Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biological Control 29 (2004) 66–72
Biological Control
www.elsevier.com/locate/ybcon

# Biocontrol of tomato wilt by plant growth-promoting rhizobacteria

Jian-Hua Guo,[a,*] Hong-Ying Qi,[b] Ya-Hui Guo,[c] Hong-Lian Ge,[a] Long-Ying Gong,[a] Li-Xin Zhang,[d] and Ping-Hua Sun[d]

[a] *Plant Pathology Department, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China*
[b] *Huaian Modern Horticulture Technology Centre, Jiangsu province 223003, China*
[c] *Food Department, Handan Agricultural College, Hebei province 057150, China*
[d] *Agricultural Technology Station, Qixia district, Nanjing 210038, China*

Received 6 August 2002; accepted 12 May 2003

**Abstract**

In greenhouse experiments, three strains of plant growth-promoting rhizobacteria (PGPR), *Serratia* sp. J2, fluorescent pseudomonad J3, and *Bacillus* sp. BB11, were evaluated for biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum*. A total of four field trials were conducted, each in a different location in Jiangsu and the Hebei provinces. Strains J2, J3, and BB11 provided disease control and increased yield. In trial one in Qixia (1999), disease was reduced 66.1, 73.6, and 68.4% by J2, J3, and BB11, respectively, compared to the control. Yield increases with bacteria in this trial ranged from 49.5 to 70.8%. In trial two in Huaian (2000), disease was reduced 78.1, 94.1, and 86.9% by J2, J3, and BB11, respectively. Yield increases ranged from 180 to 237%. In trial three in Handan (1999), biocontrol efficiencies of 71.3, 63.6, and 78.2% were achieved by J2, J3, and BB11, respectively. Yield increases ranged from 53.5 to 76.2%. In trial 4 in Handan (2000), disease was reduced 74.4, 75.1, and 81.9% by J2, J3, and BB11, respectively, compared to the control. Yield increases with these bacteria ranged from 46.3 to 78.5%. Additional tests were conducted with 1-year-old and 2-year-old formulations of each PGPR strain. Populations of PGPR in the 1-year-old formulations were nearly identical to freshly prepared PGPR formulations. Biological control efficacy was retained by the stored formulations of all three PGPR strains, with 1-year-old formulations providing 68.4–99.5% control and 2-year-old formulations providing 63.4–78.5% control. Yield increases with stored formulations ranged from 35.4 to 67.0%. We conclude that our method of formulating PGPR products provides stable formulations that retain biological control and plant growth-promoting activities.


*Keywords:* *Bacillus* sp.; *Serratia* sp.; *Pseudomonas* sp.; *Ralstonia solanacearum*; Bacterial wilt; *Lycoperscion esculentum*; Biological control; PGPR strains; Conservation; Living bacteria; Population

## 1. Introduction

*Ralstonia solanacearum* is an important soilborne bacterial phytopathogen with a worldwide distribution and a large host range of more than 200 species in 50 families (Hayward, 1995). Some of its economically important hosts include tomato, pepper, potato, tobacco, banana, cowpea, peanut, cashew, papaya, and olive. Survival of *R. solanacearum* is affected by asymptomatic hosts (Swanepoel, 1992). Various control strategies, including host–plant resistance (Dalal et al., 1999), transgenic resistant plant (Jia et al., 1999), cropping systems (Dalal et al., 1999), soil amendments (Vincent and Mew, 1998), integrated control (Katayama and Kimura, 1987), and biological control, have been developed. Potential biological agents used to control bacterial wilt of tomato (*Lycopersicon esculentum*) include vesicular–arbuscular mycorrhizae (VAM) (Halos and Zorilla, 1979), avirulent mutants of *R. solanacearum* (Dong et al., 1999), genetically engineered antagonistic bacteria (Kang et al., 1995), and some naturally occurring antagonistic rhizobacteria such as *Bacillus* spp. (Silveira et al., 1995), *Pseudomonas* spp. (Guo et al., 2001), and *Streptomyces* spp. (el Albyad et al., 1996).

Our group has been studying plant growth-promoting rhizobacteria (PGPR) as potential biocontrol agents. We have reported that PGPR strains with promising

* Corresponding author. Fax: +86-25-439-5246.
*E-mail address:* jhguo@njau.edu.cn (J.-H. Guo).

1049-9644/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S1049-9644(03)00124-5

biocontrol activity can be selected by the combination of inhibiting zones and root-colonizing capacity (Guo et al., 2001; Guo et al., 1996), and the living microbes can be stored for more than 2 years at house temperature (Guo and Fang, 2001).

The objectives of this study were to: (1) determine the efficacy of PGPR strains J2, J3, and BB11 in controlling tomato bacterial wilt under greenhouse and field condition in different environments of Jiangsu and Hebei provinces and (2) determine the efficacy of PGPR formulations stored for 1 and 2 years.

## 2. Materials and methods

### 2.1. Bacterial strains and their growth condition

*Ralstonia solanacearum* strain Tm15 was isolated from tomato in Nanjing City, Jiangsu Province, China. Potential biocontrol strains were selected according to their pathogen-inhibition zones (diameters larger than 1 cm on artificial media) and their root-colonizing population (higher than $5 \times 10^3$ cfu/g root 30 days after root-dipping and transplanting) (Guo et al., 1996). *Serratia* sp. strain J2 and *Pseudomonas* sp. strain J3 were isolated from rhizosphere soil in Xitujie of Nanjing, Jiangsu Province, in fields that had been continuously cropped with healthy tomato cultivar 'Su Kang No. 5' for 3 years. *Bacillus* sp. strain BB11 was obtained from the rhizosphere of ginger in Shucheng County, Anhui Province. Another *Bacillus* sp. Strain FH17 was obtained from the rhizosphere of hot pepper in Nanjing City. *Pseudomonas* sp. and *R. solanacearum* were grown at 30 °C and *Bacillus* sp. and *Serratia* sp. were grown at 35 °C in NA nutrient broth (beef extract 3 g, peptone 6 g, and sucrose 10 g in 1.0 L) (Fang, 1998) under vigorous aeration.

### 2.2. Growth of plants

Tomato cv 'Su Kang No. 5' seeds were obtained from the Vegetable Research Institute of Jiangsu Agricultural Scientific Academy. Tomato seeds were surface-sterilized with 2% sodium hypochlorite for 2 min, washed thoroughly with sterilized water, and planted into pots of sterilized soil. After 4 weeks, seedlings were transplanted into pots containing experimental soil amendments and grown in the greenhouse at 25–35 °C. The soil was obtained from healthy tomato fields in Xitujie, near Nanjing Agricultural University campus. The soil was a clay loam, pH 6.8, and was autoclaved before use.

### 2.3. Evaluation of PGPR strains in control of bacterial wilt under greenhouse conditions

Three PGPR strains were evaluated under greenhouse conditions for control of tomato bacterial wilt. For each strain, two kinds of treatments were conducted. First, PGPR suspensions were diluted 100 times and watered into the soil around the tomato roots using 50 ml/plant at the time of transplanting into soil containing *R. solanacearum* at $2 \times 10^5$ cfu/g soil (Ren et al., 1987). Subsequent applications were applied at 7, 15, and 30 days after transplanting. Second, seedlings in another group were treated with liquid culture medium, diluted 100 times. The culture medium was obtained by centrifuging bacterial cultures and using the supernatant. The same application times as in the first method were used. The experiment was conducted three times. Treatments were replicated eight times.

The disease incidence and yields were determined 60 days after transplanting. There were two control treatments in this experiment: seedlings that received only $H_2O$ (Control 1) and seedlings that were watered with 100-times diluted fresh culture liquid medium (Control 2).

Biological control efficacy was calculated using the following formula: biological control efficacy = ([disease incidence of control − disease incidence of treatment group]/disease incidence of control) × 100%.

### 2.4. Field experiments

A total of four field trials were conducted, each in a different location in Jiangsu and the Hebei provinces. All field trials included the four treatments: suspensions of bacterial strains J2, J3, and BB11 and a water control.

The experimental design was a complete randomized block. Each plot was 5 m in length and 2 m in width, and 60 tomato seedlings were planted in each plot. The biocontrol bacterial suspension was diluted 100 times with water, mixed evenly into the organic fertilizer, and applied into the soil. The field was raked and covered with plastic film. Tomato transplanting was conducted 7 days later. Except for the application of fungicides, standard agronomic practices were conducted to raise the crop.

Trial one was conducted in 1999 in Jiangsu province in the Qixia district of Nanjing City (32° latitude and 118.7° longitude). The tomato cultivar 'Zao Feng' was used. There were 10 replications of each treatment.

Trial two was conducted in 2000 plastic houses of Chuzhou District, Huaian City, which is 180 km from Nanjing. In the test soil, tomatoes had been planted continuously for 2–4 years and bacterial wilt incidence was higher than 30% in 1998. The tomato cultivar was 'Mao Fen 802.' There were six replications of each treatment.

Trial 3 was an in-ground test in 1999 in the plastic houses of Handan district in Hebei province (36.6°N latitude, 118.02°N longitude). The tomato cultivar was 'Zao Feng.' Ten replications of each treatment were used.

Trial 4 was conducted in 2000 in the plastic houses in the same place as trial 3. The tomato cultivar was 'Mao Fen 802.' There were 10 replications of each treatment.

The number of wilted plants was recorded 60 days after transplanting tomatoes in each field location. Disease incidence was calculated with the following formula: $100 \times$ (number of wilted plants per plot/total number of plants per plot). Tomato fruits were harvested three times in each plot and the cumulative yields were calculated.

### 2.5. Detection of viable bacteria in biocontrol products

Samples were taken to determine viable bacteria immediately after preparation of products and again at the time of application. Serial 10-fold dilutions to $10^{-10}$ were prepared. A 10 μl suspension of each dilution was spread onto nutrient agar. After incubation for 48 h at 30 °C, colony numbers were counted and the cfu/ml in biocontrol products was determined.

### 2.6. Evaluation of biocontrol efficacy of biocontrol products stored 1 and 2 years

The storage method of living bacterial products is in patent application (Guo and Fang, 2001). Each biocontrol agent was in water formulation and stored in room temperature (10–30 °C) for 1–2 years. The living bacterial concentrations were detected in plates before application to soil, when the storage time was 12 and 24 months. The concentrations of the biocontrol bacteria were all about $1 \times 10^9$ cfu/ml in subsequent field experiments.

This experiment was conducted in a plastic house in Huaian. The plot size was $5 \times 2$ m. Fresh product and product stored 1 and 2 years were applied at a rate of 500 ml/667 $m^2$. There were four replications of each treatment. Biocontrol efficacy was determined as described above.

### 2.7. Data analysis

The analysis of variance for biocontrol efficacy, yields of the tomatoes, and the population of bacteria was performed using the SAS general linear model (GLM) procedure (SAS Institute, Version 6, Cary, NC). Mean comparisons were conducted using a least significant difference (LSD) test ($P = 0.05$ or $P = 0.01$). Standard error and a LSD result were recorded.

## 3. Results

### 3.1. Evaluation of PGPR strains in the control of tomato bacterial wilt under greenhouse conditions

The average disease incidence and tomato yield are listed in Table 1. There was little difference in disease incidence and biocontrol efficacy between the two controls. Treatment with diluted liquid cultures of the PGPR did not affect disease incidence or yield. Tomatoes treated by strain J3 grew the best, and yield increase was the most obvious (91%), much more than that of strain J2 (55.6%). The disease control efficacy of strain J3 was the highest (88.1%) as well.

### 3.2. Field experiments

Results in Tables 2–5 show that all the tested strains decreased disease incidence and increased yield in the field trials in Jiangsu and Hebei provinces.

In trial one in Qixia (1999, Table 2), disease was reduced 66.1, 73.6, and 68.4% by J2, J3, and BB11, respectively, compared to the control. Yield increases with these bacteria were 49.5, 70.8, and 59.0%, respectively.

In trial two in Huaian (2000, Table 3), disease was reduced 78.1, 94.1, and 86.9% by J2, J3, and BB11, respectively. Yield increases with bacteria ranged from 180 to 237%.

In trial three in Handan (1999, Table 4), biocontrol efficiencies of 71.3, 63.6, and 78.2% were achieved by J2, J3, and BB11, respectively. Yield increases with these bacteria were 64.2, 53.5, and 76.2%, respectively.

In trial four in Handan (2000, Table 5), disease was reduced 74.4, 75.1, and 81.9% by J2, J3, and BB11, compared to the control. Yield increases with these

Table 1
Efficacy of PGPR in controlling tomato bacterial wilt and increasing yield in the greenhouse[a]

| Strains | Disease incidence (%) | Biocontrol efficacy (%) | Actual yield (kg/treatment) | Yield increase (%) |
|---|---|---|---|---|
| Control 1[b] | 47.2 ± 2.86 AB | 0 ± 6.07 CD | 4.5 ± 0.30 C | 0 ± 6.70 C |
| Control 2[b] | 51.4 ± 3.56 A | −8.81 ± 7.53 D | 4.7 ± 0.20 C | 4.44 ± 4.45 C |
| J2 | 9.7 ± 1.40 CD | 79.5 ± 2.95 AB | 7.0 ± 0.50 B | 55.6 ± 11.2 B |
| J2CL[c] | 46 ± 6.11 AB | 2.47 ± 12.9 CD | 4.53 ± 0.50 C | 0.73 ± 11.2 |
| J3 | 5.6 ± 0.78 D | 88.1 ± 1.68 A | 8.6 ± 0.60 A | 91.0 ± 13.1 A |
| J3CL | 45.4 ± 3.97 B | 3.73 ± 8.38 C | 4.63 ± 0.55 C | 2.96 ± 12.2 C |
| BB11 | 12.5 ± 2.29 C | 73.5 ± 4.83 B | 7.7 ± 1.65 AB | 71.1 ± 16.8 B |
| BB11CL | 50.7 ± 3.84 AB | −8.83 ± 7.53 D | 4.43 ± 0.50 C | −1.47 ± 11.2 C |

[a] Means followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.01$).
[b] Control 1: seedlings watered with water. Control 2: seedlings watered with 100-times diluted fresh culture liquid medium.
[c] J2CL is the culture liquid of strain J2 without bacteria. The same is true in J3CL and BB11CL.

Table 2
Efficacy of PGPR in controlling tomato bacterial wilt and increasing yield in the field of Qixia district of Jiangsu Province (1999)[A]

| Strains | Disease incidence in treatments (%) | Disease present in control (%) | Yield in control (kg/plot) | Biocontrol efficacy (%) | Yield in treatments (kg/plot) | Yield increase (%) |
|---|---|---|---|---|---|---|
| J2 | 14.9 ± 3.67 a | 43.9 ± 7.04 a | 39.7 ± 5.57 c | 66.1 ± 9.44 b | 59.3 ± 6.94 c | 49.5 ± 17.5 c |
| J3 | 7.8 ± 3.31 c | 29.5 ± 3.84 c | 67.3 ± 8.45 a | 73.6 ± 11.3 a | 113 ± 10.4 a | 70.8 ± 12.1 a |
| BB11 | 13.0 ± 3.0 b | 41.2 ± 5.68 b | 53.6 ± 9.71 b | 68.4 ± 7.3 b | 85.2 ± 3.72 b | 59.0 ± 6.92 b |

[A] Means followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$). Data represent means of experiment with 10 replications each.

Table 3
Efficacy of PGPR in controlling tomato bacterial wilt and increasing yield in the plastic house of Huaian city of Jiangsu Province (2000)[A]

| Strains | Disease incidence in treatments (%) | Disease present in control (%) | Yield in control (kg/plot) | Biocontrol efficacy (%) | Yield in treatments (kg/plot) | Yield increase (%) |
|---|---|---|---|---|---|---|
| J2 | 16.4 ± 1.47 a | | | 78.1 ± 1.96 c | 84.3 ± 5.68 b | 181 ± 18.9 ab |
| J3 | 4.4 ± 0.86 c | 75.0 ± 8.72 | 30 ± 6.87 | 94.1 ± 1.13 a | 101 ± 15.2 a | 237 ± 50.8 a |
| BB11 | 9.8 ± 1.13 b | | | 86.9 ± 1.50 b | 83.9 ± 8.98 b | 180 ± 29.9 b |

[A] Means followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$). Data represent means of the experiment with six replications each.

Table 4
Efficacy of PGPR strains in controlling tomato bacterial wilt and increasing yield in the plastic house of Hebei Province (1999)[A]

| Strains | Disease incidence (%) | Disease present in control (%) | Yield in control (kg/plot) | Biocontrol efficacy (%) | Actual yield (kg/plot) | Yield increase (%) |
|---|---|---|---|---|---|---|
| J2 | 12.5 ± 2.17 b | | | 71.3 ± 4.99 b | 98.5 ± 10.5 b | 64.2 ± 17.4 b |
| J3 | 15.8 ± 3.24 a | 43.5 ± 7.95 | 60 ± 7.81 | 63.6 ± 7.30 c | 92.1 ± 13.9 c | 53.5 ± 23.2 c |
| BB11 | 9.5 ± 2.29 c | | | 78.2 ± 5.27 a | 106 ± 10.7 a | 76.2 ± 17.8 a |

[A] Means followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$). Data represent means of the experiment with 10 replications each.

Table 5
Efficacy of PGPR strains in controlling tomato bacterial wilt and increasing yield in the plastic house of Hebei Province (2000)[A]

| Strains | Disease incidence (%) | Disease present in control (%) | Yield in control (kg/plot) | Biocontrol efficacy (%) | Actual yield (kg/plot) | Yield increase (%) |
|---|---|---|---|---|---|---|
| J2 | 14.6 ± 3.11 a | | | 74.4 ± 5.46 b | 82.0 ± 11.6 b | 57.7 ± 22.3 b |
| J3 | 14.2 ± 2.79 a | 57.0 ± 9.42 | 52 ± 10.1 | 75.1 ± 4.89 b | 76.1 ± 9.14 c | 46.3 ± 17.6 c |
| BB11 | 10.3 ± 2.23 b | | | 81.9 ± 3.91 a | 92.8 ± 10.3 a | 78.5 ± 19.8 a |

[A] Means followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$). Data represent means of the experiment with 10 replications each.

bacteria in this trial were 57.7, 46.3, and 78.5%, respectively.

For strains J2, J3, and BB11, if the disease present in the control was lower than 29%, there were some obvious differences among biocontrol efficacies in different plots of the same treatment. It seems that in order to achieve greater than 50% biocontrol efficacy, there has to be more than 29% disease in the control.

The experiment in 2000 (Table 3) was conducted in the plastic houses in Huaian City. Therefore, the effect of weather conditions such as wind and rain was not a factor. The control occurrence of wilt was very high (75%) at this time, and the biocontrol efficacies and yield increases were much higher than those of 1999.

### 3.3. Living bacterial concentration in biocontrol products

The living bacterial concentration of fresh biocontrol products was $0.99–1.02 \times 10^9$ cfu/ml and $0.95–1.01 \times 10^9$ cfu/ml before application into fields 1 year later. No obvious differences were found between fresh products and products stored for 1 year. There were

Table 6
Living bacteria concentration in biocontrol products[A]

| Strains | J2 | J3 | BB11 |
|---|---|---|---|
| Fresh products ($\times 10^9$ cfu/ml) | 1.02 ± 0.01 a | 0.99 ± 0.03 a | 1.05 ± 0.04 a |
| One-year stored products ($\times 10^9$ cfu/ml) | 0.99 ± 0.04 a | 0.95 ± 0.09 a | 1.01 ± 0.06 ab |
| Two-years stored products ($\times 10^9$ cfu/ml) | 0.83 ± 0.04 b | 0.84 ± 0.06 b | 0.96 ± 0.03 b |

[A] Data represent means of experiments with three replications each. Means followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$).

Table 7
Biocontrol efficacy of biocontrol products stored for 1 and 2 years (2001, Huaian)[A]

| Strains | Products | Disease incidence (%) | Disease present in control (%) | Yield in control (kg/plot) | Biocontrol efficacy (%) | Actual yield (kg/plot) | Yield increase (%) |
|---|---|---|---|---|---|---|---|
| J2 | Fresh | 11.9 ± 5.07 de | | | 81.1 ± 8.08 ab | 91.5 ± 14.5 b | 86.7 ± 29.5 b |
| | One* | 23.9 ± 10.0 bc | | | 61.9 ± 16.0 cd | 69.1 ± 11.7 c | 41.1 ± 23.9 c |
| | Two** | 30.5 ± 10.3 a | | | 51.4 ± 16.4 e | 66.7 ± 10.1 c | 36.1 ± 20.5 c |
| J3 | Fresh | 8.3 ± 2.24 b | | | 86.8 ± 3.58 a | 106 ± 13.5 a | 117 ± 27.7 a |
| | One | 8.6 ± 3.53 b | 62.7 ± 12.8 | 49.0 ± 9.0 | 86.3 ± 5.62 a | 87.4 ± 8.56 a | 78.4 ± 17.6 b |
| | Two | 19.9 ± 5.1 a | | | 68.2 ± 8.16 b | 69.3 ± 9.85 c | 41.4 ± 20.1 c |
| BB11 | Fresh | 14.0 ± 4.33 d | | | 77.6 ± 6.92 b | 85.8 ± 6.72 b | 75.2 ± 13.7 b |
| | One | 28.1 ± 4.17 ab | | | 55.3 ± 6.69 de | 71.3 ± 6.81 c | 45.5 ± 13.9 c |
| | Two | 29.4 ± 9.91 a | | | 53.1 ± 15.8 e | 67.9 ± 10.1 c | 38.6 ± 20.6 c |

[A] Means followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$). Data represent means of experiments with four replications in each treatment and eight replications in the control.
* Product stored for 1 year.
** Product stored for 2 years.

some differences between products stored for 2 years and other groups (Table 6).

### *3.4. Biocontrol efficacy of 1 and 2 years stored living bacterial products*

All fresh products of 3 strains and 1-year stored J3 and BB11 bacterial products achieved biocontrol efficacy of more than 70%. Two-years-stored J3 and one-year-stored J2 were nearly equal (69.3 and 69.1%). All fresh and stored products resulted in yield increases of more than 36.1% (Table 6).

Disease incidence 30 days after treatments was lower than 6% in groups treated with fresh products and lower than 15% in other treatments. From May 15 to May 22, 2001, the weather changed greatly in Huaian, with increases in temperature from 25 to 35 °C and the relative humidity from 50 to 100% in the experimental plastic house. During this 7-day period, the disease incidence greatly increased. Data from the harvest on May 22, 2001, revealed that yield was increased 36.1% with J2 and BB11 stored for 2 years.

All three strains of PGPR strains stored for 1-year retained biocontrol efficacy ranging from 68.4 to 99.4%. Those stored for 2 years showed biocontrol ranging from 63.4 to 78.5%. Both showed yield increase in the range of 35.4–67.0% (Table 7).

## 4. Discussion

Before biological control can be implemented on a practical level, it is important to establish the efficacy of the biocontrol interaction under a variety of different environmental conditions (Boland, 1997; Cook et al., 1996). This research evaluated the biocontrol efficacy of three strains in two provinces. At the same time, the influence of several different environmental conditions, such as temperature, humidity, and soil fertilizer, on biological control of bacterial wilt of tomato by strains J2, J3, and BB11 was investigated.

Biological control efficacy was significant so that these strains can be considered for registration as a new pesticide. But, the application methods of the PGPR product should be studied in detail before this process.

As for environmental conditions, it seems that the temperature and moisture are important influences on the biocontrol efficacy. The weather of Jiangsu province is very different from that of Hebei. It is wetter and warmer in Jiangsu than in Hebei, especially in the spring, summer, and autumn. This kind of weather is suitable for the occurrence of bacterial wilt. Usually, almost no bacterial wilt occurred in the open fields in Hebei province. However, in recent years, more and more plastic vegetable houses have been established in Handan city of this province. In some continuously

cropped plastic tomato houses, wilt has become more common.

Approximately 30 days after tomato were transplanted, if the temperature was higher than 30 °C and the moisture was greater than 90% for a few days in the plastic house or field, bacterial wilt developed immediately and the biocontrol efficacy decreased. We postulate that the growth and spread of *R. solanacearum* was favored by these environmental conditions, although perhaps the population of PGPR strains also decreased. The biological control treatments provided a relatively unstable suppression of disease and were usually effective only when environmental conditions were less favorable for disease development (Ciampin-Panno et al., 1989). Hence, the biocontrol agents J2, J3, or BB11 should be applied at 25–30 days after transplanting. Results in Tables 2–5 indicate that strains J2, J3, and BB11 can effectively reduce bacterial wilt of tomato under a variety of environmental conditions and have potential for further development as biocontrol agents.

One of the most important problems in biocontrol using microbial products is the storage time of living microbes. A short shelf life can be a serious obstacle in the development of biocontrol products with living microbes. Vidhyasekaran et al. (1997) found that unformulated bacterial suspensions of *Pseudomonas fluorescens* strains could not be stored even for 10 days, at which time their efficacy was completely lost.

The shelf life of a biocontrol product is mainly depended on the characteristics of the biocontrol agent itself. Connick et al. (1998) found that shelf life of *Fusarium oxysporum* samples, which were used to control the narcotic plants coca (*Erythroxylum coca*) and opium poppy (*Papaver somniferum*), was maximized by incorporating at least $5 \times 10^6$ cfu/g of chlamydospore inoculum (Connick et al., 1998).

Another important factor of shelf life is the formulation method. In the powder and granular formulations, many kinds of carriers, binders, and different temperatures were used to improve the formulation of biocontrol agents and achieve good results. Sabaratnam et al. (2002) found that at 4 °C, the powder and granular formulations were the most stable and were shown to be 100% viable after 14 and 10 weeks of storage, respectively (Sabaratnam et al., 2002). The nematophagous fungus *Verticillium chlamydosporium* retained its viability when mixed with a carrier (kaolin) and a binder (gum arabic) and stored in vacuum-sealed bags at 25 °C for 12 months (Stirling et al., 1998). Talc formulations of *P. fluorescens* strains controlled peanut leaf spot (*Cercosporidium personatum*) and rust (*Puccinia arachidis*) and effectively inhibited mycelial growth of *Fusarium udum* even after 6 months of storage, while peat formulations were effective up to 60 days of storage (Meena et al., 2002). Trigalet et al. (1997) reported that the shelf life of vermiculite, lignite, and kaolinite formulations of *P. fluorescens* that was used to control pigeon pea wilt was short.

There is less work regarding the shelf life of water formulation. Significant improvement in the efficacy of the fungus *Ascochyta caulina*, which is a plant pathogenic fungus specific to *Chenopodium album* L., was achieved in glasshouse trials with an aqueous formulation (Netland et al., 2001).

As for our biocontrol agents, we could only maintain the living bacteria concentration and efficacy of strains J2, J3, and BB11 for 10–60 days in cultural liquid. With the water formulation of the three strains, bacterial suspensions stored for 1 and 2 years all achieved biocontrol efficacy. However, the efficacy decreased as the conservation time became longer. It can be expected that this kind of biocontrol product will maintain more than 2 years' time of efficacy.

Although in J3 treatments disease incidence was very low in the groups of products stored for 1 and 2 years and there was no obvious difference between J3 (fresh) and J3 (1-year stored), yields obtained by these treatments were different. Hence, it is possible that longer storage times can decrease yield-promoting capacity of the bacteria while biocontrol efficacy is retained.

Additional information is required to understand the complex process of biological control of bacterial wilt. In future studies, we evaluate mixtures of strains J2, J3, BB11, and other PGPR strains in field trials.

## Acknowledgments

This research was supported by a Grant-in-Aid for science research from the Science and Technology Committee of Nanjing City (20011016), Jiangsu Province (BJ93072), and 863 high-tech. Program (2002AA 244041). The authors would like to thank Dr. Steve Johnson for critical review of the manuscript.

## References

Boland, G.J., 1997. Stability analysis for evaluating the influence of environment on chemical and biological bicontrol of white mold (*Sclerotinia sclerotiorum*) of bean. Biol. Control 9, 7–14.

Ciampin-Panno, L., Fernandez, C., Bustamante, P., Andrade, N., Ojeda, S., et al., 1989. Biological control of bacterial wilt of potatoes caused by *Pseudomonas solanacearum*. Am. Potato J. 66, 315–332.

Connick Jr., W.J., Daigle, D.J., Pepperman, A.B., Hebbar, K.P., Lumsden, R.D., Anderson, T.W., Sands, D.C., 1998. Preparation of stable, granular fomulations containing *Fusarium oxysporum* pathogenic to narcotic plants. Biol. Control 13, 79–84.

Cook, R.J., Bruckart, W.L., Coulson, J.R., Goettel, M.S., Humber, R.A., Lumsden, R.D., Maddox, J.V., McManus, M.L., Moore, L., Meyer, S.F., Quimby Jr., P.C., Stack, J.P., Vaughn, J.L., 1996. Safety of microorganisms intended for pest and plant disease control: a framework for scientific evaluation. Biol. Control 7, 333–351.

Dalal, N.R., Dalal, S.R., Golliwar, V.G., Khobragade, R.I., 1999. Studies on grading and pre-packaging of some bacterial wilt

resistant brinjal (*Solanum melongena* L.) varieties. J. Soils Crops 9, 223–226.

Dong, C., Zeng, X., Liu, Q., 1999. Biological control of tomato bacterial wilt with avirulent bacteriocinogenic strain of *Ralstonia solanacearum*. J. S. China Agric. Univ. 20, 1–4.

el Albyad, M.S., el Sayed, M.A., el Shanshoury, A.R., et al., 1996. Effect of culture conditions on the antimicrobial activities of UV-mutants of *Streptomyces corchorusii* and *S. spiroverticillatus* against bean and banana wilt pathogens. Microbiol. Res. 151, 201–211.

Fang, Z.D., 1998. Methods of Plant Pathology. China Agriculture Press, Beijing.

Guo, J.H., Guo, Y.H., Zhang, L.X., Qi, H.Y., Fang, Z.D., 2001. Screening for biocontrol agents against cayenne pepper bacterial wilt. Chin. J. Biol. Control 17, 101–106.

Guo, J.H., Wang, Y.L., Li, J., et al., 1996. Screen of biocontrol bacteria of plant wilt by inhibiting zones and root-colonizing capacity. Acta Phytopathol. Sin. 26, 49–54.

Guo J.H., Fang, Z.D., 2001. A conservation method of living microbes. Patent application number: 02112559.7.

Halos, P.M., Zorilla, R.A., 1979. Vesicular–arbuscular mycorrhizae increase growth and yield of tomatoes and reduce infection by *Pseudomonas solanacearum*. Philipp. Agric. 62, 309–315.

Hayward, A.C., 1995. *Pseudomonassolanacearum*. In: Singh, U.S., Singh, R.P., Kohmoto, K. (Eds.), Pathogenesis and Host Specificity in Plant Disease: Histopathological, Biochemical, Genetic and Molecular Bases, vol. 1. Elsevier, Tarrytown, pp. 139–151.

Jia, S., Qu, X., Feng, L., Tang, T., Tang, Y., Liu, K., Zheng, P., Zhao, Y., Bai, Y., Cai, M., 1999. Expression of antibacterial peptide gene in transgenic potato confers resistance to bacterial wilt. In: Chinese Agricultural Sciences, Beijing, China.

Kang, Y., Mao, G., Lu, C., He, L., 1995. Biological congtrol of bacterial wilt of tomato by extracellular protein defective mutant of *Pseudomonas solanacearum*. Acta Phytophylacica Sin. 22, 287–288.

Katayama, K., Kimura, S., 1987. Ecology and protection of bacterial wilt of potato 2. Some control methods and their integration. Bull. Nagasaki Agric. For. Exp. Stn. 15, 29–57.

Meena, B., Radhajeyalakshmi, R., Marimuthu, T., Vidhyasekaran, P., Velazhahan, R., 2002. Biological control of groundnut late leaf spot and rust by seed and foliar applications of a powder formulation of *Pseudomonas fluorescens*. Biocontrol Sci. Technol. 12, 195–204.

Netland, J., Dutton, L.C., Greaves, M.P, Baldwin, M., Vurro, M., Evidente, A., Einhorn, G., Scheepens, P.C., French, L.W., 2001. Biological control of *Chenopodium album* L. in Europe. BioControl 46, 175–196.

Ren, X.Z., Zhang, J., Fang, Z.D., 1987. On colonization of avirulent bacteriocin-*solanacearum* strain nOE-104 of *Pseudomonas solanacearum* on tomato plant. Acta Phytopathol. Sin. 17, 129–133.

Sabaratnam, S., James, A., Traquair, 2002. Formulation of a *Streptomyces* biocontrol agent for the suppression of Rhizoctonia damping-off in tomato transplants. Biol. Control 23, 245–253.

SAS Institute Inc., 2002. SAS Procedures Guide, Version 6, Third Edition, SAS Institute Inc., Cary, NC.

Silveira, E.B., Da, R., Mariano de, L.R., Michereff, S.J., et al., 1995. Antagonism of *Bacillus* spp. against *Pseudomonas solanacearum* and effect on tomato seedling growth. Fitopatol. Bras. 20, 605–612.

Stirling, G.R., Licastro, K.A., West, L.M., Smith, L.J., 1998. Development of commercially acceptable formulations of the nematophagous fungus *Verticillium chlamydosporium*. Biol. Control 11, 217–223.

Swanepoel, A.E., 1992. Survival of South African strains of biovar-2 and biovar-3 of *Pseudomonas solanacearum* in the roots and stems of weeds. Potato Res. 35, 329–332.

Trigalet, A., Trigalet-Demerym, D., Prior, P., 1997. Competition between Ralstonia solanacearum of tomato plants: application of biological control. In the 2nd *IBWS*. 22–27 June 97. Guadeloupe French West Indies.

Vidhyasekaran, P., Sethuraman, K., Rajappan, K., Vasumathi, K., 1997. Powder formulations of *Pseudomonas fluorescens* to control pigeonpea wilt. Biol. Control 8, 166–171.

Vincent, V.M., Mew, T.W., 1998. Effect of a soil amendment on the survival of *Ralstonia solanacearum* in different soils. Phytopathology 88, 300–305.