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Antibacterial activity of chitosan solution against *Xanthomonas* pathogenic bacteria isolated from *Euphorbia pulcherrima*

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Abstract

The antibacterial activity of chitosan solution against *Xanthomonas* pathogenic bacteria isolated from *Euphorbia pulcherrima* was investigated in this study. Results showed that chitosan solution at 0.10 mg/mL markedly inhibited the growth of *Xanthomonas* pathogenic bacteria from different geographical origins. The antibacterial activity of chitosan solution against the selected strain R22580 of *Xanthomonas axonopodis* pv. *poinsettiicola* increased with the increase of chitosan concentration up to 0.10 mg/mL. The antibacterial activity of chitosan solution was also enhanced by the addition of NaCl, but was unaffected by the type of nutrient and sterilization. In addition, chitosan solution exhibited strong antibacterial activity against strain R22580 over the pH range of 5.5–7.0. Overall, the results indicated that chitosan was a potential bactericide against bacterial pathogens of *E. pulcherrima* under different environmental conditions.

Keywords: Antibacterial activity; Chitosan; *Euphorbia pulcherrima*; *Xanthomonas*

1. Introduction

Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin, a major component of the shells of crustacea such as crab, shrimp, and crawfish. In recent years, applications of chitosan to the fields of medicine, food, chemical engineering, pharmaceuticals, nutrition, environmental protection and agriculture have received considerable attention (Chung, Wang, Chen, & Li, 2003; Devlieghere, Vermeulen, & Debevere, 2004; Li et al., 2007). Applications of chitosan in environmental protection and agriculture include its use as a biocontrol agent for controlling plant disease (Atia, Buchenauer, Aly, & Abou-Zaid, 2005; Khan, Fischer, Egan, & Doohan, 2006).

Chitosan has been reported to reduce the infection of *Fusarium oxysporum* f. sp. *apii* in celery (Bell, Hubbard, & Li, 1998) and inhibit the spread of *Sphaerotheca pannosa* var. *rosae*, *Peronospora sparsa* and *Botrytis cinerea* on roses (Ben-Shalom & Fallik, 2003; Wojdyła, 2001). Treating tomato plants with chitosan solution reduced mycelial growth, sporangial production, release of zoospores and germination of cysts of *Phytophthora infestans* which resulted in significant disease protection (Atia et al., 2005). In addition, chitosan seed treatment could reduce *Colletotrichum* sp. infection and improve performance of chili seedling (Photchanachai, Singkaew, & Thamthong, 2006).

Xanthomonas axonopodis pv. *poinsettiicola* or *Xanthomonas arboricola* pv. *poinsettiicola* is the causal agent of bacterial leaf spot of *Euphorbia pulcherrima*. This pathogen has been worldwide identified (Li, Xie, & Swings, 2005, 2006; Li, Xie, Zhang, Janssens, & Swings, 2006). In practice, control of *Xanthomonas* leaf spot of *E. pulcherrima* is very difficult, and is almost entirely dependent on the

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elimination of all infected plants. The bactericidal activity of chitosan has been reported in bacterial diseases in human (Simunek, Tishchenko, Hodrova, & Baptonova, 2006). However, few investigation has been generated about chitosan activity on bacterial diseases in plants.

The objective of this research was to evaluate the antibacterial activity of chitosan solution prepared under different environments against *Xanthomonas* bacterial strains of *E. pulcherrima* collected from different geographic sources.

2. Materials and methods

2.1. Preparation of chitosan stock

Chitosan (practical grade, from crab shells) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Chitosan was dissolved in 0.04 N HCl (5 mg/mL). After stirring (300 rpm) for 12 h at room temperature, the solution was then alkalized to pH 5.6 with 2 N KOH (Zuppini et al., 2003) and autoclaved at 121 °C for 20 min. Sterile deionized water of pH 5.6 was used as a control.

2.2. Bacterial strains

To obtain inocula for the examination, nine strains of *Xanthomonas* that cause leaf spot disease of *E. pulcherrima* were collected from India, New Zealand and China (Table 1). All bacterial strains involved in this study were deposited in both the culture collection of the Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium (LMG) and the Institute of Biotechnology, Zhejiang University, China. The bacterial strains were cultured for 48 h on nutrient agar medium (Schaad, Jones, & Chun, 2001) at 28 °C. After incubation, each bacterial suspension was prepared in sterilized water, and the initial concentration of bacteria was adjusted to approximately 10^8 CFU/mL.

2.3. Counting surviving cells

Bacterial suspensions were 10-fold serially diluted and 10 μL samples were inoculated on nutrient agar medium in triplicate for each dilution and were incubated for 48 h at 28 °C. After incubation, the surviving cells on the agar were counted based on the colony forming units and then the mean value of the cells at the lowest dilution was calculated. Each experiment was carried out in duplicate and was replicated twice.

2.4. Effect of chitosan against Xanthomonas sp. pathogenic to E. pulcherrima from different geographical sources

Chitosan solution at 0.10 mg/mL was prepared by adding 100 μL chitosan stock (pH 5.6) to 4.9 mL nutrient broth. Bacterial solution was added to 5 mL of chitosan solution at 0.10 mg/mL to give a final bacterial concentration of 10^7 CFU/mL and then the mixture was incubated at 28 °C on a rotary shaker (Hualida Company, Taicang, China) at 160 rpm. In the control treatment chitosan stock was replaced with sterile deionized water of pH 5.6 in order to obtain the same pH. Six hours later, samples were collected from each cell suspension and bacterial counting was followed as indicated above.

2.5. Effect of chitosan against strain R22580 of X. axonopodis pv. poinsettiicola under different environments

Strain R22580 of *X. axonopodis* pv. *poinsettiicola* was selected and inoculated into chitosan solution prepared under different environments to give a final bacterial concentration of 10^7 CFU/mL and then the mixture was incubated at 28 °C on a rotary shaker at 160 rpm. In the control treatment chitosan stock was replaced with sterile deionized water of pH 5.6 in order to obtain the same pH. Six hours later unless otherwise stated, samples were collected from each cell suspension and bacterial counting was followed as indicated above.

2.5.1. Effect of chitosan concentration and incubation time on strain R22580

Chitosan solutions of 5 mL in volume were prepared by adding chitosan stock to nutrient broth to give a final chitosan concentration of 0.05, 0.10, 0.15 and 0.20 mg/

Table 1
Effect of chitosan solution on the growth of *Xanthomonas* sp. pathogenic to *Euphorbia pulcherrima* from different geographical sources

Strains number[a]	Species identity	Strain sources	Cell numbers ($\log_{10}$ CFU/mL)[c]	
			Control	Chitosan[b]
LMG849	*X. axonopodis* pv. *poinsettiicola*	India	9.77 ± 0.12	4.26 ± 0.14
LMG5401	*X. axonopodis* pv. *poinsettiicola*	India	10.02 ± 0.24	6.16 ± 0.16
LMG5402	*X. arboricola* pv. *poinsettiicola*	New Zealand	10.71 ± 0.03	4.32 ± 0.16
LMG5403	*X. arboricola* pv. *poinsettiicola*	New Zealand	10.61 ± 0.04	5.36 ± 0.06
LMG8675	*X. arboricola* pv. *poinsettiicola*	New Zealand	10.39 ± 0.06	5.59 ± 0.15
LMG8676	*X. arboricola* pv. *poinsettiicola*	New Zealand	10.64 ± 0.01	4.82 ± 0.06
R22578	*X. axonopodis* pv. *poinsettiicola*	China	9.59 ± 0.06	5.20 ± 0.10
R22579	*X. axonopodis* pv. *poinsettiicola*	China	9.98 ± 0.03	4.10 ± 0.04
R22580	*X. axonopodis* pv. *poinsettiicola*	China	9.30 ± 0.17	3.64 ± 0.10

[a] Initial concentration of bacteria is 10^7 CFU/mL; the surviving cells in chitosan solution were counted after 6 h of incubation.
[b] Concentration of chitosan solution is 0.10 mg/mL.
[c] The data were shown as means ± standard error from a representative experiment repeated twice with similar results.

mL. Antibacterial activity of chitosan solution at the concentration of 0.20 mg/mL on the growth of strain R22580 was also determined after 2 and 4 h of incubation.

2.5.2. Effect of pH on the antibacterial activity of chitosan

Chitosan solutions of 5 mL in volume were prepared by adding 200 μL chitosan stock to 4.8 mL nutrient broth with different pH values to give a final chitosan concentration of 0.20 mg/mL. The pH of nutrient broth was adjusted to 5.5, 6.0, 6.5, 7.0 and 7.5 with a pH meter (Mettler Toledo 320, Halstead, UK) by adding dilute HCl or NaOH aqueous solution. The final pH of the chitosan solution was similar to that of nutrient broth.

2.5.3. Effect of autoclave sterilization on the antibacterial activity of chitosan

In order to explain the possible interaction between chitosan and nutrient broth at high temperature, chitosan solutions of 5 mL in volume were prepared by adding 200 μL chitosan stock to 4.8 mL nutrient broth before and after sterilization to give a final chitosan concentration of 0.20 mg/mL.

2.5.4. Effect of type of nutrient on the antibacterial activity of chitosan

In order to clarify the nutritional factor in the antibacterial activity of chitosan solution, chitosan solutions of 5 mL in volume were prepared by adding 200 μL chitosan stock to 4.8 mL growth broths with different nutrient compositions to give a final chitosan concentration of 0.20 mg/mL. Three growth broths including yeast extract-nutrient broth (YNB), yeast salts broth (YSB) and nutrient broth (NB) were prepared according to the method of (Schaad et al., 2001). In addition, NB was prepared at 10%, 50% and full strength concentrations.

2.5.5. Effect of inoculation time on the antibacterial activity of chitosan

The time sensitivity assay was determined according to the method of Liu et al. (2006) by adding chitosan at different stages of bacterial culture such as lag phase (0 h), initial log phase (14 h), middle log phase (18 h) and final log phase (22 h). Chitosan solutions of 5 mL in volume were prepared by adding 200 μL chitosan stock to 4.8 mL bacterial culture to give a final chitosan concentration of 0.20 mg/mL. Bacterial counting was followed as indicated above except that the incubation time was reduced to 2 h.

2.5.6. Effect of NaCl on the antibacterial activity of chitosan

Chitosan solutions of 5 mL in volume were prepared by adding 50 μL chitosan stock to 4.95 mL nutrient broth with different concentration of NaCl to give a final chitosan concentration of 0.05 mg/mL. The NaCl concentration in the nutrient broth was adjusted to 0.0%, 0.5% or 2.0%, respectively, by adding a NaCl aqueous solution.

2.6. Statistical analysis

The software STATGRAPHICS Plus, version 4.0 (Copyright Manugistics Inc., Rockville, MD, USA) was used to perform the statistical analysis. Levels of significance ($P < 0.05$) of main treatments and their interactions were calculated by analysis of variance after testing for normality and variance homogeneity.

3. Results and discussion

3.1. Effect of chitosan against Xanthomonas sp. pathogenic to E. pulcherrima from different geographical sources

The antibacterial activity of chitosan solution against nine strains of *X. arboricola* pv. *poinsettiicola* and *X. axonopodis* pv. *poinsettiicola* from different geographic sources is shown in Table 1. The surviving cell numbers in the chitosan solution decreased more than 3.86 $\log_{10}$ CFU/mL compared to the control after 6 h of incubation regardless of the bacterial strain, which implies that chitosan is a good bactericide. To the best of our knowledge, this is the first report about antibacterial activities of chitosan on plant pathogenic bacteria, which showed chitosan have a potential against bacterial leaf disease of *E. pulcherrima*.

Most studies on the mode of action of chitosan have been conducted with fungal pathogens, and little is known about its action on bacteria (El Ghaouth et al., 1994). Several studies have indicated that the interactions between positively charged chitosan molecules and negatively charged residues on the bacterial cell surface play an important role in the inhibitory effect of chitosan on Gram-negative bacteria (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Sudarshan, Hoover, & Knorr, 1992). In addition, it has been suggested that reduction of cell numbers is caused by cell surface alterations and loss of barrier functions (Helander et al., 2001).

3.2. Effect of chitosan concentration and incubation time on strain R22580

Since the chitosan solution at 0.10 mg/mL showed antibacterial activity against all *Xanthomonas* strains tested, we explored the effect of chitosan concentration on the antibacterial activity against strain R22580 of *X. axonopodis* pv. *poinsettiicola*. As shown in Fig. 1, chitosan at four different concentrations showed effective antibacterial activity against the strain R22580 compared to the control after 6 h of incubation. Chitosan solutions up to 0.10 mg/mL showed stronger antibacterial activity compared with the remainder treatment, which is consistent with the result of Liu et al. (2006), who found that the antibacterial activity of chitosan was influenced by its concentration in the solution. The surviving cell numbers in chitosan solution of 0.05 mg/mL decreased 2.59 $\log_{10}$ CFU/mL, while the surviving cell numbers in chitosan solution of 0.10 mg/mL decreased 4.46 $\log_{10}$ CFU/mL compared to the control.

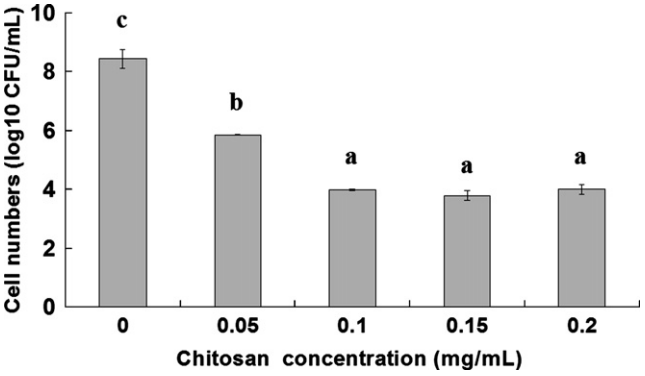

Fig. 1. Effect of chitosan concentration on the antibacterial activity of strain R22580 of *X. axonopodis* pv. *poinsettiicola*. Columns with the same letters are not significantly different ($P < 0.05$). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results.

Concentrations higher than 0.10 mg/mL were not significantly different.

The effect of the incubation time on the antibacterial activity of chitosan against strain R22580 is shown in Fig. 2. The results of the present study showed that the antibacterial activity of the chitosan solution of 0.20 mg/mL increased with the incubation time within 6 h. After 2 h of incubation, the surviving cell numbers in the chitosan solution decreased 1.32 $\log_{10}$ CFU/mL compared to the control. However, the surviving cell numbers in the chitosan solution decreased 5.00 $\log_{10}$ CFU/mL compared to the control after 6 h of incubation, which showed a certain incubation time is required for the chitosan solution to inhibit the bacterial growth. In addition, the antibacterial activity of chitosan solution was unaffected by sterilization (data not shown).

3.3. Effect of pH on the antibacterial activity of chitosan against strain R22580

The effects of pH on the inhibition of strain R22580 by the chitosan solution of 0.20 mg/mL are shown in Table 2.

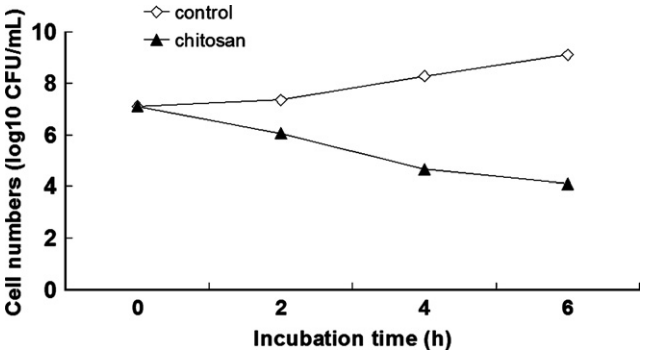

Fig. 2. Effect of incubation time on the antibacterial activity of chitosan at 0.20 mg/mL against strain R22580 of *X. axonopodis* pv. *poinsettiicola*. Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results.

Table 2
Effect of pH on the antibacterial activity of chitosan solution against strain R22580 of *X. axonopodis* pv. *poinsettiicola* [a]

pH	Cell numbers ($\log_{10}$ CFU/mL)[c]	
	Control	Chitosan[b]
5.5	9.97 ± 0.07	5.46 ± 0.09
6.0	9.92 ± 0.08	5.10 ± 0.10
6.5	10.10 ± 0.09	4.30 ± 0.17
7.0	10.06 ± 0.56	4.68 ± 0.08
7.5	10.12 ± 0.04	8.16 ± 0.05

[a] Initial concentration of bacteria is 10^7 CFU/mL; the surviving cells in chitosan solution were counted after 6 h of incubation.
[b] Concentration of chitosan solution is 0.20 mg/mL.
[c] The data were shown as means ± standard error from a representative experiment repeated twice with similar results.

In the absence of chitosan, there was no difference in bacterial growth over the pH range of 5.5–7.5. The addition of chitosan inhibited the bacterial growth compared to the control. However, the antibacterial activity of the chitosan solution was reduced when the pH is higher than 7.0. At or below pH 7.0, the surviving cell numbers in the chitosan solution decreased more than 4.50 $\log_{10}$ CFU/mL, while the surviving cell numbers decreased only 1.96 $\log_{10}$ CFU/mL at pH 7.5 compared to the control, which is a little different from the result of Chung et al. (2003), who found that the inhibition percentage of chitosan solution decreased with increasing pH and dropped dramatically when the pH was greater than 6.0.

Chitosan with positive charges easily reacts with negatively charged bacteria and further inhibits bacterial growth. Surface interference may be the possible mechanism for the bactericidal properties (Helander et al., 2001). In addition, the antibacterial action of chitosan has been associated with the pH, with higher activity at lower pH value (Fujimoto, Tsuchiya, Terao, Nakamura, & Yamamoto, 2006; No, Park, Lee, & Meyers, 2002). Since chitosan does not carry positive charges at alkaline pH, which may explain the surviving cell numbers in a chitosan solution of pH 7.5 why suddenly increased to 8.16 $\log_{10}$ CFU/mL in this study.

3.4. Effect of type of nutrient on antibacterial activity of chitosan against strain R22580

The effect of growth broths on the antibacterial activity of chitosan against strain R22580 is shown in Fig. 3. In the absence of chitosan, the bacterial population was significantly higher in NB than in YNB and YSB. In the presence of chitosan, there was no significant difference in bacterial population among YNB, YSB and NB. In addition, the surviving cell numbers decreased 4.08 $\log_{10}$ CFU/mL in YNB, decreased 3.79 $\log_{10}$ CFU/mL in YSB and decreased 5.30 $\log_{10}$ CFU/mL in NB compared to the control.

The effect of NB and its dilutions on the antibacterial activity of chitosan against strain R22580 is shown in Fig. 4. In the absence of chitosan, the bacterial population

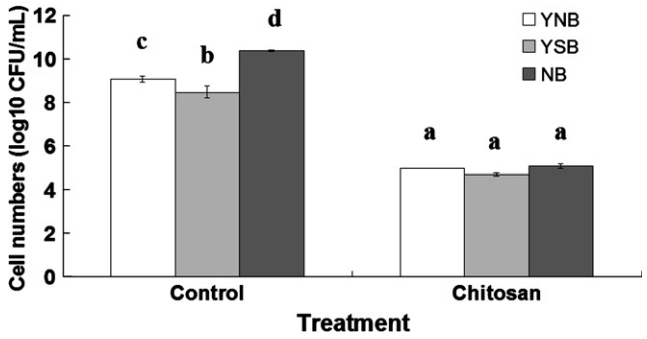

Fig. 3. Effect of type of nutrient on the antibacterial activity of chitosan at 0.20 mg/mL against strain R22580 of *X. axonopodis* pv. *poinsettiicola*. Columns with the same letters are not significantly different ($P < 0.05$). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results.

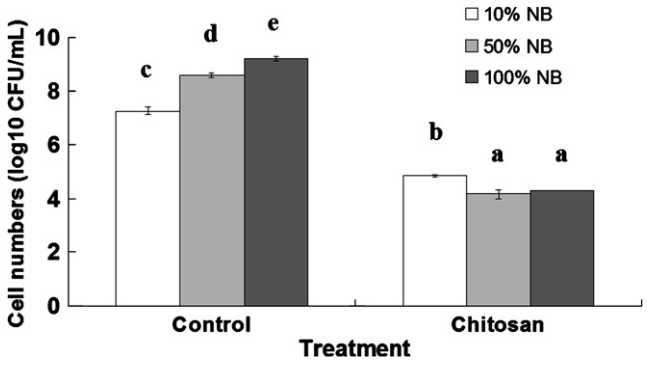

Fig. 4. Effect of strength of nutrient broth on the antibacterial activity of chitosan at 0.20 mg/mL against strain R22580 of *X. axonopodis* pv. *poinsettiicola*. Columns with the same letters are not significantly different ($P < 0.05$). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results.

increased with the increase in concentration of NB. In the presence of chitosan, bacterial growth was significantly inhibited compared to the control. However, the surviving cell numbers decreased 2.42 $\log_{10}$ CFU/mL in 10% strength NB, decreased 4.42 $\log_{10}$ CFU/mL in 50% strength NB and decreased 4.90 $\log_{10}$ CFU/mL in full strength NB compared to the control. The antibacterial activity of chitosan increased with the increase in concentration of NB may be attributed to the increase in the ionic strength of the solution, which contained NaCl. A higher ionic strength may enhance the solubility of chitosan and thus increases its antibacterial activity (Chung et al., 2003).

3.5. Effect of inoculation time on antibacterial activity of chitosan against strain R22580

Bacteria in different growth stages have different sensitivity to chitosan. With different inoculation time the effects were different. The time sensitivity of strain R22580 to chitosan at a concentration of 0.20 mg/mL is shown in Fig. 5. The results of this study indicated that bacterial growth was significantly inhibited by chitosan compared

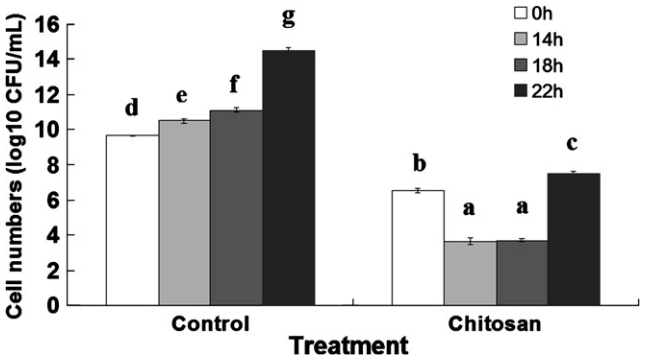

Fig. 5. Effect of inoculation time on the antibacterial activity of chitosan at 0.20 mg/mL against strain R22580 of *X. axonopodis* pv. *poinsettiicola*. Columns with the same letters are not significantly different ($P < 0.05$). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results.

to the control although chitosan was added to the bacterial culture at a different time. In addition, chitosan solution showed a stronger antibacterial activity against the strain R22580 at initial log phase (14 h) and middle log phase (18 h) of bacterial growth, which is consistent with the result of Liu et al. (2006), who found that the growth of *Escherichia coli* was markedly inhibited when chitosan was added at log phase of bacterial growth.

3.6. Effect of NaCl concentrations on antibacterial activity of chitosan against strain R22580

Effect of NaCl on antibacterial activity of chitosan is shown in Fig. 6. In absence of chitosan, bacterial growth in NB was unaffected by NaCl. In the presence of chitosan, bacterial growth was significantly inhibited compared to the control. In addition, the antibacterial activity of chitosan solution at 0.05 mg/mL was enhanced by NaCl. The surviving cell numbers decreased 1.52 $\log_{10}$ CFU/mL when no NaCl was added, while the surviving cell numbers decreased 2.34 $\log_{10}$ CFU/mL at 0.5% NaCl concentration and decreased 2.13 $\log_{10}$ CFU/mL at 2.0% NaCl concen-

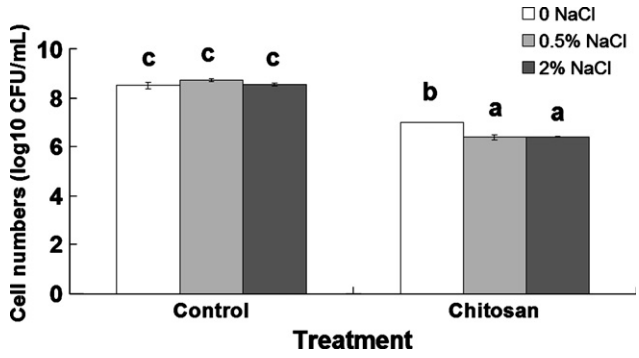

Fig. 6. Effect of NaCl concentrations on the antibacterial activity of chitosan at 0.05 mg/mL against strain R22580 of *X. axonopodis* pv. *poinsettiicola*. Columns with the same letters are not significantly different ($P < 0.05$). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results.

tration, respectively, compared to the control. Chung et al. (2003) had showed that the antibacterial activity of chitosan increased with the ionic strength of the solution. However, in our study, there was no significant difference in the antibacterial activity of chitosan when the concentration of NaCl was higher than 0.5%.

4. Conclusion

Our data demonstrated that the chitosan solution markedly inhibited the growth of *Xanthomonas* sp. pathogenic to *E. pulcherrima* from different geographical origins. Furthermore, chitosan showed antibacterial activity against strain R22580 of *X. axonopodis* pv. *poinsettiicola* under various environmental conditions. This is the first study that showed chitosan was a potential bactericide against plant pathogenic bacteria although further studies are required to evaluate the effect of chitosan solution in controlling leaf spot of *E. pulcherrima* caused by these bacteria in nursery.

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References

Atia, M. M. M., Buchenauer, H., Aly, A. Z., & Abou-Zaid, M. I. (2005). Antifungal activity of chitosan against *Phytophthora infestans* and activation of defence mechanisms in tomato to late blight. *Biological Agriculture & Horticulture, 23*, 175–197.

Bell, A. A., Hubbard, J. C., & Li, L. (1998). Effects of chitin and chitosan on the incidence and severity of Fusarium yellows of celery. *Plant Disease, 82*, 322–328.

Ben-Shalom, N., & Fallik, E. (2003). Further suppression of *Botrytis cinerea* disease in cucumber seedlings by chitosan–copper complex as compared with chitosan alone. *Phytoparasitica, 31*, 99–102.

Chung, Y. C., Wang, H. L., Chen, Y. M., & Li, S. L. (2003). Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. *Bioresource Technology, 88*, 179–184.

Devlieghere, F., Vermeulen, A., & Debevere, J. (2004). Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21*, 703–714.

El Ghaouth, A., Arul, J., Grenier, J., Benhamou, N., Asselin, A., & Bélanger, R. (1994). Effect of chitosan on cucumber plants: suppression of *Pythium aphanidermatum* and induction of defense reactions. *Phytopathology, 84*, 313–320.

Fujimoto, T., Tsuchiya, Y., Terao, M., Nakamura, N., & Yamamoto, M. (2006). Antibacterial effects of chitosan solution® against *Legionella pneumophila*, *Escherichia coli*, and *Staphylococcus aureus*. *International Journal of Food Microbiology, 112*, 96–101.

Helander, I. M., Nurmiaho-Lassila, E. L., Ahvenainen, R., Rhoades, J., & Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria. *International Journal of Food Microbiology, 71*, 235–244.

Khan, M. R., Fischer, S., Egan, D., & Doohan, F. M. (2006). Biological control of Fusarium seedling blight disease of wheat and barley. *Phytopathology, 96*, 386–394.

Li, B., Xie, G. L., & Swings, J. (2005). Differentiation of xanthomonads causing the bacterial leaf spot of poinsettia in China from the pathotype strain of *Xanthomonas axonopodis* pv. *poinsettiicola*. *Journal of Zhejiang University Science, 6B*, 451–453.

Li, B., Xie, G. L., & Swings, J. (2006). First report of leaf spot caused by *Xanthomonas campestris* on poinsettia in China. *Plant Pathology, 55*, 293.

Li, B., Xie, G. L., Zhang, J. Z., Janssens, D., & Swings, J. (2006). Identification of the bacterial leaf spot pathogen of poinsettia in China. *Journal of Phytopathology, 154*, 711–715.

Li, Y., Chen, X. G., Liu, N., Liu, C. S., Liu, C. G., Meng, X. H., et al. (2007). Physicochemical characterization and antibacterial property of chitosan acetates. *Carbohydrate Polymers, 67*, 227–232.

Liu, N., Chen, X. G., Park, H. J., Liu, C. G., Liu, C. S., Meng, X. H., et al. (2006). Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. *Carbohydrate Polymers, 64*, 60–65.

No, H. K., Park, N. Y., Lee, S. H., & Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology, 74*, 65–72.

Photchanachai, S., Singkaew, J., & Thamthong, J. (2006). Effects of chitosan seed treatment on *Colletotrichum* sp. and seedling growth of chili cv. "Jinda". *Acta Horticulturae, 712*, 585–590.

Schaad, N. W., Jones, J. B., & Chun, W. (2001). *Laborats* (3rd ed.). St Paul, MN: American Phytopathological Society.

Simunek, J., Tishchenko, G., Hodrova, B., & Baptonova, H. (2006). Effect of chitosan on the growth of human colonic bacteria. *Folia Microbiologica, 51*, 306–308.

Sudarshan, N. R., Hoover, D. G., & Knorr, D. (1992). Antibacterial action of chitosan. *Food Biotechnology, 6*, 257–272.

Wojdyła, A. T. (2001). Chitosan in the control of rose diseases – 6 year – trials. *Bulletin of the Polish Academy of Science: Biological Sciences, 49*, 243–252.

Zuppini, A., Baldan, B., Millioni, R., Favaron, F., Navazio, L., & Mariani, P. (2003). Chitosan induces Ca^{2+}-mediated programmed cell death in soybean cells. *New Phytologist, 161*, 557–568.