Eur J Plant Pathol (2008) 120:353–362
DOI 10.1007/s10658-007-9223-8

FULL RESEARCH PAPER

Induction of systemic resistance in banana (*Musa* spp.) against *Banana bunchy top virus* (BBTV) by combining chitin with root-colonizing *Pseudomonas fluorescens* strain CHA0

Mathiyazhagan Kavino · Sankarasubramanian Harish · Neelakandan Kumar · Duraisamy Saravanakumar · Ramasamy Samiyappan

Received: 10 April 2007 / Accepted: 24 September 2007 / Published online: 1 November 2007

Abstract *Pseudomonas fluorescens* strains CHA0 and Pf1 were investigated for their biocontrol efficacy against *Banana bunchy top virus* (BBTV) in banana (*Musa* spp.) alone and in combination with chitin under glasshouse and field conditions. Bioformulation of *P. fluorescens* strain CHA0 with chitin was effective in reducing the banana bunchy top disease (BBTD) incidence in banana under glasshouse and field conditions. In addition to disease control, the bioformulation increased the economic yield significantly compared to the untreated control. Increased accumulation of oxidative enzymes, peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), pathogenesis-related (PR) proteins, chitinase, β-1,3-glucanase and phenolics were observed in CHA0 bioformulation amended with chitin-treated plants challenged with BBTV under glasshouse conditions. Indirect ELISA indicated the reduction in viral antigen concentration in *P. fluorescens* strain CHA0 with chitin-treated banana plants corresponding to reduced disease ratings. The present study revealed that induction of defence enzymes by *P. fluorescens* with chitin amendment reduced the BBTD incidence and increased bunch yield in banana.

Keywords Banana bunchy top virus · Chitin · Bioformulations · Induced resistance · Defence enzymes · Bunch yield

M. Kavino (✉) · N. Kumar
Department of Fruit Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, India
e-mail: mrkavino@yahoo.co.uk

S. Harish
Molecular Plant Virology lab, Department of Plant Pathology, National Chung Hsing University, Taichung 11529, Taiwan

D. Saravanakumar
AGROINNOVA, University of Turin, Turin 10095, Italy

R. Samiyappan
Director, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 003, India

Introduction

Banana bunchy top disease (BBTD) is one of the most destructive diseases of banana in tropical Asia, Australia and the South Pacific (Dietzgen and Thomas 1991). In India, *Banana bunchy top virus* (BBTV) is widespread in all banana-growing states. BBTV is an isometric virus, 18 nm in diameter with a circular single stranded DNA genome consisting of at

least six components (BBTV DNA 1–6) (Burns et al. 1995). In the hills of lower Pulney, Western Ghats, Tamil Nadu, India hill banana cv. Virupakshi (AAB), a prized fruit, grown on more than 18,000 ha as a rainfed perennial crop, is badly devastated by bunchy top; consequently the area is reduced to 2,000 ha. The first characteristic symptoms appear as irregular, nodular, dark green streaks in the lower portion of the leaf midrib and petiole. This type of symptom is sometimes referred to as 'Morse code streaking' due to the irregularity and resemblance to the series of dots and dashes (Magee 1940). As the successive leaves of an infected plant emerge, they seem to be more and more abnormal. They appear to be bunched at the top of the plant giving the typical bunchy top appearance. Severely infected banana plants usually will not fruit, and even if they produce, the banana hands and fingers are likely to be distorted and twisted.

BBTV is endemic in lower pulney hills, India and the usual practice by the banana growers is to apparently select healthy suckers from existing perennial old farms and use the same for planting the next crop. Hence, early diagnosis and management has become indispensable, considering the severe incidence of the disease. The therapeutic approach of spraying a systemic pesticide to control the vector has been the prevailing disease control strategy for over 50 years. Though pesticides have shown some promising results in controlling the vector, phytotoxicity and pesticide residues are the major problems besides causing environmental pollution and human health hazards. In this context, biocontrol approaches help to develop ecofriendly control strategies for managing this viral disease in banana besides preserving the ecological niche in the Western Ghats. Plants have latent defence mechanisms against pathogens, which can be systemically activated upon exposure of plants to stress or infection by pathogens (Baker et al. 1997). This phenomenon is called induced systemic resistance (ISR) (Tuzun and Kuc 1991). The mechanism operates through the activation of multiple defence compounds at sites distant from the point of pathogen attack (Dean and Kuc 1985). There is growing evidence concerning the use of biocontrol agents such as *Trichoderma*, *Pseudomonas fluorescens*, *Bacillus subtilis* to control different pathogens in crop plants under glasshouse and field environments (Murphy et al. 2003).

Soil application of *P. fluorescens* CHA0 has significantly reduced the lesion number and lesion size in tobacco against *Tobacco necrosis virus* (TNV) (Maurhofer et al. 1994). Further, the development of commercial formulations required newer molecules in order to enhance the survival and efficacy of the Plant Growth-Promoting Rhizobacterial (PGPR) strains. Recently, chitin amendment in the medium has increased the chitinase production which in turn showed increased biocontrol activity against plant pathogens (Radjacommare et al. 2002; Vivekananthan et al. 2004). Similarly, application of PGPR strains GB03 (*Bacillus subtilis*) and IN937a (*Bacillus amyloliquefaciens*) with the carrier chitosan to the tomato leads to protection against *Cucumber mosaic virus* (Murphy et al. 2003). Though there are several reports with PGPR strains amended with chitin against plant pathogens, there is only little information available about the induction of defence enzymes against virus diseases. In this context, the present study was carried out to investigate the induction of defence enzymes by PGPR strains amended with or without chitin against bunchy top virus transmitted by the banana aphid (*Pentalonia nigronervosa*).

Materials and methods

Biocontrol agents

Pseudomonas fluorescens strain Pf1 was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. In addition, *Pseudomonas* strain CHA0 (Provided by Dr. Christophkeel, Institute de Biologie Vegetale, Switzerland) was also used. Bacterial cultures were stored in King's B broth (KBB) in 30% (v:v) glycerol at −80°C from which fresh cultures were prepared on plates of KB medium.

Talc-based formulation

A loopful of *P. fluorescens* was inoculated into the KBB and incubated in a rotary shaker at 150 rpm for 72 h at room temperature (28±20°C). After 72 h of incubation, the broth containing 9×10^8 CFU ml^{-1} was used for the preparation of talc-based formulation. To 400 ml of bacterial suspension, 1 kg of sterilized talc-powder, 15 g calcium carbonate (to adjust the pH to neutral) and 10 g carboxymethyl cellulose (adhesive) were mixed and dried under shade for 12 h as described by Nandakumar et al. (2001).

Chitin amendment with talc-based formulation of *P. fluorescens*

Five grams of crab shell chitin was slowly added into 100 ml of cold 0.25 N HCL with vigorous stirring and kept overnight at 4°C. The mixture was filtered through glass wool into 200 ml of ice-cold ethanol at 4°C with continuous stirring. The resultant chitin suspension was centrifuged at 1,000 rpm for 20 min and the chitin pellets were washed repeatedly with distilled water until the pH become neutral. The concentration of colloidal chitin was adjusted to 10 mg ml^{-1} by gravimetric dry method analysis. Colloidal chitin was incorporated into KBB at 1% (v:v). After sterilization, *P. fluorescens* was inoculated in KBB and incubated at room temperature (28±20°C) for 72 h. The bioformulation was prepared with the 400 ml culture grown in chitin-amended KB medium, 1 kg of talc-powder, calcium carbonate 15 and 10 g carboxymethyl cellulose as described by Radjacommare et al. (2004). Chitin amended KBB without *P. fluorescens* inoculation was mixed with talc-powder as described above and used for the chitin alone treatment.

Glasshouse study

Banana (*Musa* spp.) cv. Virupakshi (AAB) plants were planted in earthen pots (0.35 m diameter, height 4 m). Pot mixture comprising soil, sand and compost (2:1:1; v/v) and the soil mixture was pre-sterilized for 30 min in an autoclave at 121°C (to ensure elimination of any symbiont in the soil mixture) containing 0.035 m^3 soil (soil pH: 7.1, soil type: sandy clay loam, clay: 15.1%, silt: 23.6%, sand: 51.8%, organic matter: 2.2%, EC: 0.25 dsm^{-1}) and watered regularly to maintain 60% moisture content to assess the efficacy of rhizobacteria against *Banana bunchy top virus*. Antagonistic bacterial strains were prepared from fresh cultures on KB plates and were used for further studies after inoculation into KBB. The application included sucker dipping (1%) of *P. fluorescens* strains Pf1 and CHA0 (3×10^8 CFU ml^{-1}) with and without chitin at planting, and soil drenching and foliar spraying of the bacterial suspension (3×10^8 CFU ml^{-1}) (Cartwright and Benson 1995) at 3 months after planting in earthen pots. The systemic pesticide (methyl demeton-0.1%) was used as chemical control and plants without bacterial treatment (KB broth) were also maintained. There were three replications with each replicate consisting of 15 plants. The experiment was laid out in a completely randomized block design (RBD).

Mass rearing of aphids

The banana plants were raised periodically in earthen pots filled with pot mixture (comprising soil, sand and compost (2:1:1; v/v) pre-sterilized in an autoclave at 121°C for 30 min to ensure elimination of any symbiont in the soil mixture). Thirty day-old plants were used for culturing the aphids. The test insect *P. nigronervosa* collected from the fields was inoculated on the potted plants for culturing. A population of BBTV-free banana aphids cultured on a BBTV-free banana seedling placed in an insect-proof growth chamber controlled at 25°C, was used to transmit BBTV. For BBTV inoculation, the BBTV-free banana aphids were transferred to a BBTV-infected leaf in a Petri dish (15 cm diameter) for acquisition access overnight. Ten viruliferous aphids were then collected and transferred to a young healthy banana seedling (15 cm tall) for a 2-day inoculation. A fine-tip brush was used to collect and transfer aphids. All the inoculated banana seedlings were grown in an insect proof growth chamber at 25°C. The viruliferous aphids were maintained in a glasshouse and used for laboratory experiments (Su et al. 2003).

Assay of defence-related enzymes and compounds

One gram of leaf sample was homogenized with 2 ml of 0.1 M sodium citrate buffer (pH 5.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rpm. The supernatant was used as crude enzyme extract for assaying chitinase (Boller and Mauch 1988) and β-1,3-glucanase (Pan et al. 1991) activity. Enzyme extracted in 0.1 M sodium phosphate buffer (pH 7.0) was used for the estimation of peroxidase (PO) (Hammerschmidt et al. 1982), polyphenol oxidase (PPO) (Mayer et al. 1965) and phenylalanine ammonia-lyase (PAL) (Ross and Sederoff 1992). The phenol content was estimated as described by Zieslin and Ben-Zaken (1993) and expressed as catechol equivalents g^{-1} of fresh tissue.

Western blotting for chitinase

The plant samples taken from the glasshouse trial were used for the western blot analysis for chitinase

expression. The protein content of the leaf sample was determined by the Bradford method (Bradford 1976). SDS-PAGE was carried out using 12% polyacrylamide gels (Sigma) according to Laemmli (1970). A medium range protein marker (Genei, Bangalore, India) was used as a molecular mass standard. After electrophoresis, proteins were electro-transferred from the gel to a nitrocellulose membrane (Sigma) as described by Gallagher et al. (1995). The barley chitinase polyclonal antiserum (provided by Dr. S. Muthukrishnan, Kansas State University, USA) was used to detect the chitinase isoforms. The bands were visualized in an alkaline phosphatase reagent containing nitroblue tetrazolium (NBT) and 5-bromo, 4-chloro, 3-indolyl phosphate (BCIP) (Sigma). The experiment was repeated at least twice to confirm the results.

Field study

A field experiment was conducted at Pulney hills, Western Ghats, Tamil Nadu, India with banana cv. Virupakshi (AAB) suckers to test the efficacy of the bioformulations against *Banana bunchy top virus* for two seasons (Plant crop and Ratoon crop) (1,000 m MSL; rainfall: 900 mm; mean temperature: 12± 30°C; RH: 85–95%). Sword suckers of 1.5–2.0 kg weight, free from diseases and nematodes were used as planting material. Before planting, the roots and decayed portion of the corm were trimmed, the pseudostem was cut from the corm leaving 20 cm and the suckers were graded to size. The crop was grown in the rain-fed condition (soil pH:6.4, soil type: sandy clay loam, clay:15.9%, silt:25.4%, sand:50.8%, organic matter:3.1%, EC:0.22 dsm^{-1}). The experiment was laid out in a RBD with nine treatments replicated three times. In each treatment, there were 30 plants per replication and 2×2 m spacing was adopted. *Pseudomonas fluorescens* strains Pf1 and CHA0 amended with and without chitin were applied at planting and 3, 5 and 7 months after planting. Ten grams of the talc-based formulation of the rhizobacteria was added to the soil as the soil application (SA). Methyl demeton at 0.1% was used as a chemical check and sprayed at the same time as other treatments. Natural incidence of diseases was recorded in the field trial from 40 days after planting and the percent disease incidence (Reddy et al. 1983) was calculated as per the following formula: Percent Disease Incidence= (Number of plants exhibiting BBTV/Total number of plants)×100. Banana bunch yield was recorded from individual plants and mean bunch weight was calculated for individual treatments.

Virus titre using Enzyme Linked Immuno Sorbent Assay (ELISA)

The samples taken from the glasshouse and field study were used for the ELISA test. Ten samples were taken from each replication and three replications were maintained for an individual treatment. The virus titre was detected in different bacterized banana plants challenged with viruliferous aphids in this test. Antigen and antiserum dilutions of 1:10 and 1:500 were used in the assay. The antigen diluted with coating buffer was placed in wells of a microtitre plate and incubated overnight at 4°C. The antigen solution was removed and the remains were washed three times with phosphate buffered saline-Tween 20 (PBST). The plate was then supplemented with 200 µl of the polyclonal antiserum per well and incubated at 4°C overnight. The plate was washed three times with PBST and 200 µl of alkaline phosphatase (ALP) conjugate goat-anti rabbit immunoglobulin (Genei, Bangalore, India) was added. After 2 h of incubation at room temperature, the plate was then washed three times, 100 µl of ALP substrate solution (*p*-nitrophenyl phosphate (Genei, Bangalore, India) in substrate buffer at 1 mg/m^{-1}) was added and incubated for 35 min at room temperature. The reaction was stopped by adding 50 µl of 3 M NaOH and absorbance was read in Lab systems Multiscan MS ELISA reader.

Statistical analysis

The data were analyzed using the IRRISTAT version 92-1 programme developed by the biometrics unit, International Rice Research Institute, The Philippines. Percentage infection and yield data were analyzed independently by trial. Data were subjected to analysis of variance (ANOVA). Disease incidence data were arcsine-transformed before analysis. The treatment means were compared by Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

Results

Efficacy of PGPR bio formulations against *Banana bunchy top virus* under glasshouse conditions

The percent infection of BBTV-affected plants showed high variation among the treatments. The CHA0 strain either alone or in combination with chitin was very effective in recording minimum infection (20%) despite being challenged with aphids and was superior to the other strain of *Pseudomonas* Pf1 with or without chitin where 40 and 60% infection was recorded, respectively. The control plants on the other hand produced 100% infection whereas the plants receiving chitin alone showed 85% infection. In terms of percent reduction over the control, it was further evident that CHA0 + chitin and CHA0 alone caused more disease reduction. The ELISA at the final stage (6 months after inoculation) revealed that the titre value was very low with CHA0 + Chitin (0.428) closely followed by CHA0 (0.552), and these values were superior to the Pf1 strain-inoculated plants; the control had an invariably higher ELISA value (1.510) followed by the chitin (1.320) alone treatment (Table 1).

Table 1 Efficacy of different rhizobacteria treatments in banana cv. Virupakshi (AAB) against BBTD in pot culture

Treatments	Percent infection	Percent reduction over control	ELISA value (405 nm)
Pf1	60.00^{c}	40	0.920^{e}
Pf1 + chitin	40.00^{b}	60	0.650^{c}
CHA0	20.00^{a}	80	0.552^{b}
CHA0 + chitin	20.00^{a}	80	0.428^{a}
Chitin	85.00^{d}	15	1.320^{f}
Methyl demeton (0.1%)	60.00^{c}	40	0.830^{d}
Control	100.00^{e}	–	1.510^{g}
P=0.05	5.26	–	0.086

Values are the means of three replicates. For statistical analysis the disease incidence data were transformed using arc-sine. Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P=0.05.

Induction of enzymes in the phenyl propanoid pathway

The assay of defence enzymes revealed that *Pseudomonas* bioformulations induced a greater amount of enzymes in the *Pseudomonas*-treated plants than the untreated control. The induction of the PO enzyme was significantly higher and it was twice as high in banana plants treated with *P. fluorescens* CHA0 + chitin than the untreated control (Table 2). Similarly, higher induction of PPO was observed in banana plants treated with *P. fluorescens* CHA0 combined with chitin followed by CHA0. Combination of chitin with *P. fluorescens* CHA0 induced a higher accumulation of PAL enzyme in banana plants, a 48.10% increase over the untreated control. Induction of chitinase and β-1,3-glucanase enzyme activities were significantly less in the untreated control compared to banana plants treated with CHA0 + chitin, which showed a higher induction of hydrolytic enzyme activity followed by application of CHA0 alone. Accumulation of phenols in banana plants sprayed with *P. fluorescens* CHA0 bioformulation was significantly higher compared to all other treatments. The highest accumulation of phenols was noticed in banana plants treated with the bioformulation of *P. fluorescens* CHA0 + chitin compared to the untreated control. Furthermore, the *P. fluorescens* Pf1 + chitin treatment induced all the enzymes above next to *P. fluorescens* CHA0 amended with or without the chitin molecule. Interestingly, the activity of PO and PPO in plants treated with chemical pesticide was similar to the biocontrol strains. However, banana plants treated with *P. fluorescens* strain CHA0 with or without chitin showed an appreciable disease reduction and higher induction of defence related enzymes against the bunchy top disease compared to the chemical-sprayed and untreated control plants. Interestingly, PO, PPO and chitinase activity did not show significant differences from the untreated control. Furthermore, PAL and β-1, 3-glucanase activity in chitin alone-treated plants revealed significant activity compared to the untreated control, but the values were not as high as those of other treatments.

Western blot analysis

Western blots of barley chitinase antiserum showed that there was constitutive expression of one chitinase isoform with molecular weights of 42

Table 2 Changes in defense enzymes activity in bacterial strains treated banana cv. Virupakshi (AAB) and challenged with *P. nigronervosa*

Treatments	PO	PPO	Chitinase	β-1-3-glucanase	Phenol	PAL
Pf1	1.340^{bc}	1.630^{bc}	58.30^{e}	151.70^{e}	700.320^{e}	2364.23^{e}
Pf1 + Chitin	1.750^{ab}	1.820^{ab}	83.70^{c}	172.50^{c}	735.620^{c}	2785.62^{c}
CHA0	1.830^{ab}	1.900^{ab}	89.40^{b}	185.60^{b}	772.150^{b}	2865.34^{b}
CHA0 + Chitin	2.150^{a}	2.180^{a}	96.50^{a}	195.80^{a}	795.520^{a}	3052.81^{a}
Chitin	0.941^{d}	1.320^{c}	45.28^{f}	134.56^{f}	680.152^{f}	2145.26^{f}
Methyl demeton (0.1%)	1.410^{bc}	1.740^{ab}	65.70^{d}	166.40^{d}	715.520^{d}	2534.26^{d}
Control	0.793^{d}	1.240^{c}	38.40^{g}	126.80^{g}	665.270^{g}	2061.35^{g}
$P=0.05$	0.425	0.375	1.52	5.28	16.58	56.85

Values are the means of three replicates. Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at $P=0.05$.

PO (Peroxidase) – 470 n g^{-1} fw min^{-1}; PPO (Poly phenol oxidase) – 470 n g^{-1} fw min^{-1}; Chitinase – nmol of GlcNAc g^{-1} fw min^{-1}; β-1-3-glucanase – μg of glucose g^{-1} fw min^{-1}; Phenol – catechol g^{-1} fw; PAL – Phenylalanine ammonia lyase – nmol of transcinnamic acid g^{-1} fw min^{-1}

kDa in *P. fluorescens* strain CHA0 with chitin-treated BBTV-challenged plants compared to the control (Fig. 1).

PGPR bioformulation against *Banana bunchy top disease* (BBTD) under field conditions

PGPR bioformulations were prepared with or without chitin and used in this study at different stages of plant growth. Percent infection was observed after the first application of bioformulations. Among the various treatments used, *P. fluorescens* CHA0 + chitin (0.1%) concentration applied at different stages of plant growth recorded the lower mean percent infection (38.33%) which is significantly different from the chemical control (58.3%), chitin alone (81.25) and untreated control (83.33%) treatments (Table 3). However, *P. fluorescens* Pf1 with or without chitin application did not show an appreciable amount of disease reduction. During ratoon crop, *P. fluorescens* strain CHA0 with chitin bioformulation application at different growth stages significantly reduced the mean percent infection of bunchy top disease to 35.00% followed by CHA0 alone with a percent infection of 40.00%, whereas in the control and chitin treatment, 75.00 and 72.33% were recorded respectively (Table 3). Application of *P. fluorescens* strain CHA0 with chitin at different stages reduced the disease incidence of bunchy top in banana plants and was comparable with chemical pesticide treatment.

The *P. fluorescens* strain CHA0 with chitin showed a significant reduction in the virus titre value (0.38; 0.32) compared to the untreated control (1.35; 1.43) plants (Table 3) in both trials. These results were positively correlated for disease incidence and virus

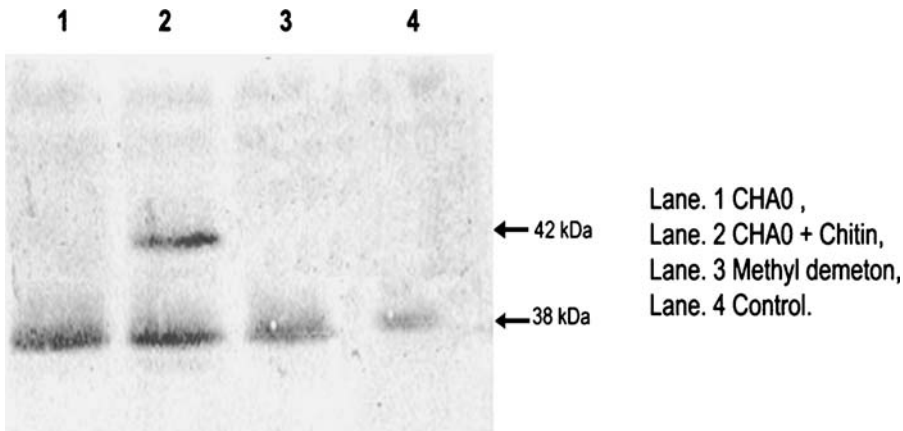

Fig. 1 Western blot analysis of chitinase isoforms in banana cv. Virupakshi treated with *Pseudomonas* against BBTV inoculated with viruliferous *Pentalonia nigronervosa*

Table 3 Efficacy of bacterial strains in banana against BBTD in the field conditions

Treatments	Plant crop				Ratoon crop			
	Percent infection	Percent reduction over control	ELISA value (405 nm)	Bunch weight (kg)	Percent infection	Percent reduction over control	ELISA value (405 nm)	Bunch weight (kg)
Pf1(P)	78.33^{g}	6.02	0.9242^{g}	9.40^{e}	66.66^{g}	11.12	0.9152^{g}	9.70^{f}
Pf1 (P,3 rd, 5th & 7th MAP)	63.33^{e}	24.09	0.7156^{e}	10.20^{d}	53.33^{e}	28.89	0.7346^{e}	10.50^{d}
Pf1 + chitin (P, 3rd, 5th & 7th MAP)	46.66^{c}	44.00	0.5248^{c}	11.10^{b}	45.00^{c}	40.00	0.5128^{c}	11.60^{b}
CHA0 (P)	70.00^{f}	15.99	0.8337^{f}	10.00^{e}	60.00^{f}	20.00	0.8151^{f}	10.10^{e}
CHA0 (P, 3rd, 5th & 7th MAP)	41.66^{b}	50.00	0.4652^{b}	10.40^{c}	40.00^{b}	44.45	0.4146^{b}	11.10^{c}
CHA0 + chitin(P, 3rd, 5th & 7th MAP)	38.33^{a}	54.21	0.3845^{a}	11.60^{a}	35.00^{a}	53.33	0.3238^{a}	12.00^{a}
Chitin (P, 3rd, 5th & 7th MAP)	81.25^{h}	2.49	1.1855^{h}	8.60^{f}	72.33^{h}	3.56	1.3841^{h}	8.80^{h}
Methyl demeton (0.1%)	58.33^{d}	30.00	0.6436^{d}	8.60^{f}	50.00^{d}	33.33	0.6852^{d}	9.00^{g}
Control	83.33^{h}	–	1.3561^{i}	8.50^{g}	75.00^{h}	–	1.4318^{h}	8.60^{h}
P=0.05	2.85	–	0.0521	0.42	3.23	–	0.0581	0.36

Values are the means of three replicates. For statistical analysis the disease incidence data were transformed using arc-sine. Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P= 0.05. *P* Planting; *MAP* Month after planting

titre value, as the treatments having lesser disease incidence recorded minimum titre values and treatments with higher percentage infection showed maximum titre values.

In the present study, PGPR bioformulation-treated plants had higher yields than the chemical and control plants. Besides reducing the bunchy top disease, *P. fluorescens* strain CHA0 with-chitin treated plants recorded higher yields (11.60; 12 kg/bunch) when compared to chemical (8.6;9.0 kg/bunch) and the untreated control (8.5;8.6 kg/bunch) in both crops (Table 3). Interestingly, *P. fluorescens* Pf1 + chitin recorded the higher bunch yield (11.10; 11.60 kg/bunch) next to CHA0 + chitin treatment. However, *P. fluorescens* CHA0 with chitin-treated plants recorded a higher bunch yield in banana than all other treatments.

Discussion

Use of chemicals to control insect vectors is important in the integrated disease management of plant viruses. However, chemicals are slowly being replaced by naturally-occurring and introduced biocontrol agents (Radjacommare et al. 2002; Saravanakumar et al. 2007b). *Pseudomonas fluorescens* has been mainly used to manage soil-borne diseases and limited success has been achieved in the management of virus diseases (Maurhofer et al. 1994; Raupach et al. 1996). So far, no resistant cultivars and economically-viable management practices are available to manage *Banana bunchy top virus.* Although the use of PGPR strains against fungal and bacterial diseases has been documented for several crops (van Loon et al. 1998; Chen et al. 2000), there is only little information available for virus disease management (Raupach et al. 1996; Zehnder 2000). However, Maurhofer et al. (1994) used the classical tobacco system to demonstrate the ability of *P. fluorescens* strain CHA0 to induce resistance against *Tobacco necrosis virus* (TNV). Cucumber and tomato plants were protected from *Cucumber mosaic cucumovirus* through ISR using different PGPR strains (Raupach et al. 1996; Zehnder 2000). The CHA0 strain used in this study had already been reported to protect tobacco plants from TNV and interestingly, the same strain significantly reduced BBTD. Although it significantly reduced BBTV infection, its biocontrol efficacy was further increased when it was applied along with

chitin. To our knowledge, our study is the first report to use a *P. fluorescens* strain in combination with chitin for BBTV management in banana. In earlier work, chitin was reported to increase the efficacy of biocontrol strains against various plant pathogens and pests (Vivekananthan et al. 2004; Nandakumar et al. 2001). Similar to these previous reports, chitin amendment increased the efficacy of PGPR strains in the present study and the chitin alone treatment did not show a significant reduction in BBTD under pot culture and field conditions. From this, it is assumed that PGPR strains are chitinolytic in nature and this could enhance the growth and survival capacity of the PGPR strains. Recently, research work has demonstrated that *P. fluorescens* strains might stimulate the production of biochemical compounds associated with the host defence (Kavino et al. 2007). Of these, the early induction of PAL is more important as it is the first enzyme in the phenylpropanoid pathway which leads to production of phytoalexin, phenolic substances leading to the formation of lignin with the help of peroxidases. Conspicuously, the present study also recorded the higher activity of PAL and PO in plants treated with CHA0 + chitin bioformulation generating the speculation of induced defence responses in plants treated with biocontrol strains.

In addition, the presence of phenolic compounds in plants or their synthesis in response to infection has often been associated with resistance (Ingham 1972). It is well known that resistant plants contain more phenols or produce polyphenols more rapidly than susceptible ones. Our results showed an increase in phenol content both in *P. fluorescens*-treated and in virus-infected plants. Also, multifold increase in phenol content was observed in *P. fluorescens*-treated plants along with pathogen inoculation compared with the infected control plants. Hence the accumulation of phenols may be due to excess production of H_2O_2 in infected plants through increased respiration (Farkas and Kiraly 1962) or due to the activation of the hexose-monophosphate shunt pathway, acetate pathway and release of bound phenols by hydrolytic enzymes (Goodman et al. 1967). It is no surprise that the phenol content increases since these compounds produced by plants are derived through phenylpropanoid metabolism. Thus, increase in peroxidase and PAL content might have frequently enhanced the phenol content in plants in the current study.

In general, *P. fluorescens* in association with the plant induces one or more biochemical compounds which act simultaneously in a synergistic manner to suppress the pathogen during infection (van Loon 1997; Ramamoorthy et al. 2002). In our study, banana plants treated with *P. fluorescens* strain CHA0 alone or in combination with chitin, showed an increase in PR-2 (β-1–3-glucanase) and PR-3 (chitinase) proteins. Thus, the induction of PR proteins corresponding to a reduction in BBTV infection in banana supports the hypothesis that the resistance induced by strains is systemic. Moreover, our results are in agreement with the previous findings of Maurhofer et al. (1994). Until recently, the role of PR proteins and their mechanisms in the reduction of viral diseases was obscure.

The induction of a new chitinase isoform in this study by *P. fluorescens* strain CHA0 with chitin-treated banana plants against BBTV identified using the Western blot technique, confirms the results obtained by Maurhofer et al. (1994) as few chitinase isoforms were observed in *P. fluorescens*-treated tobacco plants against *Tobacco necrosis virus*. Pyung-II et al. (2002) reported the induction of PR-1a and PAL in tobacco plants treated with the *P. fluorescens* strain EXTN-1 against the *Pepper mild mottle virus* (PMMoV). The role of PPO in disease resistance is to oxidize phenolic compounds to quinines, which are often more toxic to microorganisms than the original phenols; the enzyme itself is inhibitory to viruses by inactivating the RNA of the virus (Vidhyasekaran 1988a, b). Recently, Rajendran et al. (2007) demonstrated the endophytic behaviour of *Bacillus* and *Pseudomonas* strains that mediates the disease resistance in crop plants against plant pathogens. Similar to previous studies, *P. fluorescens* strain CHA0 alone and in combination with chitin-mediated induction of PPO in banana plantlets might account for the reduction of BBTD in the current study.

To study the BBTV severity in banana plants, ELISA tests were performed and results revealed that the viral antigen in *P. fluorescens* strain CHA0 with chitin-treated banana plants was less compared to chemical-treated and control plants. This result was in agreement with the previous findings of Raupach et al. (1996) who demonstrated the significance of virus titre with biocontrol application. In addition to disease management, rhizobacterial strains were found to increase plant growth after inoculation (Kloepper et al. 1980) and to

increase yield in potato, radish, mango and sugar beet (Kloepper et al. 1997; Vivekananthan et al. 2004). In the current study, chitin amendment with *P. fluorescens* strain CHA0 significantly increased the yield compared to other treatments and the untreated control. When we examined the cost of application and practicability (data not shown) of the bioformulation (*P. fluorescens* CHA0 + chitin) that we developed, it was found to have more beneficial effects than chemical application and the additional effect was attributed to the reduction in BBTD and the increased yield by the PGPR treatment. This is in agreement with the previous findings of Vivekananthan et al. (2004) who reported an increase in mango fruit yield by the application of *P. fluorescens* strains amended with chitin. Recently, Saravanakumar et al. (2007a) reported a significant increase in tea yield by the application of *Pseudomonas* strains under field conditions besides reducing blister blight disease incidence. Thus, production of phytohormones by *Pseudomonas* strains (Kloepper 1992) might have increased the banana bunch yield in the present work besides reducing BBTD incidence under glasshouse and field conditions.

In conclusion, *P. fluorescens* strains are capable of inducing high levels of defence enzymes in banana, and it could be speculated that induced enzyme activities by *P. fluorescens* may be associated with the biosynthesis of phenolic compounds that have been considered as major determinants in inducing systemic resistance against BBTV disease, besides increasing the bunch yield.

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