EFFECTS OF *PAENIBACILLUS* STRAINS AND CHITOSAN ON PLANT GROWTH PROMOTION AND CONTROL OF RALSTONIA WILT IN TOMATO

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SUMMARY

The effects of chitosan and sixteen *Paenibacillus* strains against the wilt pathogen *Ralstonia solanacearum* were evaluated *in vitro* and under greenhouse conditions. Chitosan and two *Paenibacillus* strains, in particular *Paenibacillus polymyxa* MB02-1007, were found to have strong *in vitro* antibacterial activities against *R. solanacearum.* In addition, chitosan applied as soil drench or seed treatment significantly reduced wilt incidence by 72% and 48%, respectively while *P. polymyxa* MB02-1007 as a soil drench or a seed treatment significantly reduced wilt incidence by 82% and 88%. In general, regardless of the application method, plant growth parameters as well as the activities of chitinase and β-1,3-glucanase in tomato plants were significantly increased by chitosan and *P. polymyxa* MB02-1007 as compared to the corresponding control, both in the absence and presence of *R. solanacearum*. The growth of tomatoes, however, was promoted by chitosan more as a soil drench than as a seed treatment, while *P. polymyxa* MB02-1007 as a seed treatment was more effective than as a soil drench. This is the first report on the use of chitosan for the control of tomato wilt and promotion of tomato plants. In conclusion, both chitosan and *P. polymyxa* MB02-1007 show promise for plant growth promotion and control of *R. solanacearum* in tomato. Combining chitosan and *P. polymyxa* may improve disease control, which should be examined in future studies.

Key words: *Ralstonia solanacearum,* chitosan, *Paenibacillus*, tomato, antibiosis, induced resistance.

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INTRODUCTION

Ralstonia solanacearum (Yabuuchi *et al*., 1995), causing bacterial wilt is an important soil-borne pathogen with a worldwide distribution and a host range exceeding 200 species in 50 families (Algam *et al.,* 2004; Aliye *et al*., 2008). However, bacterial wilt control approaches such as field sanitation, crop rotation, and application of resistant varieties, have shown limited success (Guo *et al*., 2004). Bacterial wilt cannot be managed with pesticides. Thus, alternative disease measures are needed (El-Mougy *et al*., 2004). Biological control involving microbial agents or biochemicals offer an eco-friendly and cost-effective alternative as an important component of an integrated disease management program (Li *et al*., 2007, 2008).

Several potential biocontrol agents against bacterial wilt of tomato have been reported (Guo *et al*., 2004; Lemessa and Zeller, 2007; Xue *et al*., 2009). However, one of the most important problems in biocontrol using microbial products is the storage time of living microbes. Interestingly, it is well known that bacteria of the genus *Paenibacillus* produce endospores that can stay dormant for extended periods. In addition, some strains of *Paenibacillus* have been successfully used to control soil-borne pathogens such as fungi and nematodes (Yang *et al*., 2004; Ryu *et al*., 2006; Khan *et al*., 2008; Timmusk *et al*., 2009). However, limited information is available on the biocontrol traits of bacteria of the genus *Paenibacillus* against bacterial wilt of tomato.

In addition to antagonistic bacteria, some biochemicals, chitosan in particular, have also been employed to control tomato diseases alone or in combination with antagonistic bacteria (Benhamou *et al*., 1998; Atia *et al*., 2005). For example, treating tomato plants with chitosan 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, Benhamou *et al.* (1998) found that the increased resistance of bacterized tomato roots to *Fusarium* infection can be triggered by specific alterations in the physiology of the host plant due to the effect exerted by chitosan.

The objective of this study was to examine the effect

of chitosan and bacteria of the genus *Paenibacillus* on plant growth promotion and control of bacterial wilt in tomato.

MATERIALS AND METHODS

Preparation of chitosan. Chitosan extract from crab shells (degree of N-deacetylation 75%; Sigma-Aldrich, USA) was solubilized in 1% acetic acid to obtain a concentration of 10 mg ml^{-1}. The solution was alkalized to pH 5.6 with 1 N NaOH and autoclaved at 121°C for 20 min (Li *et al.,* 2008). Aqueous acetic acid (1%) alkalized with 1 N NaOH pH 5.6 served as control.

Source of *R. solanacearum.* A virulent strain of *R. solanacearum* Rs-f.91 (race 1/bivar 3/phylotype 1) identified by fatty acid methyl ester analysis using the Sherlock system (MIDI, USA) whose pathogenicity on tomato plants had been confirmed in pre-experiments was deposited in the culture collections of Fujian Academic Agriculture Sciences, China and the Institute of Biotechnology, Zhejiang University, China. The pathogenic bacterium was routinely cultured on casamino acids peptone glucose (CPG) agar (Smith *et al*., 1995) and on tetrazolium chloride (TTC) agar (Kelman, 1954) at 28°C for 48 h and temporarily stored in sterile distilled water at room condition.

Source of *Paenibacillus* strains. Seven strains of *Paenibacillus polymyxa* (MB02-226, MB02-376, MB02-428, MB02-1007, MB02-1172, MB02-1202 and MB02-1265) and nine strains of *Paenibacillus macerans* (MB02-167, MB02-429, MB02-513, MB02-523, MB02-454, MB02-727, MB02-992, MB02-1125 and MB02-1180) were provided by Department of Integrated Pest Management, Faculty of Agricultural Science, Aarhus University, Denmark. All sixteen *Paenibacillus* strains were isolated from either mycorrhizal or non-mycorrhizal systems and identified by fatty acid methyl ester analysis using the Sherlock system (Mansfeld-Giese *et al*., 2002). *Paenibacillus* strains were cultured on nutrient agar (NA) supplemented with 5% sucrose and stored in 20% aqueous glycerol at -80°C for further studies.

Antibacterial activity of chitosan. The effect of chitosan against the growth of *R. solanacearum* Rs-f.91 was evaluated applying it to agar wells or paper disks. *R. solanacearum* was grown overnight in CPG broth at 30°C, 1 ml of bacterial suspension was adjusted to an optical density of OD_{600}=1.00 (approximately equal to 1.0×10^9 CFU ml^{-1}) using a spectrophotometer, was added to 15 ml of melted CPG agar in a Petri dish and allowed to solidify. For the agar well method, four holes per plate were made using a sterile cork borer of 10 mm diameter and 50 μl of chitosan solution at 10 mg ml^{-1} was added to each hole. In the paper disk test, autoclaved 5 mm diameter filter paper disks (Whatman No 1, Whatman International, UK) were dipped in chitosan solution at 10 mg ml^{-1} and then four disks were placed on each plate. Negative controls were done with alkalized acetic acid in the same way. The diameter of the zone of inhibition was measured after incubating the plates for 48 h at 30°C. Each treatment had four replicates, and the experiment was performed twice.

***In vitro* screening of antagonistic bacteria.** The antagonistic activity of sixteen *Paenibacillus* strains against *R. solanacearum* Rs-f.91 was evaluated using the dual-culture method described by Berg *et al.* (2001) with some modifications. *R. solanacearum* was applied to CPG agar as described above. Sterile toothpicks were used to transfer the test strains of *Paenibacillus* from 2-day-old cultures onto the surface of the solidified bacterial lawn. Three plates were used for each strain tested and three test spots were placed on each plate, the plates were incubated at 30°C for 48 h and possible inhibition zone was observed after 2 days. The experiment was performed twice.

Greenhouse experiment. *Tomato seeds and plant growth conditions.* The effect of chitosan and the *Paenibacillus* strain with the largest inhibition zone on bacterial wilt of tomato was evaluated by using soil drench or seed treatment methods. Tomato seeds (cv. Hezou), obtained from Horticulture Department, Zhejiang University, China, were surface sterilized with 2% sodium hypochlorite for 2 min and washed several times with sterilized water. Pre-germinated seeds were sown in pots (20 diameter × 20 height) containing unsterilized natural vegetable soil (clay-sandy soil, pH 6.6). The experiment was carried out in January-June 2009 in Hangzhou, China. Plants were maintained in a temperature-controlled glasshouse with Osram daylight lamps providing supplementary light for a 12 h photoperiod, about 70-80% humidity and 28±2°C. The pots were arranged in a randomized block design with four replicates and 5 plants per pot. The experiment was conducted twice.

Seed treatment and soil drench. Seed treatment was carried out by soaking seeds either in a chitosan solution at 10 mg ml^{-1} for 2 h or in 10 ml of antagonistic bacterial suspension for 8 h, left to dry overnight in a flow cabinet, then placed onto sterile filter paper moistened with sterile distilled water and incubated at room temperature for 5 days. Cells of the antagonistic bacteria from the plate culture were washed twice in sterile distilled water and then resuspended in sterile distilled water. The cell suspension was adjusted to an optical density of OD_{600}=1.00 (approximately equal to $1.0x10^9$ CFU ml^{-1}) using a spectrophotometer. Seeds in the con-

trol treatment of either chitosan or antagonistic bacteria were immersed in alkalized acetic acid and sterile distilled water, respectively. Soil drenching was carried out by pouring 50 ml of either chitosan solution at 10 mg ml^{-1} or antagonistic bacterial suspension (approximately equal to 1.0×10^9 CFU ml^{-1}) into each pot 3 days before sowing pre-germinated seeds. Plants treated with either sterile distilled water or alkalized acetic acid served as controls for both treatments.

Inoculation of R. solanacearum. R. solanacearum Rs-f.91 was grown for 24 h in CPG broth at 30°C on a rotary shaker (130 rpm per minute) and harvested by centrifugation for 5 min at 10,000 rpm. Cell pellets were diluted in sterile distilled water to give a final concentration of 10^9 CFU ml^{-1} using a spectrophotometer. Two weeks after sowing pre-germinated seeds, one half of the pots (5 plants per pot) were inoculated with 50 ml of *R. solanacearum* while the other half received 50 ml of sterile water only. After inoculation plants were covered with plastic bags for 24 h to maintain high humidity.

Disease assessment. Disease severity was recorded from 2 to 8 weeks after inoculation at weekly intervals based on a scale of 0 to 4 as described by Kempe and Sequeira (1983). Wilt incidence was calculated by the following formula (Song *et al*., 2004):

Wilt incidence (%) = {(scale × number of plants infected)/(highest scale × total number of plants)} ×100

Biocontrol efficacy (%) = {(Disease incidence of control - Disease incidence of antagonist-treated group) / Disease incidence of control} × 100.

Plant growth promotion. Plant growth was measured in terms of shoot height and shoot fresh and dry weight 1-2 month after sowing both in the absence and presence of *R. solanacearum.* For dry weight measurement, plants were dried in an oven at 60°C for 3 days and weights evaluated for each treatment.

The relative growth promotion efficacy (GPE) by either chitosan or antagonistic bactteria was calculated as follows:

GPE (%) = {(Plant parameter of antagonist-treated group - Plant parameter of control) / Plant parameter of control} ×100

Assay of chitinase. Tomato leaves of 3-5 cm in length were collected seven days after inoculation of *R. solanacearum* and 1 g fresh sample was ground to powder in liquid nitrogen with a prechilled pestle and mortar, and then homogenized in 5 ml sodium citrate buffer (pH 5.0) at 0.1 mol l^{-1}. The homogenate was then centrifuged for 10 min at 10,000 rpm at 4°C and the supernatant used for enzyme assay. Colloidal chitin was prepared from crab shell chitin (Sigma, USA) according to Berger and Reynolds (1958). The commercial lyophilized snail gut enzyme (Sepracor, France) was desalted as described by Boller and Mauch (1988). For the colorimetric assay of chitinase 10 µl of 1 mol l^{-1} sodium acetate buffer (pH 4.0), 0.4 ml of enzyme extract and 0.1 ml colloidal chitin (1 mg) were pipetted into a 1.5 ml eppendorf tube.

After 2 h at 37°C, the reaction was stopped by centrifugation at 1,000 rpm for 3 min. An aliquot of the supernatant (0.3 ml) was pipetted into a glass reagent tube containing 30 µl of 1 mol l^{-1} potassium phosphate buffer (pH 7.1) and incubated with 20 µl desalted snail gut enzyme for 1 hr. The resulting monomeric N-acetylglucosamine (GlcNAc) was determined according to Reissig *et al.* (1955) using internal standards of GlcNAc in the assay mixtures for calculations. Enzyme activity was expressed as mM GlcNAc equivalents $min^{-1}g^{-1}$ fresh weight. Each treatment had four replicates of 3 plants per replicate.

Assay of β*-1,3-glucanase.* Tomato leaves were collected as described above and 1 g fresh sample were extracted with liquid nitrogen and homogenized in 5 ml of 0.05 M sodium acetate buffer (pH 5.0). The extract was then centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used in the enzyme assay. The reaction mixture consisted of 62.5 µl of laminarin (Sigma, USA) solution (20 mg ml^{-1}) and 62.5 µl of enzyme extract. The reaction was carried out at 40°C for 10 min. The reaction was then stopped by adding 375 µl of dinitrosalicylic reagent and heating for 5 min in a boiling water bath. The resulting colored solution was diluted with 4.5 ml of distilled water, vortexed and its absorbance at 500 nm was determined. Enzyme activity was expressed as mmol $min^{-1}g^{-1}$ fresh weight as described by Pan *et al.* (1991). Each treatment had four replicates of three plants per replicate.

Statistical analysis. All variables measured were subjected to one-way ANOVA. Duncan's multiple range tests was applied when one-way ANOVA revealed significant differences ($P < 0.05$). All statistical analysis was performed with SPSS BASE ver.16 statistical software (SPSS, USA).

RESULTS

***In vitro* screening of antagonistic bacteria.** Two of 16 *Paenibacillus* strains showed inhibitory *in vitro* effects on the growth of *R. solanacearum* Rs-f.91. The inhibition zone of *P. polymyxa* MB02-1007 against *R. solanacearum* Rs-f.91 was 13.0 mm while that of *P. macerans* MB02-992 was 4.0 mm (Fig. 1). Thus, *P. polymyxa* MB02-1007,

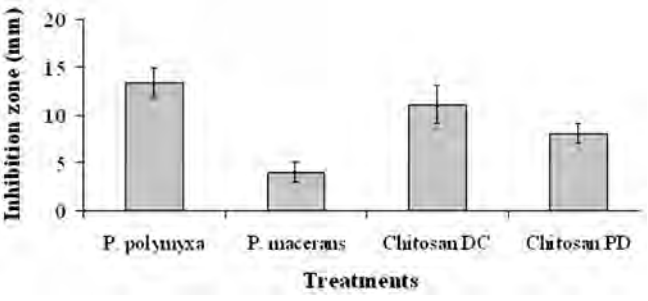

Fig. 1. *In vitro* effect of chitosan and *Paenibacillus polymyxa* MB02-1007 on the growth of *Ralstonia solanacearum* Rs-f.91 (race 1/bivar 3/phylotype 1). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results. DC = Dual Culture, PD = Paper Disc.

that gave the largest inhibition zone, was selected for further studies. None of the other 14 *Paenibacillus* strains reduced the growth of *R. solanacearum* Rs-f.91.

Antibacterial activity of chitosan. The chitosan solution at 10 mg ml^{-1} showed strong *in vitro* antibacterial activity against *R. solanacearum* Rs-f.91, regardless of the test method adopted. In the paper disk method, the zone of inhibition was 8.0 mm, whereas in agar well tests, it was 11.0 mm (Fig. 1).

Biocontrol of bacterial tomato wilt in greenhouse. Both chitosan and *P. polymyxa* MB02-1007 reduced wilt incidence of tomato plants compared to the corresponding control regardless of the application method in the presence of *R. solanacearum* (Table 1). There was no significant difference on wilt incidence between controls (alkalized acetic acid and sterile distilled water), regardless of the application method (Table 1). In general, wilt incidence was reduced more by *P. polymyxa* MB02-1007 than by chitosan. Chitosan solution (10 mg ml^{-1}) applied as a soil drench or seed treatment significantly reduced disease incidence by 72% and 48%, respectively, compared to the control (alkalized acetic acid) (Table 1). Similarly, *P. polymyxa* MB02-1007 applied as a soil drench or seed treatment significantly reduced disease incidence by 82% and 88%, respectively, compared to the control (sterile distilled water) (Table 1). In addition, the biocontrol efficacy of chitosan was increased when applied as a soil drench as compared with seed treatment. However, the biocontrol efficacy of *P. polymyxa* MB02-1007 was unaffected by the application method. Non-inoculated control tomato plants did not show wilt symptoms.

Tomato growth promotion. In the absence of *R. solanacearum*, application of chitosan and *P. polymyxa* MB02-1007 resulted in plant growth promotion compared to the corresponding non-treated control, regardless of the application method (Table 2). There was no significant difference in plant parameters between control treatments with alkalized acetic acid and sterile distilled water, regardless of the application method (Table 2). Chitosan applied as a soil drench or seed treatment significantly increased plant height by 56% and 38%, plant fresh weight by 101% and 91% and plant dry weight by 86% and 66% as compared to the control (alkalized acetic acid) (Table 2). Similarly, *P. polymyxa* MB02-1007 as a soil drench or seed treatment significantly increased plant height by 44% and 63%, plant fresh weight by 84% and 116%, and plant dry weight by 66% and 108% compared to the control (distilled water) (Table 2).

In the presence of *R. solanacearum*, application of chitosan and *P. polymyxa* MB02-1007 resulted in plant growth promotion compared to the corresponding control regardless of the application method (Table 2). Chi-

Table 1. Effect of chitosan and *Paenibacillus polymyxa* MB02-1007 on wilt incidence in tomato plants inoculated with *Ralstonia solanacearum* Rs-f.91 (race 1/bivar 3/phylotype 1) under greenhouse conditions.

Treatments	Wilt incidence (%)[a]	Biocontrol efficacy (%)
Rs + Chitosan SD	17.55 ± 5.72 [ab]	71.99 [c]
Rs + Chitosan ST	33.33 ± 7.70 [b]	48.01 [b]
Rs + *P. polymyxa* SD	13.33 ± 3.85 [a]	81.82 [d]
Rs + *P. polymyxa* ST	8.88 ± 2.22 [a]	87.89 [d]
Positive control		
Rs + Alkalized acetic acid SD	62.66 ± 4.98 [c]	0.00 [a]
Rs + Alkalized acetic acid ST	64.11 ± 4.11 [c]	0.00 [a]
Rs + Distilled water	73.32 ± 6.67 [c]	0.00 [a]

[a]Disease incidence was scored at 21, 35 and 50 days after inoculation of *R. solanacearum*.
[b]Data are presented as means ± standard error of three readings from a representative experiment repeated twice with similar results. Means in a column followed by the same letter are not significantly different (P<0.05). Tomato plants uninoculated with *R. solanacearum* were free of symptoms and not included in statistical analysis. Rs = *Ralstonia solanacearum*, SD = Soil Drench, ST = Seed Treatment.

tosan solution (10 mg ml^{-1}) applied as a soil drench or seed treatment significantly increased plant height by 68% and 39%, plant fresh weight by 98% and 82%, and plant dry weight by 76% and 55% compared to the control (alkalized acetic acid) (Table 2). Similarly, *P. polymyxa* MB02-1007 as a soil drench or seed treatment significantly increased plant height by 54% and 79%, plant fresh weight by 76% and 103%, and plant dry weight by 56% and 84% compared to the control (distilled water) (Table 2).

Activity of chitinase. The chitinase activity of tomato plants was increased by both chitosan and the antagonistic bacteria regardless of the application method both in the presence and absence of *R. solanacearum* (Fig. 2). Chitosan as a soil drench or seed treatment increased chitinase activity by 13% and 11%, respectively, while *P. polymyxa* MB02-1007 as a soil drench or seed treatment increased chitinase activity by 10% and 11%, respectively, as compared to the corresponding control in the absence of *R. solanacearum* (Fig. 2a). Chitosan as a soil drench or seed treatment increased chitinase activity by 96% and 62%, respectively, while *P. polymyxa* MB02-1007 as a soil drench or seed treatment increased chitinase activity by 68% and 78%, respectively, compared to the corresponding control in the presence of *R. solanacearum* (Fig. 2b).

Activity of β-1.3-glucanase. Chitosan as a soil drench or a seed treatment increased β-1,3-glucanase activity by

Table 2. Effect of chitosan and *Paenibacillus polymyxa* MB02-1007 on height, fresh weight and dry weight of tomato plants under greenhouse conditions.

Treatments	Height (cm)	GPE (%)	Fresh weight (g)	GPE (%)	Dry weight (g)	GPE (%)
-R. solanacearum						
Chitosan SD	36.33 ± 0.69 ga	55.72 d	28.47 ± 0.32 d	100.92 ef	5.70 ± 0.20 f	85.67 e
Chitosan ST	29.33 ± 1.15 de	37.51 b	27.20 ± 0.67 d	91.15 d	5.57 ± 0.41 ef	66.27 c
P. polymyxa SD	34.66 ± 0.51 ef	44.07 c	26.63 ± 0.70 d	84.04 c	5.17 ± 0.27 e	65.71 c
P. polymyxa ST	36.99 ± 0.51 g	63.17 e	31.27 ± 0.38 d	116.10 g	6.50 ± 0.17 g	108.33 f
Negative control						
Alkalized acetic acid SD	23.33 ± 1.20 c	0.00 a	14.17 ± 0.50 b	0.00 a	3.07 ± 0.09 ab	0.00 a
Alkalized acetic acid ST	21.33 ± 0.33 abc	0.00 a	14.23 ± 0.67 b	0.00 a	3.35 ± 0.20 b	0.00 a
Distilled water	22.67 ± 1.20 bc	0.00 a	14.47 ± 0.61 b	0.00 a	3.12 ± 0.44 ab	0.00 a
+R. solanacearum						
Chitosan SD	30.77 ± 1.44 e	67.87 e	22.60 ± 0.59 c	97.73 e	4.88 ± 0.30 d	76.17d
Chitosan ST	27.89 ± 0.44 d	39.45 b	20.37 ± 0.78 c	81.88 bc	4.23 ± 0.47 c	54.95 b
P. polymyxa SD	28.89 ± 1.79 d	53.92 d	20.47 ± 0.43 c	76.01 b	4.20 ± 0.07 c	55.55 b
P. polymyxa ST	33.55 ± 0.73 f	78.74 f	23.63 ± 0.65 c	103.18 ef	4.98 ± 0.07 d	84.44 e
Positive control						
Alkalized acetic acid SD	18.33 ± 0.88 a	0.00 a	11.43 ± 1.13 a	0.00 a	2.77 ± 0.03 a	0.00 a
Alkalized acetic acid ST	20.00 ± 0.58 ab	0.00 a	11.20 ± 0.65 a	0.00 a	2.73 ± 0.11 a	0.00 a
Distilled water	18.77 ± 0.78 a	0.00 a	11.63 ± 0.78 a	0.00 a	2.70 ± 0.32 a	0.00 a

[a]The data were shown as means ± standard error from a representative experiment repeated twice with similar results. Means in a column followed by the same letter are not significantly different ($P<0.05$). GPE = Growth Promotion Efficacy, SD = Soil Drench, ST = Seed Treatment.

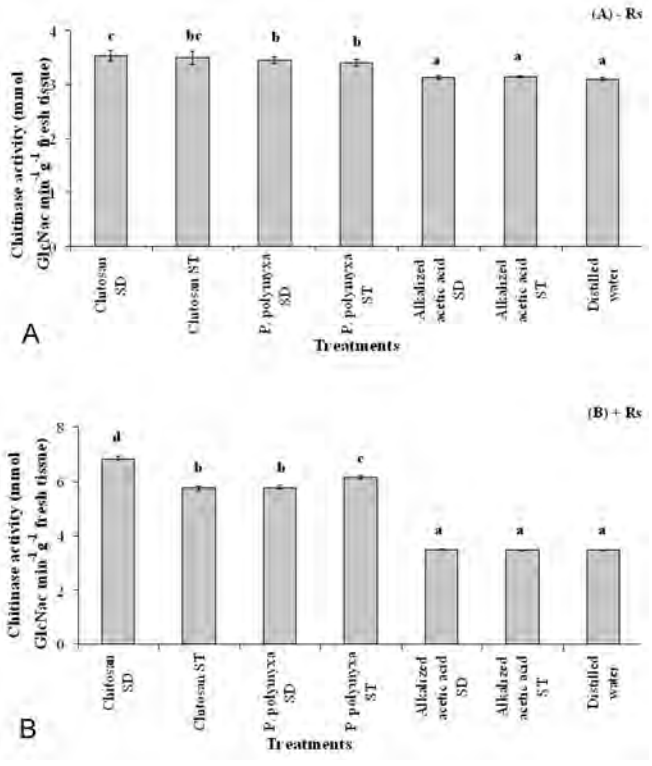

Fig. 2. Effect of chitosan and *Paenibacillus polymyxa* MB02-1007 on chitinase activity of tomato plants in the absence (a) or presence (b) of *Ralstonia solanacearum* Rs-f.91 (race 1/bivar 3/phylotype 1). Columns with the same letters are not significantly different (P≤0.05). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results. Rs = *Ralstonia solanacearum*, SD = Soil Drench, ST = Seed treatment.

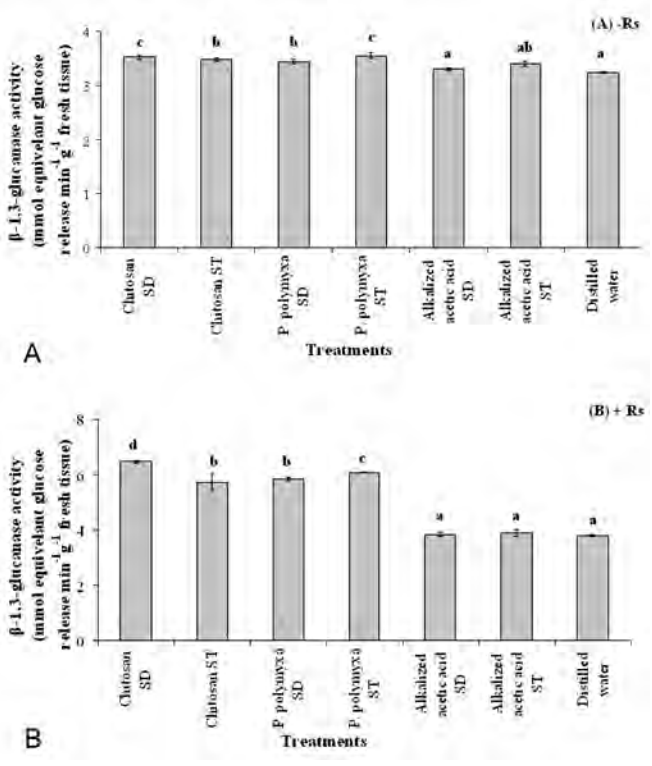

Fig. 3. Effect of chitosan and *Paenibacillus polymyxa* MB02-1007 on β-1,3-glucanases activity of tomato plants in the absence (a) or presence (b) of *Ralstonia solanacearum* Rs-f.91 (race 1/bivar 3/phylotype 1). Columns with the same letters are not significantly different (P≤0.05). Error bars represent the standard error of the mean. Data are from a representative experiment repeated twice with similar results. Rs = *Ralstonia solanacearum*, SD = Soil Drench, ST = Seed treatment.

7% and 2%, respectively, while *P. polymyxa* MB02-1007 as a soil drench or seed treatment increased β-1,3-glucanase activity by 10% and 14%, respectively, as compared to the corresponding control in the absence of *R. solanacearum* (Fig. 3a). Chitosan as a soil drench or seed treatment increased β-1,3-glucanase activity by 68% and 47%, respectively, while *P. polymyxa* MB02-1007 as a soil drench or seed treatment increased β-1,3-glucanase activity by 54% and 56%, respectively, compared to the corresponding control in the presence of *R. solanacearum* (Fig. 3b).

DISCUSSION

In the present study, chitosan and *P. polymyxa* MB02-1007 showed strong antagonistic *in vitro* effects against *R. solanacearum* and when applied as a seed coat or a soil drench in greenhouse pot experiments both reduced tomato wilt incidence and promoted plant growth. In particular, chitosan as soil drench was more effective than seed treatment and *P. polymyxa* was more effective as seed treatment than as soil drench. To our knowledge, this is the first report on the potential use of chitosan and *P. polymyxa* to control bacterial wilt of tomato.

In general, plant defense reactions in terms of chitinase and β-1,3-glucanase activity were also increased by both chitosan and *P. polymyxa* MB02-1007, suggesting that, apart from the direct antagonistic effect of chitosan and *P. polymyxa*, induced resistance may also be involved in the mode of action of these control measures against bacterial wilt in tomato.

Chitosan had a strong antibacterial activity against *R. solanacearum*, which is in agreement with the result of Li *et al.* (2008, 2009), who found that chitosan could inhibit the growth of *Xanthomonas axonopodis* pv. *poinsettiicola* strains under different environments. Several studies have shown 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 *et al*., 2001). Furthermore, Chung and Chen (2008) found that the inactivation of *Escherichia coli* by chitosan occurs via a two-step sequential mechanism: initial separation of the cell wall from its cell membrane, followed by destruction of the cell membrane.

Only two of the sixteen *Paenibacillus* strains included in the present study inhibited the *in vitro* growth of *R. solanacearum*, which is partially consistent with the result of Li *et al.* (2007), who found that the *in vitro* growth of *Pythium* was unaffected by the same sixteen *Paenibacillus* strains.

The plant growth promoting effects of chitosan observed in the present study are consistent with the result of Hilal *et al.* (2006), who found that chitosan was able to enhance the growth of many crops. The underlying mechanisms for this plant growth promoting action may be attributed to effects on plant physiological processes such as nutrient uptake, cell elongation, cell division, enzymatic activation and protein synthesis (Amin *et al.*, 2007). Like chitosan, also *P. polymyxa* MB02-1007 promoted tomato growth, in agreement with studies by Khan *et al.*, (2008) and Lal and Tabacchioni (2009), who reported that *P. polymyxa* enhanced growth of many different plants. This may be attributed to the fact that *P. polymyxa* can synthesize plant hormones (auxin and cytokinin), solubilize soil phosphorus and enhance soil porosity (Raza *et al.*, 2008).

In our study, chitosan and *P. polymyxa* MB02-1007 increased the activity of chitinase and β-1,3-glucanase, both of which are pathogenesis-related proteins (Van Loon, 1997), thus likely playing a role in the suppression of bacterial wilt. These findings agree with those of studies showing that chitosan treatment can cause induced resistance and increase enzyme activities in many plants (Abd-El-Kareem, 2002). In particular, Lafontaine and Benhamou (1996) reported that pretreatment with chitosan enhanced the resistance response of tomato seedlings by restricting pathogen growth to the outer root tissues and eliciting a number of defense reactions including structural barriers. Significant increase in the activity of plant defense enzymes including β-1,3-glucanase and chitinase were recorded in treated roots of peanut inoculated with *P. polymyxa* (Haggag, 2007). Thus, induction of systemic resistance in plants with either antagonistic bacteria or natural compounds, in particular chitosan, is a promising approach to disease control (Gozzo, 2003).

Overall, our study clearly demonstrates that bacterial wilt of tomato plants can be inhibited by either chitosan or *P. polymyxa* MB02-1007 irrespective of the mode of application. However, growth promotion of tomato plants was more pronounced when chitosan was applied as a soil drench than as a seed treatment, while *P. polymyxa* MB02-1007 was more effective as a seed treatment than as a soil drench.

In conclusion, both chitosan and *P. polymyxa* MB02-1007 show promise for plant growth promotion and control of *R. solanacearum* in tomato. Their combination may improve disease control, which should be examined in future studies.

ACKNOWLEDGEMENTS

This project was supported by 863 Project (2006AA10A211), Science Foundation of Chinese University (KYJD09022), Zhejiang Provincial Science and Technology Projects of China (2008C22072), and Zhejiang Provincial Natural Science Foundation of China (Y3090150).

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Received November 4, 2009
Accepted March 11, 2010