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RESEARCH ARTICLE

Microbe-enriched farm yard manure (MFYM) approach for the suppression of *Ralstonia solanacearum* Yabuuchi (Smith) inciting bacterial wilt disease in eggplant (*Solanum melongena* L.)

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Abstract

Purpose Soil-borne bacterial wilt disease caused by the bacterial plant pathogen *Ralstonia solanacearum* is a serious concern worldwide, resulting in huge economic losses in solanaceous vegetables. This study confirms the field efficacy of microbe-enriched organic fertilizer application in successful management of eggplant bacterial wilt disease.

Methods Four potential *Bacillus* strains isolated from diverse soils of Andaman Islands were evaluated using the microbe-enriched farmyard manure (MFYM) approach for bacterial wilt suppression and yield increase in both the greenhouse and field conditions. Also, changes in the bacterial wilt pathogen and putative *Bacillus* counts on MFYM applications were studied.

Results *Bacillus* strains used in this study were confirmed for their indole acetic acid (IAA) production, phosphate solubilization, and siderophore production traits in vitro. In addition, species identity was confirmed through rpoB gene sequences analysis. In both greenhouse and field experiments, MFYM treatments showed better biological control potential and increase in yield compared to farmyard manure (FYM) alone and unamended control treatments. In comparison to control and other treatments, the MIC-Consortia demonstrated the highest biocontrol potential across all treatments in pot culture (90.6%), first field (78.8% and 72.1%), and second field (61.5% and 75%) studies, respectively. Similarly, the MIC-Consortia showed highest yield in the first field (61.5% and 75%) and second field (60% and 48.1%), respectively, when compared to

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other treatments. Further, the studies of microbial counts in treated soils revealed that the pathogen population increased throughout the cropping season, but the rate of increase was found slower in MFYM treatments.

Conclusion This study showed that the MIC-Consortia when applied along with FYM could effectively suppress eggplant bacterial wilt disease incidence and enhance yield performance in two different field conditions over two successive years. The results could be used in devising efficient eco-friendly management strategies for bacterial wilt disease in organic farming practices.

Keywords Eggplant · Wilt · *Ralstonia solanacearum* · Microbial enrichment · FYM management

Introduction

Ralstonia solanacearum Yabuuchi (Smith) is a gram-negative, soil-borne bacterium that belongs to the *R. solanacearum* species complex in the family Burkholderiaceae, order Burkholderiales and the class Betaproteobacteria (Yabuuchi et al. 1995; Paudel et al. 2020) with high phenotypic and genotypic diversity (Palleroni and Doudoroff 1971). It has a wide host range of more than 450 plant species in about 50 families (Hayward 1991; Lei et al. 2020). The bacterium normally enters the plant roots through wounds and spreads to the xylem vessels, where it multiplies (10^{10} cells/cm of stem) and produces a large number of exo-polysaccharides, which leads to clogging of vessels and thereby death of host cells (Denny 2006).

The bacterial wilt disease is a major threat for solanaceous vegetable production worldwide. In the eggplant, bacterial wilt can cause up to 100% yield loss, resulting in significant economic losses for farmers (Sakthivel et al. 2016). Achieving complete control of bacterial wilt is a serious challenge due to the complex nature of the pathogen as it could survive in soil for several years in association with infested plant debris from a wider range of plant hosts (Yuan et al. 2014; Messiha et al. 2009). Till date, various strategies have been recommended for the management of bacterial wilt disease. These include crop rotation with alternate hosts and use of chemical fumigants, soil solarization, biological soil disinfestation, resistant cultivars, transgenic plants, and naturally or genetically modified microbes (Gamliel et al. 2000; Boulter et al. 2000; Brimner and Boland 2003; Yi et al. 2007; Messiha et al. 2007a, b, c; Lemessa and Zeller 2007; Yadessa et al. 2010).

However, the usage of genetically modified materials may raise ethical and environmental health issues (Lemessa and Zeller 2007). Soil solarization suggested by Tamietti and Valentino (2006) and biological soil disinfestations are other effective strategies, but these may not be economically feasible under larger field conditions. Other management strategies such as resistant varieties, soil drainage, and external application of nutrients were also reported to be less effective under practical field conditions.

Biological control using bacteriophages and beneficial microbes (BMs) such as *Pseudomonas* spp., *Stenotrophomonas* spp., *Streptomyces* spp., *Acinetobacter* spp., *Enterobacter* spp., *Bacillus* spp., and *Paenibacillus* (Messiha et al. 2007b; Ji et al. 2008; Zhang et al. 2008; Ramesh et al. 2009; Xue et al. 2009; Ling et al. 2010; Liu et al. 2013; Sakthivel et al. 2017) were suggested for effective suppression of plant pathogen under field conditions. However, efficiency of these BMs was found to be lower in field experiments compared to in vitro studies (Lugtenberg et al. 2001; Kamilova et al. 2005; Liu et al. 2013). This might be due to not only poor colonizing ability of the antagonistic strains under different field conditions but also other factors such as environmental conditions (e.g., soil temperature, moisture, pH), competition by other already available microorganisms, development of resistance in pathogenic microbes, and improper delivery system for BMs in the rhizosphere environment (Yuan et al. 2014; Hu et al. 2021).

Studies showed that application of organic fertilizers combined with antagonistic BMs as bio fortified organic fertilizers were very effective in controlling bacterial wilt in field conditions (Hao et al. 2009; Huang et al. 2011; Yang et al. 2011; Yuan et al. 2014). In agreement with this, Liu et al. (2013) documented that BMs such as *Trichoderma* spp., *Bacillus* spp., and *Streptomyces* spp. could suppress the bacterial wilt pathogen very effectively in combination with any organic amendments. According to Wu et al. (2021), organic fertilizers increase soil nutrient recycling by promoting the growth of other beneficial microbial populations and by changing the soil microbial community and structure. As a result, the application of biofortified BMs may support more effective rhizosphere or root surface colonization, leading to higher antibiotic production and improved control of bacterial wilt.

Andaman and Nicobar Islands, India, are an important biodiversity hotspot and well known for microbial richness due to their wide range of ecosystems and habitats, including tropical rainforests, mangrove swamps, coral reefs, and marine environments, which offer a variety of niches for microbial organisms to thrive in. However, despite of potential for agricultural applications, the microbial richness is not yet harnessed judiciously (Sakthivel et al. 2017). Therefore, the present study was conducted with the main objective of developing efficient and eco-friendly bacterial wilt disease management using multi-potential antagonistic *Bacillus* from rhizosphere and non-rhizosphere soils of the Andaman Islands, India, in the form of a MFYM.

Materials and methods

Pathogen and antagonists

The pathogen and antagonist cultures were obtained from the culture collection center, Plant Pathology Lab, ICAR-Central Island Agricultural Research Institute, Port Blair, India. For in vitro screening of antagonism and greenhouse studies, a highly virulent *R. solanacearum* strain (BRs_Ch), as mentioned earlier in Sakthivel et al. (2016), was used. Similarly, in case of antagonists, multipotential *Bacillus* strains (Table 1), as already reported by Sakthivel et al. (2018), were used in greenhouse and field experiments. The cultures were properly maintained by subculturing on a semi-specific medium amended with 1% 2,3,5-triphenyl tetrazolium chloride for *R. solanacearum* and nutrient agar slants for *Bacillus* strains, respectively.

Confirmation of in vitro potential and functional traits of bacterial antagonists

Four *Bacillus* cultures obtained were studied again for their antagonistic ability against *R. solanacearum* strain (BRS_Ch), as described by Ramesh and Phadke (2012), with slight modifications. Briefly, pure colonies of *R. solanacearum* and antagonist bacteria were grown in 5 ml CPG broth (casein hydrolysate, 1.0 g/L; peptone, 10.0 g/L; and glucose, 5.0 g/L) and King's B broth (peptone, 20.0 g/L; K_2HPO_4, 1.5 g/L; $MgSO_4 \cdot 7H_2O$, 1.5 g/L; and glycerol, 10.0 ml/L), respectively. These cultures were incubated at 28 ± 2 °C for 48 h at 140 rpm. Then, 150 µL *R. solanacearum* bacterial suspension was mixed with 100 ml molten and cooled King's B agar. The mixture was then poured onto plates and allowed to solidify. After solidification, three wells were made on each plate by removing circular agar pieces with the help of a sterile cork borer with a diameter of 8 mm. To each of the wells, 20 µL freshly prepared *Bacillus* suspension (1×10^8 cfu/ml) was added and the plates were incubated at 28 ± 2 °C for 96 h. The inhibition zones observed on the plates were measured in millimeters in two directions.

The functional traits such as production of siderophore (Schwyn and Neilands 1987), IAA (Maor et al. 2004), and phosphate solubilization (Nautiyal 1999) ability were reconfirmed prior to greenhouse and field evaluation studies. In addition, the endospore-forming

Table 1 Details of *Bacillus* strains used in the present study

Isolate ID with 16S rRNA GenBank Acc. No.	Place of Collection	Niche of collection	Crop/source	rpoB gene identification	rpoB GenBank Acc. No.
*Ls*_Agu - KP864632	Guptapara	Rhizosphere	Eggplant	*Lysinibacillus sphaericus*	OP891222
*Ba*_Abi - KP864633	Barren island	Soil	Volcano	*Bacillus amyloliquefaciens*	OP891223
*Bs*_Ane - KP864636	Neil Island	Rhizosphere	Chilli	*Bacillus subtilis*	OP891224
*Bs*_Asi - KP864637	Sippighat	Rhizosphere	Coconut	*Bacillus subtilis*	OP891225

ability of all the *Bacillus* was confirmed as per Mormak et al. (1985).

Molecular-based confirmation of genetic identity of bacterial antagonists

In a previous study, Sakthivel et al. (2023) documented the genetic identity of four multipotential *Bacillus* strains using the 16S rRNA gene (Table 1). In this study, these strains were subjected to sequencing of *Bacillus*-specific rpoB gene segments, to further establish the species-level genetic identity. For the sequencing process, primer pairs suggested by Qiu et al. (2018) were used. The 50 µL reaction mixture consisted of 100 ng template DNA, 1 × PCR buffer, 1.5 mM MgC_{12}, 50 µM of each dNTP, 5 pmol of each primer, and 1 U of Taq DNA polymerase. PCR was performed in an Eppendorf thermocycler with an initial denaturation at 96 °C for 2 min, followed by 35 cycles of 94 °C for 30s, 60 °C for 1 min, and 72 °C for 1 min. A final extension step was performed at 72 °C for 10 min. All PCR products amplified were analyzed by resolving them on 1.5% agarose gel and bi-directionally sequenced to obtain complete coverage. Contigs were assembled in DNA Baser software (version 4) and compared with GenBank sequences through NCBI-BLAST analysis.

Preparation of microbe-enriched farm yard manure

Microbe-enriched farm yard manure was prepared by using well-decomposed farm yard manure (FYM), which is composed of approximately 38.9% organic matter, 3.5% N, 2.9% P_2O_5, 0.9% K_2O, 4.0% amino acids, and 25.4% H_2O. To prepare MFYMs (MIC-Ls_Agu, MIC-Ba_Abi, MIC-Bs_Asi, and MIC-Bs_Ane), 24 to 48 hrs old bacterial suspensions (~10^9 cfu ml^{-1}) of each *Bacillus* strain were used. Each *Bacillus* strain suspension (200 ml) was mixed with 1 kg sterilized talc power and shade-dried. Then, the freshly prepared talc formulations were mixed into 50 kg of well-decomposed FYM. The mixture was kept under shaded conditions for 10 days with intermittent manual turning every 3–4 days to enrich *Bacillus* population within the FYM substrate. For MIC-Consortia treatment, a consortium of all four potentials *Bacillus* strains was mixed at a volume of 50 ml in 1 kg sterilized talc power, shade dried and mixed with well-decomposed FYM. The sole FYM amended treatment was used as positive control and treatment with no any amendment as the regular negative control.

Evaluation of biocontrol potential of MFYMs in pot cultures

Thirty-day-old eggplant seedlings (cv. TN-Co1) were transplanted into sterilized pots containing a sterile mixture of red soil, sand, and FYM in a 2:1:1 ratio. All the MFYM/FYM were applied around the root zone of eggplant seedlings and after 24 h of application, pathogenic *R. solanacearum* suspension (~1×10^6 cfu/ml) was inoculated, as described by Kumar (2012). All the pots were incubated at 28–32 °C under controlled conditions and proper care was taken during watering to avoid cross-contaminations. Each treatment was replicated three times with minimally three seedlings each in a randomized block design. The seedlings were scored for wilt symptoms at regular weekly intervals from the date of transplanting until 8 weeks. The wilting percentage and biocontrol efficacy were calculated as per Guo et al. (2004).

$$\text{Wilting percentage} = [\text{no. of plants wilted}/\text{total no. of plants inoculated}] \times 100$$

$$\text{Biocontrol efficacy} = [\text{total no. of plants in treatment} - \text{total no. of plant in control}]/\text{total no. of plants in control}] \times 100$$

Evaluation of biocontrol potential of MFYMs under field conditions

Field experiments were carried out at two locations: Garacharma (11° 36′39.39″N and 09211° 36′03.375″E) and Chouldhari (11° 36′39.39″N and 09211° 36′03.375″E), during two successive cropping years, 2015 and 2016, in farmer fields of Andaman Nicobar Islands, India. Eggplant fields with high (>50%) incidence of bacterial wilt in previous cropping seasons were selected for experiments. In the first year, seven treatments were tested: (1) Control, (2) OF: FYM alone, (3) MIC-Ls_Agu, (4) MIC-Ba_Abi, (5) MIC-Bs_Ane, (6) MIC-Bs_Adg, and (7) MIC-Consortia. In the second year, the best treatment identified (MIC-Consortia) in the initial pot culture studies and first year-field experiment results was evaluated along with OF and control. All field experiments were conducted using a randomized block design with three plots per treatment in, with each plot containing a minimum of 12 eggplant seedlings planted with a spacing of 45×60 cm. The application rate of FYM/MFYM was about 0.5 kg/plant in each respective treatment. Standard agronomic practices were followed in all experimental plots without use of any other chemicals and pesticides. The disease incidence was recorded regularly and the wilt incidence was recorded at 12th week post transplantation. The plot yield was recorded for each harvest and the total yield was calculated. Wilt incidence and biocontrol potential of each MFYM treatment were calculated as described above. The yield increase was calculated using the formula Yield increase by BOF = [(Average yield of eggplant treated with MFYM – Average yield of eggplant in FYM)/Average yield of eggplant in control] × 100, following methodology of Guo et al. (2004).

Soil sampling and microbial populations

To analyze the interactions between pathogen and beneficial *Bacillus* in both pot culture and field conditions during MFYM applications, sampling within each treatment was performed. It was performed in a non-destructive manner by collecting rhizosphere soil from root zone area of the three plants of eggplant in each treatment using metallic auger (5 mm diameter) to a depth of about 15 cm from the soil surface. Two random soil samples were collected from each plant in a treatment, mixed thoroughly and subsequently the pooled soil samples were used for further analysis. Sampling in potting experiments was carried out at intervals of 0, 1, 2, 4, 6, and 8 weeks post transplantation. In case of field experiment during 2015, sampling of rhizosphere soil surrounding primary root zone performed after seedling transplantation at regular weekly intervals was from 1 to 12 weeks. The bacterial wilt pathogen count in rhizosphere soil samples was performed in semi- selective SMSA medium (French et al. 1995). Briefly, 1 g rhizosphere soil was added to 9 ml sterile distilled water and shaken in a rotary shaker for 30 min. Serial dilutions up to 10^{-6} were made and 0.1 ml aliquots were spread on the surface of the SMSA medium. The typical colonies of *R. solanacearum* were counted after incubation of 2–3 days at 28 ºC incubation based on morphology. Similarly, antagonistic *Bacillus* population was counted in a semi-selective medium, as suggested by Travers et al. (1987). Colony-forming units (cfu) were calculated per gram (dry weight) of soil at 10^{-6} dilutions. All the microbial count experiments were repeated three times and the results were documented.

Data analysis

The experiments were performed with three replications and repeated twice for confirmation. The results were expressed as the mean ± SE of different independent replicates. Analysis of variance was performed in WASP software (version 2.0). *P* values ≤ 0.05 were considered as statistically significant.

Results

Characterization and identification of multipotential *Bacillus*

Four multipotential *Bacillus* strains obtained from culture collections of ICAR-CIARI, Port Blair, India, were used in the study. The results of in vitro antagonistic studies revealed that all four strains tested were highly effective against virulent eggplant *R. solanacearum* strain (BRs_Ch) with mean inhibition zone ranging from 9.3 to 15.8 mm diameter (Table 2). The rpoB gene sequence analysis confirmed the bacterial identity and same as submitted to the NCBI GenBank with accession numbers KP864634–KP864637, as shown in Table 1. In case of functional traits, all the four *Bacillus* strains exhibited IAA production, P solubilization, and siderophore production whereas Ls_Agu expressed high (30.7 µg/ml) level of in vitro IAA and P solubilization index (68.43 µg/ml). The siderophore production was higher in the strains Bs_Ane (10.39 mm) and Bs_Asi (10.37 mm) (Table 2). All four *Bacillus* strains were revealed as endospore producers through staining (Figure not shown).

Pot experiments

Evaluation of biocontrol potential of MFYM

The results showed that MFYMs could effectively suppress eggplant bacterial wilt pathogen in greenhouse conditions when compared to the control and FYM treatments. First incidence of bacterial wilt disease was recorded at 51, 44, 43, and 48 days post transplantation in the MIC-consortia-treated plants. Similarly, biocontrol potential was recorded in the order MIC-consortia (96.9%) > MIC-Ba_Abi (90.6%) > MIC-Bs_Ane (81.3%), MIC-Bs_Asi (69.6%) > MIC-Ls_Agu (59.3%), whereas in case of control and FYM treatments, wilt incidence was recorded at 16th and 18th days after transplantation with 33.3% and 71.1% biocontrol potential, respectively. These results indicate that the application of the MFYM treatments delayed the onset and progress of eggplant wilt disease from 14 to 26 days with increased biocontrol potential till yield stage (Table 3).

Microbial composition in pot soil

The changes in the *R. solanacearum* population in the rhizosphere soil of pot experiments after transplantation are shown in Fig. 1a. In due course of time after soil application, the pathogen population in all the treatments increased invariably, but in case of MIC-Consortia and MIC-Ba_Abi treatments, the increase in pathogen population was comparatively slow and pathogen population attained stability after 4 weeks, whereas in other treatments the increase in pathogen population was steady till the end of experiment, i.e., up to 60 days.

The population dynamics of the *Bacillus* count in the pot soil revealed that the populations of antagonists in MIC-consortia and MIC-Ba_Abi were increased initially to approximately 11×10^6 cfu per dry gram weight of soil and approximately 10×10^6 cfu per dry gram weight of soil, respectively, during the first 2 weeks after application (Fig. 1b) and then decrease in population of antagonists were recorded, but in case of MIC-Bs_Ane, MIC-Ls_Agu, and MIC-Bs_Asi treatments, the population of antagonists decreased continuously during the entire experimental period starting from the date of application.

Field experiment

Biocontrol and fertilization effect of MFYMs during first year

All the MFYM treatments showed higher biocontrol efficacy than FYM and control treatments. In the first field, the biocontrol effect at 80th day after the application of MIC-Consortia and MIC-Ba_Abi was 84.8% and 80.8%, respectively (Table 4). The highest yield increase was also recorded in MIC-Consortia (61.5%) treatment compared to other MFYM treatments, control, and FYM treatments (Table 4). Similarly, in the second field, the maximum biocontrol efficacy and yield potentials were recorded in MIC-Consortia (76.6%; 6.43 kg/plot) and MIC-Ba_Abi (73.7%; 6.17 kg/plot) treatments, respectively, followed by other MFYM treatments. The control treatment showed the highest disease incidence of 43.3% and 45.3% in the first and second fields, respectively.

Biocontrol and fertilization effect of MFYMs during second year

On the basis of the results obtained in the first year, three treatments (MIC-Consortia, FYM alone, and control) were chosen for validation in the second year. The biocontrol efficacies of MIC-Consortia were 78.8% in the field I and 72.1% in the field II, which were significantly higher than those of the FYM and control treatments (Table 4).

Furthermore, higher per plot yield was also recorded for the treatment MIC-Consortia in both the fields (61.5% and 60.0%) compared to other treatments, and the minimum yield was recorded for control (3.50 and 3.60 kg/plot).

Microbial composition in field soils

At the onset of the experiment, the population count of *R. solanacearum* in the soil was ~ 2.1×10^6 cfu per dry gram weight of soil. However, it increased continuously over the plant growth period in all treatments (Fig. 2a). In case of MIC-Consortia and MIC-Ba_Abi treatments, pathogen population was stable for first 2 weeks after transplanting, compared to that of the control (Fig. 2b). But after 2 weeks, the pathogen population increased gradually in all the treatments but the rate of increase was comparatively lower in MIC-Ba_Abi and MIC-Consortia treatments.

The *Bacillus* counts in both the fields also changed in an oscillatory manner over time, with an initial increase followed by a decrease after a certain time. However, the rate of decrease varied within the treatments in both the fields. Among the treatments, the decrease in abundance of antagonists was slow in the MIC-Consortia treatment and remained above 7.0×10^6 cfu per dry gram weight of soil until 7

Table 2 In-vitro characterization of *Bacillus* strains for plant growth promotion and antagonistic activity

Isolates	IAA production (µg/ml)	Phosphate solubilization capacity		Siderophore production	Antagonistic activity	
		pH value	Solubilization index		Mean diameter of inhibition zone (mm)	Degree of antagonism
*Ls*_Agu	9.5	6.5	35.37	+++	9.33	++
*Ba*_Abi	30.7	5.4	68.43	++	15.83	+++
*Bs*_Ane	16.4	5.8	30.45	++	10.39	++
*Bs*_Asi	18.9	5.6	32.12	++	10.17	++

'-' no production, '+' - medium (0.3-0.5 cm); ' ++ ' - strong (0.6-0.9 cm), '+++ ' - very strong (> 1 cm)

weeks after transplantation in both field experiments (Fig. 2).

Table 3 Efficacy of microbe fortified farmyard manure (MFYMs) treatments on eggplant bacterial wilt disease incidence in greenhouse experiments

Treatments	Days for first wilt	Disease incidence (%)	Biocontrol efficacy (%)
MIC-*Ls*_Agu	48	28.9 ± 6.40^{bc}	59.3
MIC-*Ba*_ Abi	43	6.7 ± 3.56^{de}	90.6
MIC-*Bs*_Asi	43	22.2 ± 5.31^{bc}	69.6
MIC-*Bs*_Ane	43	13.3 ± 6.34 ^{cd}	81.3
MIC-Consortia	51	2.2 ± 2.22^{e}	96.9
OF	16	33.3 ± 6.51^{b}	53.1
Control	18	71.1 ± 8.53^{a}	-
CD (0.05)	-	12.14	-

Disease incidence (%) was calculated at eighth week after transplantation and represented as mean ± SD (standard deviation)

Mean values in each column with the different letters ^{a,b,c} etc., showed the significant difference between each treatments by Duncan multiple comparison test ($P \leq 0.05$)

Discussion

Eggplant (*Soalnum melongena*) is an important solanaceous vegetable crop grown worldwide but bacterial wilt disease caused by pathogen *R. solanacearum* is a huge constraint throughout the world. It causes huge economic losses every year and the yield loss is estimated about 20–50% in the Andaman and Nicobar islands (Sakthivel et al. 2016).

Environmentally-friendly crop protection approaches are being recommended all over the world in view of growing concern for minimal chemical usage and environmental safety, but there are huge practical difficulties in supplementing the effect of inorganics considering both the growth promotion and crop protection. In general, strong and consistent invitro inhibition potential against highly virulent pathogen strains and in vitro nutrient mobilization properties are essential prerequisites for PGPRs/antagonists to be utilized as effective bioformulations in field conditions. Most of the microbes derived from different rhizosphere and non rhizosphere soil ecosystems showed better in vitro plant growth promoting and antagonistic potential. However, antagonists with good in vitro biocontrol and plant growth promotion (PGP) potential often failed to prove their efficacy in field conditions (Kumar et al. 2013). This reduced field performance might be due to poor colonizing ability of antagonistic strains in different environmental conditions. In addition, several factors that limit the effectiveness of biocontrol agents have been previously reported, including physical and chemical characteristics of the soil, variable temperature and moisture of soil and external environments, competition imposed by high pathogen inoculum, competition for space and nutrients, availability of organic matter in the soil; use of chemical fertilizers, pesticides, and other soil amendments (Abd-Elgawad and Askary 2020; Bonaterra et al. 2022; Sun et al. 2022). Therefore, the careful selection of microbial strains and implementation of an effective delivery system play a major role in successful integration of biocontrol agents for disease suppression in organic farming approaches.

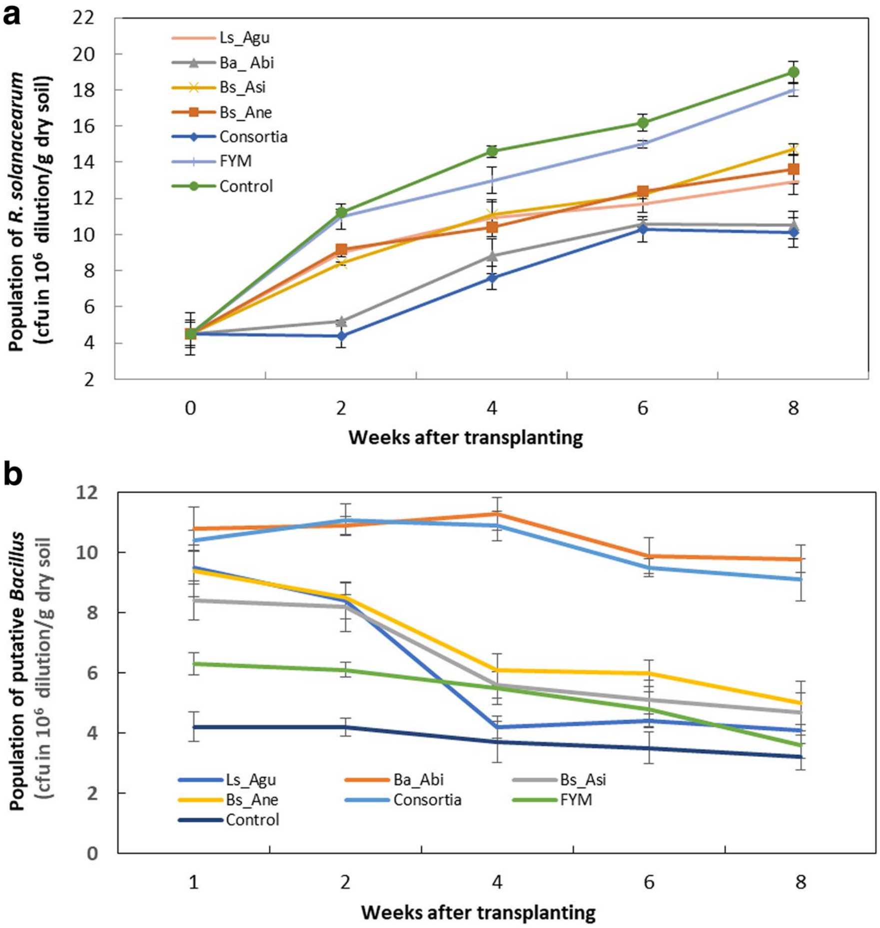

Fig. 1 Population dynamics of pathogen and *Bacillus* in pot experiments. **a** Population dynamics of *R. solanacearum* in the rhizosphere soil in pot experiments; **b** Population dynamics of *Bacillus* in the rhizosphere soil in pot experiments

In our studies, four native multipotential *Bacillus* strains isolated from diverse niches and its consortia were studied in a biofortified MFYM approach to suppress eggplant bacterial wilt disease and plant growth promotion under both greenhouse and field conditions. In addition, the changes in microbial load with respect to pathogen and antagonist populations were also studied. In general, most of the bacteria belonging to the *Bacillus* genus are considered environmentally safe and represented as "Generally recognized as safe—GRAS" by European Union (Elshaghabee et al. 2017). Members of *Bacillus* have spore-forming capacity when exposed to unfavorable environments, which increases their potential under different environments.

All the four *Bacillus* strains were assessed for their in vitro PGP traits viz., IAA production, P solubilization, and siderophore production (Table 1). IAA is an important precursor of phytohormone auxin and those bacterial strains could produce IAA in the rhizospheric soil and thus could favor good root structure in many plants (Han et al. 2009). P solubilization of bacteria increases the availability of phosphorous in rhizosphere soils, promoting plant growth (Rodriguez and Fraga 1999). Also, the production of siderophore is believed to be beneficial for drought resistance in host plants (Arzanesh et al. 2011).

Application of composts along with BMs favored better nutrient source and shelter and suppressed bacterial wilt pathogen *R. solanacearum* for a long period (Wu et al. 2014; Semenov et al. 2019). Our study results also showed that antagonistic *Bacillus* strains when applied in the form of MFYM could effectively suppress the eggplant bacterial wilt in both pot and field conditions. This effect might be due to increase in the initial surface colonization ability of antagonists and

Table 4 Effect of microbe fortified farmyard manure (MFYMs) on the suppression of eggplant bacterial wilt incidence and yield increase at two field locations in two different years

Treatments	1st Year (2015)							
	Disease incidence^{x} (%)		Biocontrol effect (%)		Yield (kg) /plot^{y}		Yield increase (%)	
	Field 1	Field 2	Field 1	Field 2	Field 1	Field 2	Field 1	Field 2
MIC-*Ls*_Agu	21.6 ± 2.0^{c}	26.6 ± 2.1^{c}	50.1	41.2	6.03 ± 1.16^{ab}	4.2 ± 0.82	50.3	25.0
MIC-*Ba*_ Abi	8.3 ± 5.2^{e}	11.9 ± 2.4^{e}	80.8	73.7	6.17 ± 0.26^{b}	4.6 ± 0.67	53.6	37.5
MIC-*Bs*_Asi	$26.6\pm3.1b^{c}$	29.3 ± 1.6^{c}	38.6	35.3	5.37 ± 0.24^{ab}	4.0 ± 0.64	29.3	18.8
MIC-*Bs*_Ane	14.9 ± 4.0^{d}	18.6 ± 3.8^{d}	65.6	58.9	5.97 ± 1.02^{ab}	4.4 ± 0.82	47.6	31.3
MIC-Consortia	6.6 ± 4.0^{e}	10.6 ± 2.6^{e}	84.8	76.6	6.43 ± 0.09^{a}	5.8 ± 0.71	61.5	75.0
OF	31.6 ± 4.8^{b}	33.3 ± 3.6^{b}	27.0	26.5	$4.40\pm0.46b^{c}$	3.4 ± 0.67	-	-
Control	43.3 ± 4.8^{a}	45.3 ± 1.3^{a}	-	-	3.30 ± 0.66^{c}	3.2 ± 0.23	-	-
CV	16.241	7.344			20.253	21.818		
CD (0.05)	6.316	3.279			1.965	NS		
2nd Year (2016)								
MIC-Consortia	9.9 ± 4.0^{c}	11.9 ± 2.4^{b}	78.8	72.1	6.28 ± 0.21^{b}	6.63 ± 0.73^{c}	60.0	48.1
OF	33.2 ± 5.2^{b}	38.6 ± 2.4^{a}	28.7	9.4	4.18 ± 0.39^{b}	4.90 ± 0.10^{b}	-	-
Control	46.6 ± 4.2^{a}	42.6 ± 2.6^{a}	-	-	3.50 ± 0.50^{a}	3.60 ± 0.60^{a}	-	-
CV	10.791	10.432			12.909	6.199		
CD (0.05)	7.311	7.330			1.359	0.709		

Values presented with disease incidence and yield with superscripts ^{x,y} in different columns are the mean ± SD (standard deviation) values of independent treatments

Mean values in each column with the different letters a,b,c etc., showed the significant difference between each treatments by Duncan multiple comparison tests ($P \leq 0.05$)

Disease incidence was recorded at 12th week after transplantation

its functional potential when applied along with organic fertilizers.

In particular, the bioefficacy of the treatment Mic-Consortia was 84.8% and 76.6% in the first year and 78.8% and 72.1% in the second year in two field conditions. Similarly, the yield increase in two fields was 61.5% and 75.0% in first year and 60.0% and 48.1% in the second year in Mic-Consortia treatments when compared to other treatments. This might be due to increased root colonization behavior and growth suppression of pathogen by consortia of antagonists than individual cultures. Ding et al. (2013) reported that the bio-organic fertilizers (BOF) treatment could effectively suppress the high-density *R. solanacerum* colonies in the pot soil and reduce the disease incidence in potato plants. Similarly, Wu et al. (2014) reported that the integrated approach of BMs and organic fertilizers could effectively alter the soil microbial structure and suppress the bacterial wilt of tobacco in pot conditions.

The observed oscillatory dynamics in counts of pathogen populations and competing or predatory microorganisms, as shown in Fig. 2a and b, were reported by Zelenev et al. (2006). Similar to our observations, an increase in antagonistic microbial populations was associated with a simultaneous decrease in plant pathogen populations when organic fertilizers were amended with microbial bioformulations. These oscillatory dynamics in microbial populations may be due to various factors such as predatory interactions among microorganisms, competition for easily available carbon sources (Zelenev et al. 2006), or interactions between microbial populations and soil physicochemical parameters. Understanding these oscillatory dynamics is important for developing effective strategies for managing diseases in agricultural soils.

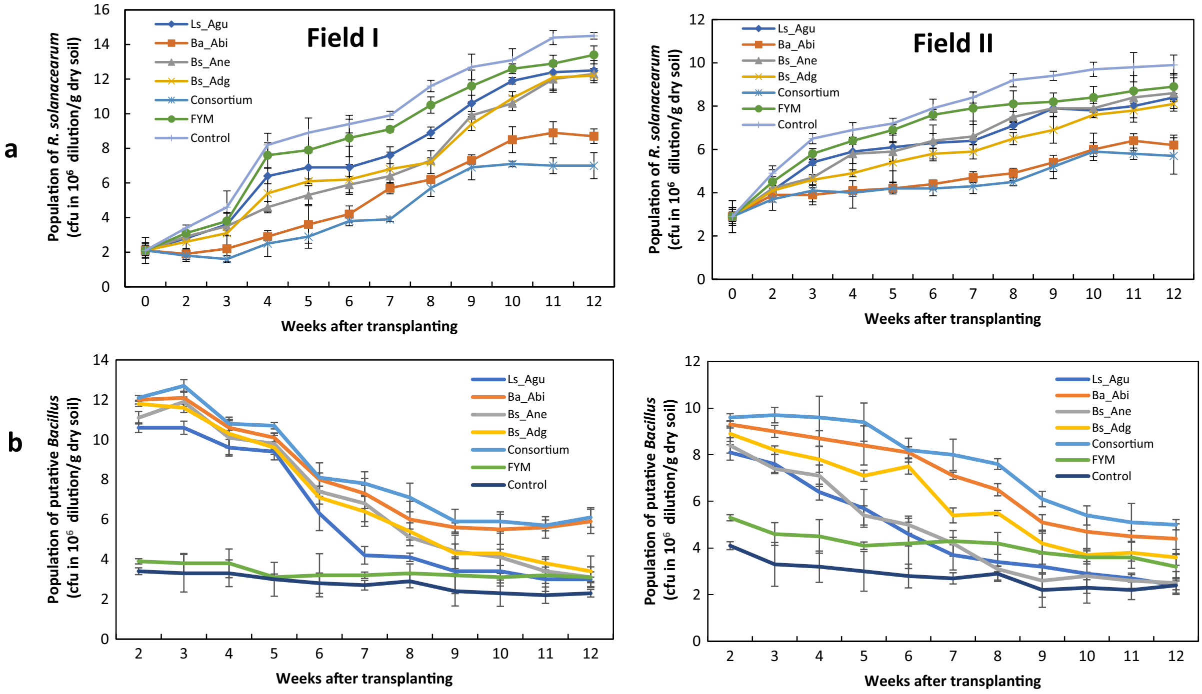

Fig. 2 Population dynamics of pathogen and *Bacillus* colonies in field conditions. **a** Population dynamics of *R. solanacearum* in eggplant rhizosphere soils in field conditions. **b** Population dynamics of putative antagonistic *Bacillus* in eggplant rhizosphere soils in field conditions

In both pot and field experiments, it was observed that the pathogen counts increased over time while the antagonist counts decreased. However, the rate of increase in pathogen population was much lower in MFYM treatments when compared to FYM and control treatments in both the field experiments from two places in two successive years. Although the putative *Bacillus* abundance in MFYM treatments decreased over time, the rate of decrease in soil *Bacillus* spp. was very slow in MIC-Consortia and MIC_Abi treatments compared to other treatments (Fig. 1a and b). This shows the importance of the periodical application of antagonists in the form of MFYM formulations at regular intervals to maintain antagonist population around the root zone, thereby facilitating diseases management.

The higher disease suppression and yield increase in MIC-Consortia and other MFYM treatments might be due to initial increase of *Bacillus* counts in the rhizosphere soil when compared to FYM and control treatments. The increase in beneficial *Bacillus* might have resulted in increase in root surface colonization and increased antibiosis toward bacterial pathogen in the rhizosphere soil. Liu et al. (2013) and Ge et al. (2004) also reported that increase in population of BMs reduces the population counts of bacterial wilt in tobacco rhizosphere, resulting in disease suppression.

The results of this study showed that eggplant crop when treated with the consortium of antagonistic *Bacillus* strains in the form of MFYM approach could effectively suppress the bacterial wilt disease incidence, leading to consistently better yield performance in two field conditions over two successive years.

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Author contributions KS, KM, AB performed the lab, pot culture studies and analyzed the data; PK supervised field experiments and RKG, AK, SKS supervised the overall work, drafted, and edited the manuscript.

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Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Abd-Elgawad MMM, Askary TH (2020) Factors affecting success of biological agents used in controlling the plant-parasitic nematodes. Egypt J Biol Pest Control 30:17

Arzanesh MH, Alikhani HA, Khavazi K, Arzanesh MH, Alikhani HA, Khavazi K, Rahimian HA, Miransari M (2011) Wheat (*Triticum aestivum* L.) growth enhancement by Azospirillum sp. under drought stress. World J Microbiol Biotechnol 27:197–205

Bonaterra A, Badosa E, Daranas N, Bonaterra A, Badosa E, Daranas N, Francés J, Roselló G, Montesinos E (2022) Bacteria as biological control agents of plant diseases. Microorganisms 10:1759

Boulter JI, Boland GJ, Trevors JT (2000) Compost: a study of the development process and end-product potential for suppression of turfgrass disease. World J Microbiol Biotechnol 16:115–134

Brimner TA, Boland GJ (2003) A review of the non-target effects of fungi used to biologically control plant diseases. Agric Ecosyst Environ 100:3–16

Denny TP (2006) Plant pathogenic *Ralstonia* species. In: Gnanamanickam SS (ed) Plant-associated bacteria, 573–644

Ding C, Shen Q, Zhang R, Chen W (2013) Evaluation of rhizosphere bacteria and derived bio-organic fertilizers as potential biocontrol agents against bacterial wilt (*Ralstonia solanacearum*) of potato. Plant Soil 366:453–466

Elshaghabee FMF, Rokana N, Gulhane RD, Elshaghabee FMF, Rokana N, Gulhane RD, Sharma C, Panwar H (2017) *Bacillus* as potential probiotics: status, concerns, and future perspectives. Front Microbiol 8:1490

French ER, Gutarra L, Aley P, Elphinstone J (1995) Culture media for Pseudomonas solanacearum isolation, identification and maintenance. Fitopatologia 30(3):126–30

Gamliel A, Austerweil M, Kritzman G (2000) Non-chemical approach to soilborne pest management–organic amendments. Crop Prot 19:847–853

Ge C, Liu B, Zhu Y et al (2004) Study on the methods on identifying the virulence of *Ralstonia solanacearum* using tissue cultured tomatoes. Wuyi Sci J 20:13–16

Guo J-H, Qi H-Y, Guo Y-H, Guo J-H, Qi H-Y, Guo Y-H, Ge H-L, Gong L-Y, Zhang L-X, Sun P-H (2004) Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. Biol Control 29:66–72

Han H, Zhang S, Sun X (2009) A review on the molecular mechanism of plants rooting modulated by auxin. Afr J Biotechnol 8

Hao Y, Wei J, Liu C et al (2009) Effect of biological soil amendment reducing on replant diseases of cucumber. Acta Agric Boreali-Sin 24:231–234

Hayward AC (1991) Biology and epidemiology of bacterial wilt caused by Pseudomonas solanacearum. Annual Rev Phytopathol 29(1):65–87

Hu Y, Li Y, Yang X, Hu Y, Li Y, Yang X, Li C, Wang L, Feng Ji, Chen S, Li X, Yang Y (2021) Effects of integrated biocontrol on bacterial wilt and rhizosphere bacterial community of tobacco. Sci Rep 11:2653

Huang X, Chen L, Ran W, Huang X, Chen L, Ran W, Shen Q, Yang X (2011) *Trichoderma harzianum* strain SQR-T37 and its bio-organic fertilizer could control *Rhizoctonia solani* damping-off disease in cucumber seedlings mainly by the mycoparasitism. Appl Microbiol Biotechnol 91:741–755

Ji X, Lu G, Gai Y et al (2008) Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. FEMS Microbiol Ecol 65:565–573

Kamilova F, Validov S, Azarova T, Kamilova F, Validov S, Azarova T, Mulders I, Lugtenberg B (2005) Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environ Microbiol 7:1809–1817

Kumar A (2012) Methods for screening ginger (*Zingiber officinale* Rosc.) for bacterial wilt resistance. Indian Phytopathol 59(3):281–286

Kumar A, Munder A, Aravind R et al (2013) Friend or foe: genetic and functional characterization of plant endophytic *Pseudomonas aeruginosa*. Environ Microbiol 15:764–779

Lei N, Chen L, Kiba A, Lei Ni, Chen Li, Kiba A, Hikichi Y, Zhang Y, Ohnishi K (2020) Super-multiple deletion analysis of type III effectors in *Ralstonia solanacearum* OE1-1 for full virulence toward host plants. Front Microbiol 11:1683

Lemessa F, Zeller W (2007) Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. Biol Control 42:336–344

Ling N, Xue C, Huang Q, Ling N, Xue C, Huang Q, Yang X, Xu Y, Shen Q (2010) Development of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy to Fusarium wilt. Biocontrol 55:673–683

Liu Y, Shi J, Feng Y, Liu Y, Shi J, Feng Y, Yang X, Li X, Shen Q (2013) Tobacco bacterial wilt can be biologically controlled by the application of antagonistic strains in combination with organic fertilizer. Biol Fertil Soils 49:447–464

Lugtenberg BJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by pseudomonas. Annu Rev Phytopathol 39:461–490

Maor R, Haskin S, Levi-Kedmi H, Sharon A (2004) In planta production of indole-3-acetic acid by Colletotrichum gloeosporioides f. sp. aeschynomene. Appl Environ Microbiol 70:1852–1854

Messiha NA, van Bruggen AH, van Diepeningen AD et al (2007a) Potato brown rot incidence and severity under different management and amendment regimes in different soil types. Eur J Plant Pathol 119:367–381

Messiha NA, van Diepeningen AD, Wenneker M et al (2007b) Biological soil disinfestation (BSD), a new control method for potato brown rot, caused by *Ralstonia solanacearum* race 3 biovar 2. Eur J Plant Pathol 117:403–415

Messiha NAS, Van Diepeningen AD, Farag NS, Messiha NAS, van Diepeningen AD, Farag NS, Abdallah SA, Janse JD, van Bruggen AHC (2007c) Stenotrophomonas maltophilia: a new potential biocontrol agent of *Ralstonia solanacearum*, causal agent of potato brown rot. Eur J Plant Pathol 118:211–225

Messiha NAS, van Bruggen AHC, Franz E, Messiha NAS, van Bruggen AHC, Franz E, Janse JD, Schoeman-Weerdesteijn ME, Termorshuizen AJ, van Diepeningen AD (2009) Effects of soil type, management type and soil amendments on the survival of the potato brown rot bacterium *Ralstonia solanacearum*. Appl Soil Ecol 43:206–215

Mormak DA, Casida LE Jr (1985) Study of *Bacillus subtilis* endospores in soil by use of a modified endospore stain. Appl Environ Microbiol 49:1356–1360

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270

Palleroni NJ, Doudoroff M (1971) Phenotypic characterization and deoxyribonucleic acid homologies of *Pseudomonas solanacearum*. J Bacteriol 107:690–696

Paudel S, Dobhal S, Alvarez AM, Arif M (2020) Taxonomy and phylogenetic research on *Ralstonia solanacearum* species complex: a complex pathogen with extraordinary economic consequences. Pathogens 9:886

Qiu Z, Lu X, Zhang LN, Qiao MX (2018) Characterization of garlic endophytes isolated from the black garlic processing. Microbiologyopen 7(1):e00547

Ramesh R, Joshi AA, Ghanekar MP (2009) Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). World J Microbiol Biotechnol 25:47–55

Ramesh R, Phadke GS (2012) Rhizosphere and endophytic bacteria for the suppression of eggplant wilt caused by *Ralstonia solanacearum*. Crop Prot 37:35–41

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Sakthivel K, Gautam RK, Kumar K, Sakthivel K, Gautam RK, Kumar K, Dam Roy S, Kumar A, Devendrakumar C, Vibhuti M, Neelam S, Vinatzer BA (2016) Diversity of *Ralstonia solanacearum* strains on the Andaman Islands in India. Plant Dis 100:732–738

Sakthivel K, Manigundan K, Gautam RK, Sakthivel K, Manigundan K, Gautam RK, Jaisankar I, Sharma SK, Singh R, Roy SD (2017) Detection of antimicrobial peptide genes from antagonistic *Bacillus subtilis* (Bs_Ane) isolated from Neil Islands of Andaman, India. J Environ Biol 38:75–80

Sakthivel K, Manigundan K, Gautam RK, Sakthivel K, Manigundan K, Gautam RK, Singh PK et al (2018) Bioefficacy of antimicrobial peptide biosynthesis-gene-linked antagonistic *Lysinibacillus sphaericus* strains for management of bacterial plant diseases in Andaman and Nicobar Islands, India. J Environ Biol 39:517–521

Sakthivel K, Manigundan K, Sharma SK et al (2023) Diversity of antimicrobial peptide genes in *Bacillus* from the Andaman and Nicobar Islands: untapped island microbial diversity for disease management in crop plants. Curr Microbiol 80:45

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56

Semenov MV, Nikitin DA, Stepanov AL, Semenov VM (2019) The structure of bacterial and fungal communities in the Rhizosphere and Root-free Loci of gray forest soil. Eurasian Soil Sc 52:319–332. https://doi.org/10.1134/S1064229319010137

Sun Y, Züst T, Silvestro D, Sun Y, Züst T, Silvestro D, Erb M, Bossdorf O, Mateo P, Robert C, Müller-Schärer H (2022) Climate warming can reduce biocontrol efficacy and promote plant invasion due to both genetic and transient metabolomic changes. Ecol Lett 25:1387–1400

Tamietti G, Valentino D (2006) Soil solarization as an ecological method for the control of Fusarium wilt of melon in Italy. Crop Prot 25:389–397

Travers RS, Martin PA, Reichelderfer CF (1987) Selective process for efficient isolation of soil *Bacillus* spp. Appl Environ Microbiol 53:1263–1266

Wu L, Li Z, Zhao F et al (2021) Increased organic fertilizer and reduced chemical fertilizer increased fungal diversity and the abundance of beneficial fungi on the grape berry surface in arid areas. Front Microbiol 12:628503

Wu K, Yuan S, Wang L (2014) Effects of bio-organic fertilizer plus soil amendment on the control of tobacco bacterial wilt and composition of soil bacterial communities. Biol Fertil Soils 50:961–971

Xue Q-Y, Chen Y, Li S-M et al (2014) (2009) Evaluation of the strains of Acinetobacter and Enterobacter as potential biocontrol agents against *Ralstonia* wilt of tomato. Biological Control 48:252–258

Yabuuchi E, Kosako Y, Yano I et al (1995) Transfer of two *Burkholderia* and an Alcaligenes species to *Ralstonia* gen. nov., *Ralstonia solanacearum* (Smith, 1986) comb. nov. Microbiol Immunol 39:897–904

Yadessa GB, van Bruggen AHC, Ocho FL (2010) Effects of different soil amendments on bacterial Wilt caused by *Ralstonia Solanacearum* and on the yield of Tomato. J Plant Pathol 92:439–450

Yang X, Chen L, Yong X, Shen Q (2011) Formulations can affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against Fusarium wilt of cucumbers. Biol Fertil Soils 47:239–248

Yi Y-J, Liu R-S, Yin H-Q et al (2007) Isolation, identification and field control efficacy of an endophytic strain against tobacco bacterial wilt (*Ralstonia solanacarum*). Ying Yong Sheng tai xue bao = the. J Appl Ecol 18:554–558

Yuan S, Wang L, Wu K, Yuan S, Wang L, Wu K, Shi J, Wang M, Yang X, Shen Q, Shen B (2014) Evaluation of *Bacillus*-fortified organic fertilizer for controlling tobacco bacterial wilt in greenhouse and field experiments. Appl Soil Ecol 75:86–94

Zelenev VV, Van Bruggen AHC, Leffelaar PA, Zelenev VV, van Bruggen AHC, Leffelaar PA, Bloem J, Semenov AM (2006) Oscillating dynamics of bacterial populations and their predators in response to fresh organic matter added to soil: the simulation model 'BACWAVE-WEB'. Soil Biol Biochem 38:1690–1711

Zhang S, Raza W, Yang X, Zhang S, Raza W, Yang X, Hu J, Huang Q, Xu Y, Liu X, Ran W, Shen Q (2008) Control of *Fusarium* wilt disease of cucumber plants with the application of a bioorganic fertilizer. Biol Fertil Soils 44:1073–1080