiol. Mol. Plant Pathol. 22 (1983) 331–338.

[21] Deepak S., Niranjan S., Shailasree S., Kini R.K., Boland W., Shetty H.S., Mithofer A., Induction of resistance against downy mildew pathogen in pearl millet by a synthetic

jasmonate analogon, Physiol. Mol. Plant Pathol. 71 (2007) 96–105.

[22] Zhao X.M., She X.P., Yu W., Liang X.M., Du Y.G., Effects of oligochitosans on tobacco cells and role of endogenous nitric oxide burst in the resistance of tobacco to tobacco mosaic virus, J. Plant Pathol. 89 (2007) 55–65.

[23] Kasprzewska A., Plant chitinases-regulation and function, Cell. Mol. Biol. Lett. 8 (2003) 809–824.

[24] Wang J., Wang B., Jiang W., Zhao Y., Quality and shelf life of mango *(Mangifera indica* L. cv. Tainong) coated by chitosan and polyphenols, Food Sci. Technol. Int. 13 (2007) 317–322.

Efecto del oligoquitosano en el desarrollo de *Colletotrichum musae in vitro* e *in situ* y su papel para la protección de las bananas.

Resumen — Introducción. Las preocupaciones que provocan los potenciales efectos nocivos de los fungicidas en la salud humana y en el medioambiente fomentan que se investigue en tratamientos alternativos para el control de enfermedades posteriores a la cosecha de los frutos perecederos. Con este fin, se estudió para las bananas del grupo Cavendish (genoma AAA) el uso potencial de oligoquitosano, como compuesto antifúngico natural contra la antracnosis posterior a la cosecha, causada por *Colletotrichum musae*. **Material y métodos**. Se determinó *in vitro*, por análisis micrográfico, la influencia del oligoquitosano en el crecimiento de *C. musae*, mientras se hizo el seguimiento *in situ* de su actividad antifúngica en bananas inoculadas artificialmente por heridas con *C. musae*. Se midieron las actividades de varias enzimas de defensa. **Resultados y discusión**. El oligoquitosano de (4 y 8) $g{\cdot}L^{-1}$ inhibió considerablemente el crecimiento micelial radial de *C. musae in vitro*. La micrografía electrónica de *C. musae* tratado con oligoquitosano en concentraciones inhibidoras mostró una distorsión y un adelgazamiento de la pared de las hifas y la reducción de diámetro de las colonias del hongo. La inmersión de las bananas en una solución de oligoquitosano (de 5 a 20) $g{\cdot}L^{-1}$ redujo significativamente el diámetro de la lesión de la antracnosis, y una inmersión con 20 g oligoquitosano$\cdot L^{-1}$ ofreció casi el mismo efecto inhibidor que 0.5 $g{\cdot}L^{-1}$ de Sportak®, fongicida de síntesis. Las actividades de las enzimas relacionadas con la defensa, tales como la fenilanalina amonio-liasa, la β-1, 3-glucanasa y la quitinasa aumentaron en las bananas tratadas con 0.5 g oligoquitosano$\cdot L^{-1}$, mientras que la de la polifenol oxidasa no lo hizo. **Conclusión**. El efecto inhibidor del oligoquitosano en el desarrollo de la antracnosis es debido a la combinación de efectos antifúngicos directos e indirectos en el patógeno, de modo que las actividades de las enzimas de defensa de la banana se han mejorado. Para controlar la antracnosis en las bananas cosechadas, el uso de fungicidas de síntesis podría ser reemplazado por un tratamiento con una concentración de oligoquitosano superior a 20 $g{\cdot}L^{-1}$.

China / *Musa* (bananos) / pérdidas postcosecha / control de enfermedades / control biológico / antracnosis / oligoquitosano / propiedades antimicosicas / resistencia inducida