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RESEARCH PAPERS

Field suppression of Fusarium wilt disease in banana by the combined application of native endophytic and rhizospheric bacterial isolates possessing multiple functions

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Summary. To develop effective biological control methods for management of Fusarium wilt of banana, 71 endophytic bacteria were isolated from root and corm tissues and 37 bacteria were isolated from the rhizospheres of 21 different banana accessions. The *in vitro* screening of these microbes against *Fusarium oxysporum* f.sp. *cubense* (causal agent of Fusarium wilt in banana) demonstrated that six endophytic and four rhizospheric bacterial isolates reduced the severity of Fusarium wilt while not totally suppressing the disease. These effective bacteria were mixed in different combinations and evaluated in cv. Grand Naine. Soil applications of five different combinations resulted in complete suppression of Fusarium wilt and increased plant growth parameters and number of roots compared to control plants. Field evaluation of these five combinations of bacterial isolates in an area of heavy inoculum and using cv. Grand Naine indicated that all the bacterial combinations suppressed Fusarium wilt and increased the number of banana hands (up to 155%) and bunch weight (up to 214%) as compared to untreated controls. These bacterial antagonists survived in the rhizosphere for up to 35 d and completely colonized roots, corms, pseudo stems and petioles 15 d after inoculation. The combination of bacterial antagonists could provide sustainable management of Fusarium wilt in banana under field conditions.

Key words: combined application, multiple functions.

Introduction

Among the various production constraints affecting banana cultivation, Fusarium wilt is considered as one of the most important destructive diseases in the tropical and subtropical regions of the world (Ploetz *et al.*, 2003). This diseases is caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*). In India, the disease is widespread in almost all banana growing regions, affecting most of the commercial cultivars grown except Red Banana (AAA) and Nendran (AAB) (Thangavelu and Mustaffa, 2010).

Use of biocontrol agents to protect and promote plant growth is generally considered as a potential approach for management of plant diseases (Harish *et al.*, 2009). This method also offers an attractive and environmentally sound alternative for the control of Fusarium wilt of banana (Berg *et al.*, 2001). Smith *et al.* (2003) reported that the introduction of *Pseudomonas* strains 84 and 4B to banana roots of tissue-cultured plants at de-flasking stage improved plant growth and reduced infection of *Foc* in rhizomes under greenhouse conditions. Several soil antagonistic bacteria, including *Pseudomonas fluorescens* (Saravanan *et al.*, 2003), *P. aeruginosa* (Ayyadurai *et al.*, 2006), *Burkholderia cepacia* (Pan *et al.*, 1997), and *Streptomyces* sp. (Getha *et al.*, 2005) have also been assessed for management of the disease. However, none of these have completely suppressed the disease, possibly because single antagonists are not effective against the Fusarium wilt pathogen (de Boer *et al.*, 2003). This has led to inconsistent performance of biocontrol agents and

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poor activity in all soil environments in which they are applied or against all pathogens that attack the host plants. To overcome these problems, application of combinations of biocontrol agents having multiple traits is advocated, particularly under field conditions where they are highly influenced by abiotic and biotic conditions (Duffy *et al.*, 1996; Raupach and Kloepper 1998; Guetsky *et al.*, 2001). Increasing the genetic diversity of the biological control system through the use of mixtures of microbes may result in treatments that persist longer in host rhizospheres and utilize several biocontrol mechanisms under a broad range of environmental conditions. Pierson and Weller (1994) showed that combinations of several fluorescent Pseudomonads have the potential for greater biocontrol activity against take all of wheat as compared to same strains applied individually. Similarly, Lemanceau *et al.* (1993) described increased suppression of Fusarium wilt of carnation by combining *Pseudomonas putida* WCS358 with nonpathogenic *Fusarium oxysporum* Fo47.

Hence, in the present study, an attempt was made to evaluate combinations of endophytic and rhizospheric bacteria for effective management of Fusarium wilt in banana. The objectives formulated were: i) to isolate native endophytic and rhizospheric bacteria; ii) to identify the effective bacteria having multiple functions against *Foc* under *in vitro* conditions; and iii) to assess the efficacy of individual and combined applications of these bacteria against Fusarium wilt of banana under *in vivo* conditions.

Materials and methods

Isolation and maintenance of the fungal pathogen

From naturally wilt affected banana samples (cv. Cavendish), *Foc* (VCG 0124 of race-1) was isolated and purified by single conidium isolation (Riker and Riker, 1936). Single conidium cultures were maintained in Potato Dextrose Agar (PDA) slants at 25ºC. Filter paper cultures of *Foc* were also prepared and stored at 4ºC.

Isolation of endophytic bacteria

Endophytic bacteria were isolated from different plant parts of banana germplasm accessions, which are maintained at the farm of the National Research Centre for Banana, Tiruchirapalli, Tamil Nadu, India. The samples of roots, corms, stems and petioles were washed thoroughly with tap water to remove dust and soil particles. Portions (5 g) of these samples were separately and individually immersed in 70% ethanol for 3 min, followed by washing with fresh sodium hypochlorite solution (2.5% available chlorine) for 5 min, rinsed with 70% ethanol for 30 s and subsequently washed five times with sterile distilled water. Aliquots of the distilled water used in the final rinse were spread on tryptic soy agar (TSA) in Petri plates to confirm the successful sterilization process, and that the isolates were of endophytic origin. The samples were then each macerated with 1 mL of sterile distilled water in a sterile mortar and pestle, and each sample was serially diluted in test tubes containing 9 mL of sterile distilled water. The dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were plated separately on King's B (KB) agar medium. The plates were then incubated at 28°C for 3 d and observed for bacterial growth. Each colony obtained was sub-cultured on KB medium and maintained at –80°C as a glycerol stock culture until further use.

Isolation of bacteria from rhizospheres

One gram of rhizospheric soil was collected from each different banana cultivar and transferred to a 250 mL conical flask containing 100 mL of sterile distilled water. The flask was placed in rotary shaker for 10 min at 120 rpm. From the soil solution, 1 mL of the supernatant was taken and serially diluted to 10^{-5} dilutions. One mL from 10^{-3}, 10^{-4}, 10^{-5} dilutions was poured separately at the centre of sterilized Petri plates. Sterilized KB agar medium was cooled to 45°C and 15 mL of the medium was poured into each plate and rotated clockwise and anticlockwise. The plates were then incubated at 28°C for 2 d and observed for bacterial colonies. Individual colonies were then purified by streaking onto plates containing KB agar medium. Glycerol stock cultures of the bacterial isolates were maintained at -80°C.

In vitro screening of endophytic and rhizospheric bacterial isolates for multiple biocontrol activity

The bacterial isolates were assessed for the following biocontrol activities against *Foc* (VCG0124): conidium germination (CSFT, 1943), mycelial growth inhibition in dual culture plates (Dennis and Webster, 1971) and production of volatiles (Dennis and Web-

ster, 1971), antifungal activity using the agar well diffusion method (Mehmood *et al.*, 1999), production of fungal cell wall degrading enzymes including proteases (Simbert and Krieg, 1994) and chitinase (Renwick *et al.*, 1991), siderophore production (Schwyn and Neilands, 1987), and HCN production. In addition, the plant growth promotion capabilities, including indole acetic acid (IAA) production (Brick *et al.*, 1991) and phosphate solubilization (Gaur, 1990), were also assessed for all of the bacterial isolates.

Pot trial evaluations of bacterial isolates for the suppression of Fusarium wilt

The six endophytic and four rhizospheric bacterial isolates which showed multiple functions such as biocontrol activities (inhibition of conidium germination and mycelial growth, production of volatile metabolites, chitinase, protease enzymes, HCN, and siderophore production) and plant growth promoting characters (phosphate solubilization and IAA) were selected for further screening for suppression of Fusarium wilt under pot culture conditions. Tissue-cultured banana plants (cv. Grand Naine), obtained from Jain irrigation, Udumalpet, Tamil Nadu, India, were used for all the pot experiments. The bacterial isolates were evaluated individually and in combinations, as described below.

Evaluation of individual bacterial isolates

Suspensions of individual endophytic bacterial isolates were prepared by centrifuging 48 h cultures at 10,000 rpm for 15 min. and adjusting to 10^8 cells mL^{-1} using distilled water. The roots of tissue-cultured banana plants (cv. Grand Naine) were immersed in the 500 mL of each bacterial suspension for 90 min and then planted into pots containing sand and soil (1:1 ratio). After 10 d, a sand/maize culture of *Foc* was applied at 30 g $plant^{-1}$. Five replications were maintained for each treatment, and *Foc* alone-inoculated plants were used as untreated experimental controls.

Similarly, for the evaluation of rhizospheric bacterial antagonists, talc-based formulations (as described by Vidhyasekaran and Muthamilan, 1995) of individual isolates (applied at 50 g $plant^{-1}$) were applied to the plants 1 week after planting. Sand/maize culture of *Foc* (30 g $plant^{-1}$) was applied 10 d after bacterial application. Five replications were maintained for each treatment, and *Foc* alone-inoculated plants were used as untreated experimental controls.

Three months after planting, the plants were assessed for height, girth, number of leaves, leaf area and the number of roots. Wilt severity (vascular discolouration) was assessed using a 1–6 score (Orjeda, 1998) (1 = corm completely clean, no vascular discolouration; 2 = isolated points of discolouration in vascular tissue; 3 = discolouration of up to one-third of vascular tissue; 4 = discoloration of below one-third and two-thirds of vascular tissue; 5 = discolouration of greater than two-thirds of vascular tissue; 6 = total discolouration of vascular tissue).

Evaluation of combined effects bacterial antagonists

Roots of tissue-cultured banana plants (cv. Grand Naine) were dipped in bacterial suspensions of effective endophytic bacterial isolates (10^8 cells mL^{-1}) for 90 min and the plants were then planted into pots. After 7 d, 50 g of talc-based formulation of bacterial isolates were applied. The plants were inoculated with *Foc* sand/maize culture (30 g $plant^{-1}$) 10 d after bacterial treatment. For each treatment, five replications were used, and *Foc* alone-inoculated plants were taken as untreated experimental controls. The assessments of plant growth parameters and wilt severity were recorded as described above.

Field evaluation of combined applications bacterial isolates

A field experiment was conducted in a wilt-sick field in the Theni district of Tamil Nadu, India, using cv. Grand Naine. Bacterial isolates found effective under pot culture conditions were assessed for suppression of Fusarium wilt. The soil was a red loam with a pH of 6.2. Average annual rainfall at the site is 1000 mm, average temperature ranges from of 20.3 to 38.6°C and relative humidity from 37 to 80%. The field was abandoned for commercial production due to severe wilt incidence. Tissue-cultured plants obtained from Jain Irrigation Pvt. Ltd. were planted (August 19, 2012) in the field with 2 × 2 m spacings between the plants. The talc powder formulations of five combinations of bacterial isolates were applied. These were: endophytic *Pseudomonas putida* (C4r4) + rhizospheric *Bacillus cereus* (Jrb1); endophytic *Acromobacter* spp. (Gcr1) + rhizospheric *Bacillus cereus* (Jrb5); endophytic *Rhizobium* spp.

(Lpr2) + rhizospheric *Bacillus* cereus (Jrb1); endophytic *Bacillus flexus* (Tvpr1) + rhizospheric *Bacillus cereus* (Jrb1) and endophytic *Bacillus flexus* (Tvpr1) + rhizospheric *Pseudomonas putida* (Jrb2). These treatments were applied (50 g plant^{-1} containing 10^8 cells g^{-1}) around the plants on three occasions, at the time planting, 2 months after planting and 4 months after planting. Control plants were treated only with talc powder formulation. Ten replications per treatment with four plants per replication were maintained. Timely applications of fertilizers, manures, water and other cultural operations were followed according to standard production practices. Observations on proportions (%) of infected plants, yield parameters including total number of banana hands, bunch weights, percentage plants yielding bunches and internal wilt disease scores (described above) were made at the time of banana harvest.

Population dynamics of bacteria in plant tissues and soil

Colonization of banana plant tissues

All the effective endophytic and rhizospheric bacterial isolates were developed as rifamycin resistant mutants as described by Xianling *et al.* (2010). Colonization of plant tissues by the rifamycin resistant mutants of endophytes were assessed at 0, 3, 5, 10, or 15 d after the application of bacterial antagonists to banana plants (cv. Grand Naine) under pot culture conditions. Whole plants were uprooted at each sampling time and the roots, corm, pseudostem and petiole of each plant were individually washed with tap water. One g of tissues taken from the roots, corm, pseudostem and petiole of each plant were surface sterilized with 3% sodium hyphochlorite for 1 min and then in 70% ethanol for 1 min. The samples were washed three times with sterile distilled water. To confirm that the sterilization process was successful, aliquots of the distilled water used in the final rinse were applied to KB plates. The plates were examined for bacterial growth after incubation at 28°C for 3 d. The samples were each macerated in 3 mL of sterile distilled water in a sterile mortar and pestle. Each sample was then serially diluted up to 10^{-5} and plated on KB medium in Petri plates. The plates were then incubated at 28 °C for 3 d and observed for bacterial growth, and each colony obtained was sub-cultured onto KB medium for reconfirmation (Sun *et al.*, 2011).

Quantification in soil

From pots where bacteria were applied, 1 g of rhizospheric soil was collected separately and added to 10 mL sterile distilled water in a 100 mL conical flask. The flask was placed on a rotary shaker for 10 min at 120 rpm. One mL of the soil suspension was then serially diluted up to 10^{-5} dilutions, and 1 mL from the 10^{-3}, 10^{-4}, or 10^{-5} dilution was added separately at the centre of sterilized Petri plates. Sterilized KB agar medium was cooled to 45°C and 15 mL was poured into each plate and which was then rotated clockwise and anticlockwise. The plates were then incubated at 28°C for 2 d and observed for bacterial colonies (King *et al.*, 1954).

Molecular identification of bacterial isolates

The genomic DNA from cultures of bacteria which showed complete suppression of Fusarium wilt was extracted using the method of Moore *et al.* (1987). The nearly full-length 16S rRNA gene of each isolate was amplified using primer FD1- 5'- AGT TTG ATC CTG GCT CA-3', RP2, - 5'-ACG GCT ACC TTG TTA CGA CTT-3', and the band of approx. 1400 bp size was excised from an agarose gel and purified using a Gen elute kit (Sigma laboratories, USA). Each purified gene was then sequenced. Forward and reverse sequences were then assembled within Bioedit and compared to sequences already available in the GenBank database using the BLAST algorithm (Altschul *et al.*, 1990). The sequence results showed that the 16S rRNA sequences of the endophytic bacterial isolates had BLAST homology as follows: C4r4, *Pseudomonas putida*; Gcr1, *Achromobacter* sp.; Lpr2, *Rhizobium* sp.; and Tvpr1, *Bacillus flexus*. The rhizospheric isolates had BLAST homology as follows: Jrb1, *Bacillus cereus*; Jrb2, *Pseudomonas putida*; and Jrb5, *Bacillus cereus*. The sequences of these potential biocontrol agents were deposited in GenBank (Accession numbers KJ131490 to KJ131496), and cultures of these isolates were deposited at Indian Type Culture Collection, New Delhi (*Pseudomonas putida* (C4r4)- ITCCBG0002; *Achromobacter* sp. (Gcr1)- ITCCBO0001; *Rhizobium* spp. (Lpr2)- ITCCBP0001; *Bacillus flexus* (Tvpr1)- ITCCBJ0004; *Bacillus cereus* (Jrb1)- ITCCBJ0006; *Pseudomonas putida* (Jrb2) –ITCCBG0003 and *Bacillus* cereus (Jrb5)- ITCCBJ0006.

The sequence of the *Foc* culture (VCG 0124 of race 1) isolated from banana cv. Cavendish used in this study was also deposited in GenBank (KJ131497) and in the ITCC (ITCC7441).

Statistical analyses

All the experiments were repeated at least once for confirmation of the results. All the data on the effect of bacterial isolates against *Foc* pathogen were analyzed by multivariate analysis (MANOVA). The effects of bacterial isolates on plant growth parameters and root numbers were analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. The effect of bacterial isolates on disease severity was analyzed by chi-square tests and the treatment means were compared by DMRT at $P \leq 0.05$. The data of inhibition of conidium germination, percentage colonization, percent of plants infected with *Foc* and percent of plants yielding good bunches were arcsine transformed before analysis (Gomez and Gomez, 1984). Percentage tissue colonization was further subjected to analysis of variance (ANOVA) for roots, corms, pseudostems and petioles. The package used for statistical analyses was IBM SPSS statistics version 21 developed by the International Business Machines Corporation.

Results

Isolation and screening of bacterial isolates

A total of 71 endophytic (57 from roots and 14 from corms), and 37 rhizospheric bacterial isolates, belong to different genomic groups, were obtained from different banana accessions. All of these isolates were screened for bio-control activity against *Foc* and also for plant growth promoting characters under *in vitro* conditions.

Endophytic bacteria

The *in vitro* screening of endophytic bacteria against *Foc* revealed that only six out of the 71 isolates were effective. These were *Pseudomonas putida* (C4r4), *Achromobacter* spp. (Gcr1), *Rhizobium* sp. (Klr4), *Ochromobactrum* sp. (Klc2), *Rhizobium* sp. (Lpr2) and *Bacillus flexus* (Tvpr1). These six isolates gave 98–100% inhibition of *Foc* conidium germination, 68–83% inhibition of mycelial growth of *Foc* in dual culture plates and 100% inhibition of *Foc* mycelial growth in agar well diffusion tests. All these six isolates also produced siderophores (5–10 mm lytic zone) and protease enzymes (5–13 mm lytic zone). However, only four of these isolates, *P. putida* (C4r4), *Ochromobactrum* sp. (Klc2), *Rhizobium* sp. (Lpr2) and *B. flexus* (Tvpr1), were able to produce chitinase (10–12 mm lytic zone), while three, *P. putida* (C4r4), *Achromobacter* sp. (Gcr1), and *Bacillus flexus* (Tvpr1), produce hydrogen cyanide. With regard to plant growth promoting characters, five of the isolates, including *P. putida* (C4r4), *Achromobacter* sp. (Gcr1), *Ochromobactrum* sp. (Klc2), *Rhizobium* sp. (Lpr2), and *B. flexus* (Tvpr1) produced IAA (5–40 $\mu g\ mL^{-1}$), and two of the isolates, *Achromobacter* sp. (Gcr1) and *B. flexus* (Tvpr1), solubilized phosphate producing lytic zones of 5 mm diameter (Table 1).

Rhizospheric bacteria

The results of *in vitro* screening of rhizospheric bacterial isolates for multiple functions (biocontrol activity and plant growth promotion) indicated that only four isolates (*B. cereus* Jrb1, *P. putida* Jrb2, *Bacillus* sp. Jrb6 and *Bacillus* sp. Jrb7) were effective against *Foc* (data not presented). These four isolates gave 95–100% inhibition of conidium germination, 30–50% inhibition of mycelial growth of the *Foc* in dual plate assays and 100% inhibition of mycelial growth in antifungal assays. All of these isolates also produce siderophores, proteases and chitinase. Three of these isolates (*B. cereus* (Jrb1), *P. putida* (Jrb2) and *Bacillus* sp. (Jrb6)) produced IAA (1-3 $\mu g\ mL^{-1}$), and one isolate (*P. putida* (Jrb2)) produced hydrogen cyanide. None of the rhizospheric isolates solubilized phosphate (Table 2).

Pot culture evaluation of bacterial isolates

Individual effects

Individual soil application of four rhizospheric bacterial isolates significantly decreased mean internal wilt severity scores compared to *Foc* alone-inoculated control plants. Among these isolates, *B. cereus* (Jrb1) gave the lowest internal wilt score of 2.0, while isolate *P. putida* (Jrb2) and *Bacillus* sp. (Jrb7) each gave internal wilt scores of 3.0. *Foc* alone-inoculated plants had a mean score of 5.0. These bacterial isolates also significantly increased the plant growth parameters, including plant height (92% increase), girth (up to +80%), number of leaves (up to +52%) and number of roots (up to +101%) compared to the *Foc* alone-inoculated control plants (data not presented).

In the case of endophytic bacterial isolates, the individual applications of *B. flexus* (Tvpr1) and *Rhizo-*

Table 1. *In vitro* screening of endophytic bacterial isolates against *Fusarium oxysporum* f.sp. *cubense* (VCG-0124).

Endophytric bacterial strain	Inhibition of conidium germination (%)	Mycelial inhibition by dual culture (%)	Siderophore prodn- lytic zone (mm)	Chitinase prodn- lytic zone (mm)	Mycelial inhibition by antifungal assay (%)	HCN prodn	IAA prodn (μgmL^{-1} of culture filtrate)	Protease prodn. (mm)	Po4 solubilization lytic zone (mm)
Pseudomonas putida (C4r4)	100a	83.3a	5c	11a-b	100	+ve	12c	13a	0b
Achromobacter sp (Gcr1)	100a	70b-c	8b	0c	100	+ve	12c	10b	5a
Rhizobium sp (Klr4)	100a	67.8c	5c	0c	100	-ve	0e	13a	0b
Ochrobactrum sp (Klc2)	100a	67.8c	10a	12a	100	-ve	5d	10b	0b
Rhizobium sp (Lpr2)	100a	70b-c	7b	10b	100	-ve	40a	10b	0b
Bacillus flexus (Tvpr1)	98b	71.4b	10a	12a	100	+ve	18b	5c	5a
Control	0c	0d	0d	0c	0	0	0e	0d	0b

Values are means of three replications. Means within a column that are followed by the same letter are not significantly different (DMRT at $P \leq 0.05$).

Table 2. *In vitro* screening of rhizospheric bacterial isolates against *Fusarium oxysporum* f.sp. *cubense* (VCG-0124).

Rhizospheric bacterial isolates	Inhibition of conidium germination (%)	Inhibition of mycelial growth by dual culture (%)	Siderophore prodn - lytic zone (mm)	Chitinase prodn- lytic zone (mm)	Mycelial inhibition by antifungal assay	HCN	IAA prodn. (μgmL^{-1} of cultute filtrate)	Protease prodn. (mm)	Po4 solubilization (mm)
Bacillus cereus (Jrb1)	100a	50.0a	10b	10b	100	-ve	1b	3b	0
Pseudomonas putida (Jrb2)	100a	46.6b	15a	10b	100	+ve	1b	12a	0
Bacillus cereus (Jrb5)	95.3c	30.0d	10b	15a	100	-ve	3a	3b	0
Bacillus sp (Jrb7)	98.0b	43.3c	13a	10b	100	-ve	0c	12a	0
Control	0d	0e	0c	0c	0	0	0c	0b	0

Values are means of three replications. Means within a column that are followed by the same letter are not significantly different (DMRT at $P \leq 0.05$).

bium sp. (Klr4) gave the lowest mean internal wilt score of 1.4, followed by the four other isolates *P. putida* (C4r4), *Achromobacter* sp. (Gcr1), *Ochromobactrum* sp. (Klc2) and *Rhizobium* sp. (Lpr2) which gave mean scores of 2.0, when compared to *Foc* alone-inoculated plants (mean score of 5.0). These isolates also significantly increased plant growth parameters, including plant height (up to +70%), girth (up to +197%), number of leaves (up to +70%) and number of roots (up to +180%), compared to *Foc* alone-inoculated control plants (data not shown).

Combined effects

Among different combinations of bacterial isolates tested, the combined application of endophytic bacteria *P. putida* (C4r4) + rhizospheric *B. cereus* (Jrb1); endophytic *Acromobacter* sp (Gcr1) + *B. cereus* (Jrb5); endophytic *Rhizobium* sp (Lpr2) + rhizospheric *B. cereus* (Jrb1); endophytic *B. flexus* (Tvpr1) + rhizospheric *B. cereus* (Jrb1) and endophytic *B. flexus* (Tvpr1) + rhizospheric *P. putida* (Jrb2) recorded total suppression (100%) of Fusarium wilt (mean severity score 1.0 = healthy). These combinations also gave significant increases in the plant growth parameters, including plant height (up to +103%), girth (up to +116%), number of leaves (up to +107%) and number of roots (up to +99%) compared to *Foc* alone-inoculated control plants (Table 3).

Field evaluation

In general, soil application of all the endophytic and rhizospheric bacterial isolate combinations gave significant reductions of Fusarium wilt (mean disease score 1.8 to 2.3) as compared to untreated control plants [mean score 5.1 (Figure 1)]. Treatment of all these combinations also significantly increased the banana yield parameters, including

Table 3. Mean host plant parameters and Fusarium wilt severity scores from combined applications of endophytic and rhizospheric bacterial isolates in banana cv. Grand Naine (VCG-0124), assessed in pot experiments.*

Endophytic + rhizospheric bacterial isolates	Height (cm)	Girth (cm)	Total No. of leaves	Total No. of roots	Internal wilt disease score (1–6 scale)
Pseudomonas putida (C4r4) + *Bacillus cereus* (Jrb1)	26.2abc (72.37)	10.4a (108.0)	6.2a (106.67)	20.2abc (44.29)	1.0a
Achromobacter sp. (Gcr1) + *Bacillus cereus* (Jrb1)	26.8ab (76.32)	10.0ab (100.0)	4.6bc (53.33)	20.0abc (42.86)	1.2b
Achromobacter sp. (Gcr1) + *Bacillus cereus* (Jrb5)	22.2bcde (46.05)	8.5bc (70.0)	4.2cd (40.00)	14.6c (4.29)	1.0a
Rhizobium sp. (K lr4) + *Pseudomonas putida* (Jrb2)	24.8abcd (63.16)	9.2abc (84.0)	5.2ab (73.33)	26.2a (87.14)	1.0a
Rhizobium sp. (Lpr2) + *Bacillus cereus* (Jrb1)	18.8de (23.68)	7.8c (56.0)	5.4a (80.00)	23.4ab (67.14)	1.0a
Bacillus flexus (Tvpr1) + *Bacillus cereus* (Jrb1)	30.2a (98.68)	9.0abc (80.0)	3.6de (20.00)	19.2abc (37.14)	1.0a
Bacillus flexus (Tvpr1) + *Pseudomonas putida* (Jrb2)	25.8abcd (69.74)	9.0abc (80.0)	4.0cd (33.33)	22.0ab (57.14)	1.0a
Control (without anything)	19.4 cde (27.63)	5.4d (8.0)	6.0a (100.00)	17.0bc (21.43)	1.0a
Foc alone inoculated control	15.2e (0.0)	5.0 d (0.0)	3.0e (0.0)	14.0c (0.0)	5.0c

Values are means of five replications. Figures in parenthesis are percent increases over *Foc* pathogen alone-inoculated experimental controls.

Means within a column that are followed by the same letter are not significantly different (DMRT at $P \leq 0.05$).

* Data in this table are for the effective bacterial isolates and not for all the bacterial combinations tested.

Figure 1. The internal symptom of vascular discolouration in corm tissues of field-grown banana plants of cv. Grand Naine, in untreated control plants (A) and bacterial isolates-treated plants (B). The plant in B was treated with endophytic *Bacillus flexus* (TvPr1) + rhizospheric *Bacillus cereus* (Jrb1).

Table 4. Field evaluation of combined applications of endophytic (endo.) and rhizospheric (rhizo.) bacterial isolates for the suppression of Fusarium wilt in banana cv. Grand Naine

Treatment details	Internal wilt disease score (1–6 scale)	Plants infected with Fusarium wilt (%)	Plants harvested with good bunches (%)	No. of hands/ bunch	Bunch weight (kg)
Endo. *Pseudomonas putida* (C4r4) + rhizo. *Bacillus cereus* (Jrb1)	2.2 a [a]	72.2 b	88.9 b	12.3 a (151) [b]	26.4 a (210.6)
Endo. *Achromobacter* sp. (Gcr1) + *Bacillus cereus* (Jrb5)	2.0 a	57.8 a	84.5 b	12.5 a (155.1)	26.1 a (207)
Endo. *Rhizobium* sp. (LPr2) + *Bacillus cereus* (Jrb1)	2.1 a	61.1 a	88.9 b	12.5 a (155.1)	26.7 a (214.1)
Endo. *Bacillus flexus* (TvPr1) + rhizo. *Bacillus cereus* (Jrb1)	1.8 a	61.1 a	94.4 a	12.2 a (148.9)	26.0 a (205.8)
Endo. *Bacillus flexus* (TvPr1) + rhizo. *Pseudomonas putida* (Jrb2)	2.3 a	70.5 b	82.3 b	12.5 a (155.1)	26.3 a (209.4)
Control (untreated)	5.1 b	100.0 c	52.6 c	9.7 b (0.0)	8.5 b (0.0)

[a] Values are means of 10 replications. Means within a column that are followed by the same letter are not significantly different (DMRT at $P \leq 0.05$).
[b] Figures in parentheses are the percent increases over untreated experimental control plants

number of banana hands (up to 155% more), bunch weight (up to 214% greater) and percent of plants yielding good bunches (82 to 94% increase) than the untreated control plants. The percentage of plants affected by Fusarium wilt was also significantly less (61 to 72% fewer) when compared to control plants,

of which all of the plants were affected by the disease (Table 4).

Colonization of banana tissues and quantification of rifampicin resistant bacterial isolates

The colonization study clearly showed that among the endophytic bacterial isolates, *P. putida* (C4r4), *Achromobacter* sp. (Gcr1) colonized root tissues after 3 d, corms after 10 d, and stem and petiole tissues after 15 d. *Rhizobium* sp. (Lpr2) and *B. flexus* (Tvpr1) colonized only root and corm tissues, respectively, after 3 and 10 d. The rhizospheric bacterial isolates did not colonize root, corm, stem or petiole tissues. In general, the population of these endophytes was 10^5 cfu g^{-1} of tissues up to 20 to 35 d after application, and thereafter the bacteria were mostly not detected. The exception was *P. putida* (Jrb2), which was detected up to 40 d in root, corm and stem tissues (data not presented).

With regard to quantification of rifampicin resistant bacterial isolates in sterile soil, the endophytic and rhizospheric bacteria were detected at up to 10^5 cfu g^{-1} of soil until 30 d after inoculation. Thereafter, the populations decreased to 10^4 cfu g^{-1} of soil at 40 d after application, and no bacteria were detected after this period. (data not presented).

Discussion

The Fusarium wilt pathogen of banana can survive in soil for several years as chlamydospores, and enters host plants through roots. It is therefore important to identify a mixture of antagonists against the pathogen, which can reside and act both in the host rhizospheres and endophytically in plants. Several previous studies have been carried out on the use of mixtures of microbes possessing multiple functions, but very little research has been done on the selection of mixtures of antagonists, which can act both in rhizospheres and endophytically. Hence, the present study, attempted to identify mixtures of microbes, which could act in both domains, with multiple functions (including biocontrol activity and plant growth promotion), and to evaluate these organisms for suppression of Fusarium wilt in banana.

Six endophytic and four rhizospheric bacterial isolates were shown to be effective *in vitro* inhibitors of mycelial growth and conidium germination of *Foc*. This could be due to direct action by production of antibiotics, lytic enzymes such as chitinase and protease, and/or hydrogen cyanide by these isolates. The presence of plant growth promoting characters such as phosphate solubilization and IAA production was also demonstrated. This is in accordance with the findings of Akila *et al.* (2011) who reported that the plant growth promoting rhizobacterial (PGPR) strains Pf1 and TRC 54 were effective in reducing the mycelial growth of *Foc*. They concluded that the observed inhibition might be due to direct action of the enzymes and antibiotics produced by PGPR. O'Sullivan *et al.* (1992) and Nagrajkumar *et al.* (2004) also reported that the production of siderophores, other secondary metabolites and lytic enzymes by *Pseudomonas* strains may be responsible for the effective control of plant root pathogens including *F. oxysporum* and *R. solani*.

Successful colonization of host rhizospheres and plant tissues by introduced microbes is very important for effective control of soilborne pathogens, including the Fusarium wilt pathogen of banana (*Foc*) (Ploetz, 2005). The present study revealed that the endophytic bacterial isolates colonized plant tissues (roots, corms, pseudostems and petioles) within 15 d after inoculation, whereas the rhizospheric isolates did not colonize plant tissues. However, all the endophytic and rhizospheric isolates survived in rhizospheres and maintained populations of 10^5 cfu g^{-1} of soil for up to 35 d after application. Similarly, Sun *et al.* (2011) demonstrated that the *Bacillus subtilis* strain KY-21 was detected in banana root, corm and pseudostem tissues until 20 d after inoculation.

The pot trial evaluation in cv. Grand Naine showed that only the endophytic *B. flexus* (Tvpr1) and *Bacillus* sp. (Klr4) recorded maximum reduction of wilt severity (disease score 1.4), and also enhanced plant growth parameters compared to *Foc* alone-inoculated plants. This could be due to the greater biocontrol activities (inhibition of conidium germination and mycelial growth, production of volatile metabolites, hydrogen cyanide, siderophores and/or lytic enzymes) and plant growth promoting traits (IAA production and phosphate solubilization) exhibited by these isolates. Several reports have previously demonstrated the use of different species of *Trichoderma, Pseudomonas, Streptomyces*, or non-pathogenic *Fusarium* (np*Fo*), of both rhizospheric and endophytic origin, against Fusarium wilt under glasshouse and field conditions (Sivamani and Gnana-

manickam, 1988; Lemanceau and Alabouvette, 1991; Thangavelu *et al.*, 2001; Nel *et al.*, 2006; Fishal *et al.*, 2010; Gopalakrishnan *et al.*, 2011; Sun *et al.*, 2011). However, none of these studies have reported complete suppression of Fusarium wilt by employing these antagonistic microbes.

Similarly in the present study although significant suppression of Fusarium wilt was achieved by employing the effective bacterial isolates individually, the ultimate aim of complete control of the disease could not be achieved. Therefore, combinations of bacterial strains containing both rhizospheric and endophytic bacterial isolates were tested after confirming their compatibility *in vitro*. Compatible interactions are an important pre-requisite for successful development of an integrated approach for the control of plant diseases (Baker, 1990). Moreover, by combining antagonistic microbes, multiple antifungal traits and plant growth promoting traits can also be combined. Under these circumstances, at least one biological control mechanism is likely to be functional under the varied environmental conditions faced by the released biocontrol agents. It is also possible that by combining both rhizospheric and endophytic microbes, the antagonists can reside and act against the Fusarium wilt pathogen in host rhizospheres and also as endophytes inside host tissues. In this study, a total of 24 different combinations of rhizospheric and endophytic bacterial isolates were evaluated under pot trial conditions. Results showed that the treatment of banana plants with the endophytic bacterial isolate *Pseudomonas putida* (C4r4) + rhizospheric *Bacillus cereus* (Jrb1); endophytic *Acromobacter* spp. (Gcr1) + *Bacillus cereus* (Jrb5); endophytic *Rhizobium* spp. (Lpr2) + rhizospheric *Bacillus cereus* (Jrb1); endophytic *Bacillus flexus* (Tvpr1) + rhizospheric *Bacillus cereus* (Jrb1) and endophytic *Bacillus flexus* (Tvpr1) + rhizospheric *Pseudomonas putida* (Jrb2) gave complete suppression of Fusarium wilt. Similarly, the field evaluation of all these five combinations of bacterial isolates, in an area of severe disease risk, demonstrated that soil application of these bacterial combinations gave effective suppression of Fusarium wilt in cv. Grand Naine. The treatments also increased banana yield parameters including bunch weight. These bacterial combinations decreased the percent of Fusarium wilt infected plants, and also increased the proportion of banana plants yielding good bunches in the field. This is similar to the findings of Sundaramoorthy *et al.* (2012), who reported that among different bacterial bio-formulations, the combined application of rhizospheric and endophytic bacterial strains (talc-based bio-formulation of *P. fluorescens* (Pf1) and *B. subtilis* (EPCO16 and EPC5) strains) reduced Fusarium wilt incidence in chilli pepper by 17–30% compared to untreated plants. Similarly, the production of siderophores, other secondary metabolites and lytic enzymes by *Pseudomonas* strains was most effective in controlling plant root pathogens including *F. oxysporum* and *R. solani* (O'Sullivan *et al.*, 1992; Nagarajkumar *et al.*, 2004). Several other reports have documented that the use of biocontrol agents in combinations were more effective than individual agents for management of plant diseases (de Boer *et al.*, 2003; Domenech *et al.*, 2006; Thilagavathi *et al.*, 2007; Ganeshmoorthi *et al.*, 2008; Latha *et al.*, 2009).

In the present investigation, soil application of the effective bacterial combinations also promoted plant growth parameters compared to other bacterial combinations. This could be due to combined effects of suppression of Fusarium wilt by different biocontrol activities and plant growth promoting characters. Similarly, Ayyadurai *et al.* (2006) also demonstrated that the strain FP10 increased banana plant height and reduced vascular discoloration, compared with *Foc* alone-inoculated plants. Although the combined application of endophytic and rhizospheric bacterial antagonists have resulted in the complete suppression of Fusarium wilt, the modes of action of these biocontrol agents both in soil and in plants have yet to be fully assessed. This will provide increased understanding of the interactions between biocontrol agents and *Foc* in banana.

We have demonstrated that the combined application of endophytic and rhizospheric bacterial strains possessing multiple functions of both biological control activity and plant growth promotion characters resulted in effective suppression of Fusarium wilt of banana, both in pot trials and in the field. The field trial was in an area of severe wilt potential, in the Tamil Nadu region where the disease incidence was very high in cv. Grand Naine. It was also observed that these bacterial antagonists were able to survive in the rhizosphere for up to 35 d, and colonized root, corm, stem and petiole tissues within 15 d after inoculation. These studies have therefore provided useful information to assist determination of the intervals required to replenish biocontrol agents,

as well as the selection of biocontrol agents for the successful suppression of a very important disease of banana.

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Literature cited

Akila R., L. Rajendran., S. Harish., K. Saveetha., T. Raguchander and R. Samiyappan, 2011. Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) causing Fusarium wilt in banana. *Biological control* 57, 175–183.

Altschul S.F., W. Gish, E.W. Miller and D.L. Myers Lipman, 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215, 403–410.

Ayyadurai N., P. Ravindra Naik., M. Sreehari Rao., R. Sunish Kumar., S.K.M. Samrat Manohar and N. Sakthivel, 2006. Isolation and characterization of a novel banana rhizosphere bacterium as fungal antagonist and microbial adjuvant in micro propagation of banana. *Journal of Applied Microbiology* 100, 926–937.

Baker P., 1990. Biological control of the coffee berry borer, *CARAPHIN News*, No. 2., IICA, Trinidad and Tobago.

Berg G., A. Fritze., N. Roskot and K. Smalla, 2001. Evaluation of potential biocontrol rhizobacteria from different host plants of *Verticillium dahliae* Kleb. *Journal of Applied Microbiology* 91, 963–971.

Brick J.M., R.M. Bostock and S.E. Silverstone, 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Applied Environmental Microbiology* 57, 535–538.

CSFT, 1943. Committee on standardization of Fungicidal test. Test tube dilution for uses with the slide germination method of evaluating protectant fungicides *The American Phytopathological technique* 37, 354–356.

de Boer M., P. Bom., F.J.J.B. Kindt Keurentjes., I. Vandersluis., L.C. Van Loon and P.A.H.M. Bakker, 2003. Control of Fusarium wilt of radish by combining *Pseudomonas putida* strains that have different disease- suppressive mechanisms. *Phytopathology* 93, 626–632.

Dennis C. and J. Webster, 1971. Antagonistic properties of species groups of *Trichoderma* III. Hyphal interaction. *Transactions of British Mycological Society* 57, 363–369.

Domenech J., M.S. Reddy., J.W. Kloepper., B. Ramos and J. Gutierrez-Manero, 2006. Combined application of the biological product LS213 with *Bacillus, Pseudomonas* or *Chryseobacterium* for growth promotion and biological control of soil-borne diseases in pepper and tomato. *BioControl* 51, 245–258.

Duffy B.K., A. Simon and D.M. Weller, 1996. Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. *Phytopathology* 86,188–194.

Fishal E.M.M., S. Meon and W.M. Yun, 2010. Induction of tolerance to Fusarium wilt and defense-related mechanisms in the plantlets of susceptible Berangan banana pre-Inoculated with *Pseudomonas* sp. (UPMP3) and *Burkholderia* sp. (UPMB3). *Agricultural Sciences in China* 9, 1140–1149.

Ganeshmoorthi P., T. Anand., V. Prakasam., M. Bharani and N. Ragupathi, 2008. Plant Growth Promoting Rhizobacteria (PGPR) bioconsortia mediates induction of defense related proteins against infection of root rot pathogen in mulberry plants. *Journal of Plant Interactions* 3, 233–244.

Gaur A.C, 1990. *Phosphate solubilizing microorganisms as biofertilizer*. New Delhi, Omega Scientific Publications, 176 pp.

Getha K., S.Vikineswary., W.H. Wong., T. Seki., A. Ward and M. Goodfellow, 2005. Evaluation of *Streptomyces* sp. for suppression of *Fusarium* wilt and rhizosphere colonization in pot grown banana plantlets. *Journal of Microbiology and Biotechnology* 32, 24–32.

Gomez XX and XX Gomez, 1984. Statistical procedures for agricultural research. John Wiley and Sons, New York.

Gopalakrishnan S., P. Humayun., B.K. Kiran., G.K. Kannan., M., SreeVidya., K. Deepthi and O. Rupela, 2011. Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot sorghum caused by *Macrophomia phaseolina* (Tassi) Goid. *World Journal of Microbiology and Biotechnology* 72, 1313–1321.

Guetsky R., D. Shtienberg., Y. Elad and A. Dinoor, 2001. Combining biocontrol agents to reduce the variability of biological control. *Phytopathology* 91, 621–627.

Harish S., M. Kavino., N. Kumar., P. Balasubramanian and R. Samiyappan, 2009. Induction of defense-related proteins by mixtures of plant growth promoting endophytic bacteria against Banana bunchy top virus. *Biological Control* 51, 16–25.

King E.O., M.K.Ward and D.E. Raney, 1954. Two simple media for demonstration of pycocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine* 14, 301–307.

Latha P., T. Anand., N. Ragupathi., V. Prakasam and R. Samiyappan, 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biological Control* 50, 85–93.

Lemanceau P and C. Alabouvette, 1991. Biological control of Fusarium diseases by fluorescent *Pseudomonas* and non-pathogenic Fusarium. *Crop Protection* 10, 279–286.

Lemanceau P., P.A. Bakker., H.M. Dekogel., W.J. Alabouvette and B. Schippers, 1993. Antagonistic effect of non pathogenic *Fusarium oxysporum* Fo47pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*. *Applied Environmental Microbiology* 59, 74–82.

Mehmood Z., I. Ahmad., F. Mohammad and S. Ahmad, 1999. Indian medicinal plants: a potential source of anticandidal drug. *Pharmaceutical Biology* 37, 237–242.

Moore R.E., C. Cheuk., X.Q.G. Yang., G.M.L. Patterson., R. Bonjouklian., T.A. Smitka., J.S. Mynderse., R.S. Foster.,

N.D. Jones., J.K. Swartzendruber and J.B. Deeter, 1987. Hapalindoles, antibacterial and anti-mycotic alkaloids from the Cyanophyte *Hapalosiphon fontinalis*. *Journal of Organic Chemistry* 52, 1036–1043.

Nagarajkumar M., R. Bhaaskaran and R. Velazhahan, 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath of blight pathogen. *Microbiology Research* 159, 73–81.

Nel B., C. Steinberg., N. Labuschage and A. Viljoen, 2006. Isolation and characterization of nonpathogenic *Fusarium oxysporum* isolates from the rhizosphere of healthy banana plants. *Plant Pathology* 55, 207–216.

Orjeda G, 1998. *Evaluation of Musa germplasm for resistance to Sigatoka diseases and Fusarium wilt*. INIBAP Technical Guidelines 3. International Plant Genetic Resources Institute, Rome, Italy, 29 pp.

O'Sullivan M., P.M. Stephens and F. O'Gara, 1992. Extracellular protease production by fluorescent *Pseudomonas* spp. and the colonization of sugar beet roots and soil. *Soil Biology and Biochemistry* 23, 623–627.

Pan M.J., S. Rademan., K. Kunert and J.W. Hastings, 1997. Ultrastructural studies on the colonization of banana tissue and *Fusarium oxysporum* f.sp. *cubense* race 4 by the endophytic bacterium *Burkholderia cepacia*. *Journal of phytopathology* 145, 479–486.

Pierson E.A and D.M. Weller, 1994. Use of mixtures of fluorescent Pseudomonads to suppress take-all and improve the growth of wheat. *Phytopathology* 84, 940–947.

Ploetz R.C., J.E. Thomas and W. Slaubaugh, 2003. Diseases of banana and plantain. In: *Diseases of tropical fruit crops* (R.C. Ploetz, ed.). CABI Publishing. Wallingford, Oxon, UK. pp. 73–134.

Ploetz R.C, 2005. Panama disease, an old enemy rears its ugly head: Parts 1 and 2. In: *Plant Health Progress*, APSnet: Online doi: 10.1094/PHP-2005-1221-01-RV

Raupach G.S and J.W. Kloepper, 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology* 88, 1158–1164.

Renwick A., R. Campbell and S. Coe, 1991. Assessment of *in vivo* screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. *Plant Patholology* 40, 524–532.

Ricker A.J and R.S. Ricker, 1936. *Introduction to research on plant diseases*. Johns Swift Co. Mc., New York, pp.117.

Saravanan T., M. Muthusamy and T. Marimuthu, 2003. Development of integrated approach to manage the fusarial wilt of banana. *Crop Protection* 22, 1117–1123.

Schwyn B. and J.B. Neilands, 1987. Universal chemical assay for the detection and determination of siderophores. *Annals of Biochemistry* 160, 47–56.

Simbert R.M and N.R. Krieg, 1994. Phenotypic characterization. In: *Methods for general and molecular bacteriology Vol. 5*, (Gerhardt P., Murray R.G., Wood W.A., Krieg N.R., eds). American Society for Microbiology, Washington DC. pp. 611–654.

Sivamani E. and S. Gnanamanickam, 1988. Presence of race-4 of *Fusarium oxysporum* f.sp. *cubense* in India. *Current Science* 57, 1251–1252.

Smith L., D.O. Keef., M. Smith and S. Hamill, 2003. The benefits of applying rhizobacteria to tissue cultured bananas. *Banana Topics Newsletter* 33, 1–4.

Sun J.B., M. Peng., Y.G. Wang., P.J. Zhao and Q.Y Xia, 2011. Isolation and characterization of antagonistic bacteria against Fusarium wilt and induction of defense related enzymes in banana. *African Journal of Microbiology Research* 5, 509–515.

Sundaramoorthy S., T. Raguchander., N. Ragupathi and R. Samiyappan, 2012. Combinatorial effect of endophytic and plant growth promoting rhizobacteria against wilt disease of *Capsicum annum* L. caused by *Fusarium solani*. *Biological Control* 60, 59–67.

Thangavelu R. and M.M. Mustaffa, 2010. First report on the occurrence of a virulent strain of Fusarium wilt pathogen (Race-1) infecting Cavendish (AAA) group of bananas in India. *Plant Disease* 94, 1379.

Thangavelu R., A. Palaniswami., G. Ramakrishman., S. Doraiswamy., S. Muthukrishnan and R. Velazhahan, 2001. Involvement of Fusaric acid detoxification by *Pseudomonas fluorescens* strain Pf10 in the biological control of Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense*. *Journal of Plant Disease and Protection* 108, 433–445.

Thilagavathi R., D. Saravanakumar., N. Ragupathi and R. Samiyappan, 2007. A combination of biocontrol agents improves the management of dry root rot (*Macrophomina phaseolina*) in greengram. *Phytopathology Mediterranea* 46, 157–167.

Vidhyasekaran P. and M. Muthamilan, 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Disease* 79, 782–786.

Xianling J.I., L.U. Guobing., Yingping Gai., L.U. Huijv Gao., Baoyun Lingrang Kong and M.U. Zhimei, 2010. Colonization of *Morus alba* L. by the plant-growth-promoting and antagonistic bacterium *Burkholderia cepacia* strain Lu 10 – 1. *BMC Microbiology* 10, 243.

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