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Full Length Research Paper

Effects of two different soil amendments on the biocontrol efficacy of biological control agents (BCA) against Ralstonia wilt on ginger

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In this research, we developed an integrated approach to control Ralstonia wilt on ginger. Two organic composts (maize powder and soybean residue) were combined with one combination (the aqueous solution of *Bacillus subtilis* and *Bacillus cereus*) and two single (*B. subtilis* 1JN2 and *Bacillus megaterium* B1301) biological control agents (BCA) which together showed significant biocontrol efficacies in our previous study. Biocontrol efficacy of each treatment was measured. According to the results, a 3 - 30% biocontrol efficacy improvement was obtained by addition of the two organic composts compared to the BCA treatment. The best biocontrol activity (73.7%) was obtained from the combination of *B. megaterium* B1301 and maize powder. Colonization ability and the impacts on the bacterial community structure of each BCA were determined by denaturing gradient gel electrophoresis (DGGE). The DGGE fingerprints gave us an insight into the rhizosphere of the plant. And there is no noticeable bacterial structure shift, which means the exotic BCAs by artificial inoculation have little impacts on the local organism community. Additionally, the colonization ability of the tested BCAs was excellent and they could be detected at 120 days post inoculation. Furthermore, the available N, P, K and organic matter of rhizosphere soil from each treatment was measured. The organic matter did not show significant change among each treatment, while maize powder, soybean residue and BCA 1JN2 enhanced the available N, P and K significantly ranging from 79 - 133%, 166 - 251% and 252 - 368%, respectively compared to the blank control.

Key words: Integrated management, biological control agents (BCA), organic compost, ginger wilt.

INTRODUCTION

Ralstonia solanacearum (Smith) (Yabuuchi et al., 1995), which causes bacterial wilt on plants, is widespread in tropical, subtropical and warm temperate regions with more than 450 host species – both monocots and dicots – from 54 families (Hayward, 2000; Smith et al., 1995). Ginger (*Zingiber officinale* Rosc.) is an important horticultural crop in South-east Asia. It produces pungent, aromatic rhizomes, which are valuable both as a seasoning and as an herbal medicine. Ginger wilt caused by *R. solanacearum* was first reported in the 1950s and became increasingly serious in China (Ren and Fang, 1981). Disease incidence in the field usually ranges from 10 to 40%, but sometimes was reported to destroy the crop completely (Zhang et al., 2001). This disease could reduce the quantity of the ginger as well, resulting in huge economic losses.

Strategies for the biological control of *R. solanacearum* are still in a developmental stage (van Overbeek et al., 2002). Potential biological control agents (BCAs) explored so far to control Ralstonia wilt include avirulent

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mutants of *R. solanacearum* from bean (Xiao et al., 2008), genetically engineered antagonistic bacteria on banana (Kang et al., 1995), arbuscular mycorrhizal (AM) fungus *Glomus versiforme* (Zhu et al., 2004) on pepper, and antagonistic rhizobacteria such as *Bacillus* spp. (Silveira et al., 1995), *Pseudomonas* spp. (Guo et al., 2001; Götz et al., 2006) and *Streptomyces* spp. (El Albyad et al., 1996) on tomato. However, ginger wilt continues to be a serious problem in China and other countries, which has spurred research on the specificity of the pathogen to ginger.

The use of compost as a peat substitute to control root pathogens was first suggested by Hoitink et al. (1975). Since then, several soil-borne plant pathogens have been reduced by using composts made of different raw materials (Borrero et al., 2004; Cotxarrera et al., 2002; Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Litterick et al., 2004). The organic fertilizer cannot only change the physical and chemical characters of the soil, but also alter the microorganisms' community structures, which showed negative correlation with the infections of *R. solanacearum* (Masaya et al., 1999; Yoshitaka et al., 1999). It also showed that the pathogen is difficult to survive in the high abundant of microorganisms environment which resulted from adding organic fertilizers (Ritchie et al., 2000). According to the reports, most available approaches for biocontrol of plant diseases involved the use of a single BCA to a single pathogen (Raupach and Kloepper, 1998). This may lead to inconsistent performance of biocontrol agents and poor activity in all soil environments in which they are applied. In order to overcome these problems, some integrated approaches were attempted to enhance the biocontrol activity of the BCAs by adding some organic amendments (Saravanan et al., 2003).

In an earlier study, we obtained several BCAs that showed significant biocontrol efficacies against Ralstonia wilt of ginger (Yang et al., submitted). The aim of the current study was to develop a suitable integrated biocontrol strategy against ginger wilt, and to test whether the organic amendments could improve the biocontrol and colonization activity of the biocontrol agents.

MATERIALS AND METHODS

Bacteria, plants and organic compost

The virulent *R. solanacearum* strain SD2808 (Race 4 Biovar 4, isolated from wilted ginger plants from Shandong province of China) was cultured on YGPA (yeast extract 5 g, glucose 10 g, peptone 5 g, and agar 15 g in 1 L of water) at 30°C for 2 to 3 days. The biocontrol agents (*Bacillus subtilis*: 1JN2, *Bacillus megaterium*: B1301 and an aqueous solution of *B. subtilis* SM21and *Bacillus cereus* AR156) (Table 1) were cultured on Luria-Bertani (LB) agar at 28°C for 1 to 2 days. Ginger (*Z. officinale* Rosc. 'Shandong DaJiang') used for *in-vivo* antagonistic test was grown in pots 16 cm deep and 16 cm in diameter filled with 1.5 kg soil. Two of the most common organic fertilizer used in the field in Shandong province: maize powder and soybean residue were tested in this study.

In vivo antagonistic test

The greenhouse experiment consisted of eleven treatments including: 1JN2; the aqueous solution of *B. subtilis* and *B. cereus*; B1301; 1JN2 and maize powder; 1JN2 and soybean residue; the aqueous solution with maize powder; the aqueous solution with soybean residue; B1301 and maize powder; B1301 and soybean residue; maize powder; soybean residue. All the treatments were inoculated with the virulent *Ralstonia* strain. One blank control was set, which was only inoculated with the pathogen. Each treatment had 24 plants and the experiment was repeated three times. The process was described followed: First, the soil was mixed with the fertilizer completely before filling into the pot. The amount of the maize powder and soybean residue in each pot was adjusted to 10 g. The ginger rhizomes were cut into small (about 50 g) pieces each with at least one bud and planted in the pots (only one piece in each pot) after soaking in the suspension of one antagonistic strain /combination (10^8 CFU/ml) for 30 min. Subsequently, the pots were drenched with 20 ml of the same suspension (10^8 CFU/ml). One week later, 20 ml of *R. solanacearum* suspension (5×10^7 CFU/ml) was treated as the same way. The pots were placed in a greenhouse and the following conditions were maintained: temperature range of 25 top 30°C, 60% humidity, and 16 h of light alternating with 8 h of darkness. Thirty days after the pathogen treatment, the plants were scored for the disease index (DI) on a scale of 0 to 4 as follows: DI 0, no visible symptoms; DI 1, up to 25% of the leaves wilted; DI 2, 25 to 50% of the leaves wilted; DI 3, 50 to 75% of the leaves wilted; DI 4, 75 to 100% of the leaves wilted (Kempe and Sequeria, 1983). Disease severity and biocontrol efficacy were calculated as follows:

Disease severity (%) = [∑(The number of diseased plants in each grade × grade) / (Total number of plants investigated × The highest disease index)] × 100.

Biocontrol efficacy (%) = [(Disease severity in the control – Disease severity in the antagonist-treated group) / Disease severity in the control] ×100.

SAS statistical software version 8 (SAS Institute, 1999) was used for the analysis. Data from the two experiments were pooled after variance homogeneity was confirmed using the HOVTEST = LEVENE option of the ANOVA procedure. Whenever ANOVA detected significant differences between treatment means, Duncan's Multiple Range Test (DMRT) was used for comparing the mean values. For correlation analysis, the PROC CORR option of the ANOVA procedure was used. A significance level of $p = 0.05$ was used in all analyses.

Rhizosphere soil collection

Rhizosphere soil samples were collected at one, three, eight and fourteen weeks after treatment with BCAs and organic compost. At each time, three pots of plants were collected. Each sample consisted of the whole root system with tightly adhering soil of three individual plants. The plants were taken out from the plastic pot without disturbance, then plant root were shaken softly to remove the root-zone soils. We collected the soil adhering to the surroundings of a root by a small brush and kept in -70°C until proceed.

DGGE analysis

The rhizosphere soil samples were analyzed by denaturing gradient gel electrophoresis (DGGE) to determine the colonization ability of the BCAs and the impacts on the bacterial community structure. Genome DNA of the soil was extracted following the instructions of

Table 1. Strains used in this study.

Bacterial strain	Species	Source
B1301	*Bacillus megaterium*	SDAS
1JN2	*Bacillus subtilis*	Our laboratory
AR156	*Bacillus cereus*	Our laboratory
SM21	*Bacillus subtilis*	Our laboratory

B1301 was provided by Yang Hetong from SDAS; BBS combination includes AR156 and SM21 with 1:1 in volume. 1JN2 was isolated in our previous study with biocontrol efficacy against ginger wilt more than 60%.

Table 2. Biocontrol efficacy of *in vivo* antagonistic test in greenhouse.

Treatment	Disease severity (%)	Biocontrol efficacy (%)
M+B1301	15.63 ± 3.13^{c}	73.68
M+BBS	16.67 ± 1.80^{c}	71.93
S+B1301	17.71 ± 3.61^{c}	70.18
M +1JN2	17.71 ± 1.80^{c}	70.18
B1301	18.75 ± 5.41^{c}	68.42
S+BBS	19.79 ± 3.61^{c}	66.67
BBS	20.83 ± 4.77^{c}	64.91
S+1JN2	22.92 ± 4.77^{c}	61.40
1JN2	23.96 ± 3.61^{c}	59.65
M	43.75 ± 8.27^{b}	26.32
S	46.88 ± 5.41^{b}	21.05
CK	59.38 ± 0.00^{a}	0

M, Maize powder; S, soybean residue; B1301, *Bacillus megaterium*; 1JN2, *Bacillus subtilis*; BBS, AR156 and SM21 combination with volume 1:1 as earlier described.

the FastDNA Spin Kit for Soil (Mpbio industry, USA). A nested-PCR was applied to amplify the 16S rRNA gene region (V6–V8 variable regions of the 16S rRNA gene) as used for the DGGE bacterial profiles. On the first round, the 16S rRNA gene of the total genome was amplified as followed: 16S rDNA primers, U8-27(F) 5'-AGA GTT TGA TC(AC) TGG CTC AG-3'; L1494-1514(R) 5'-CTA CGG (AG)TA CCT TGT TAC GAC-3' 0.2 μmmol·L-1; 1× PCR buffer; 0.2 mmol·L-1 dNTPs; 3.75 mmol·L-1 $MgCl_2$; Taq polymerase enzyme 2U was added in 25 μL system.

Polymerase chain reaction (PCR) was performed using BIO-RAD DNA Engine Peltier Thermal Cycle with initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 56°C for 2 min, and 72°C for 2 min, and the final extension at 72°C for 10 min. The products were diluted 20 fold for the second round amplification used primer F984GC: 5'-AAC GCG AAG AAC CTT ACC GCC CGG GGC GCG CCC CGG GCG GGG CGG GGG CAC GGG GGG-3' and R1378: 5' -CGG TGT GTA CAA GGCCCGGGAACG-3'. The reaction mixture (25 μL) composed of 1 μL template DNA, 1× PCR buffer, 0.2 mM dNTPs, 3.75 mM MgCl2, 4% (w/v) acetamide, 0.2 mM each primer, and 2.5 U Taq DNA polymerase. After 5 min of denaturation at 94°C, 30 cycles of 1 min at 95°C, 1 min at 53°C and 2 min at 72°C were carried out. A final extension step of 10 min at 72°C was used to finish the reaction. Products were checked by electrophoresis in 1% agarose gels and ethidium bromide staining. All the strains inoculated were amplified V6–V8 variable regions of the 16S rRNA gene as described above and loaded on DGGE as makers.

Denaturing gradient gel electrophoresis analysis was performed with the IngenyPhorU apparatus (Ingeny Inc., Leiden, Netherlands). Gel casting was performed as described by Heuer and Smalla (1997). A double gradient consisting of 46.5 to 65% denaturants (100% denaturants defined as 7 M urea and 40% formamide) and 6 to 9% acrylamide was prepared. Gels were silver stained according to Heuer et al. (2001) and air-dried. A mixture of the DGGE-PCR products from 11 bacterial species was applied at the extremities of the gels as a marker to check the electrophoresis run and to compare fragment migration between gels, as described by Smalla et al. (2001).

Soil analysis

The organic matter, available N, P and K of the rhizosphere soil samples were analyzed. Analytic methods were based on the handbook for the chemical analysis (Professional Committee of Agricultural Chemistry of Chinese Society for Soil Science, 1989).

RESULTS

Biocontrol efficacy of *in vivo* antagonistic test

Among the eleven treatments, biocontrol efficacies from BCA and organic compost combinations were all higher than the single BCA treatment (Table 2). Maize powder showed higher improvement than soybean residue, although there is no significant difference. *B. megaterium*

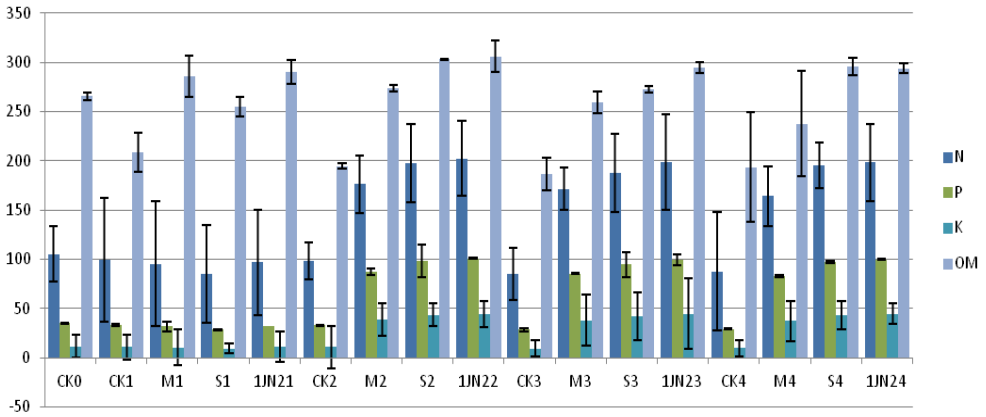

Figure 1. Concentration of available N, P, K and organic matter after treated by 1JN2 and the two fertilizers. M, Maize powder; S, soybean residue; 1JN2, *Bacillus subtilis*. 1, 2, 3 and 4 represent samples collected 1, 3, 8 and 14 weeks after treatment. N, Available N; P, available P; K, available K; OM, organic matter.

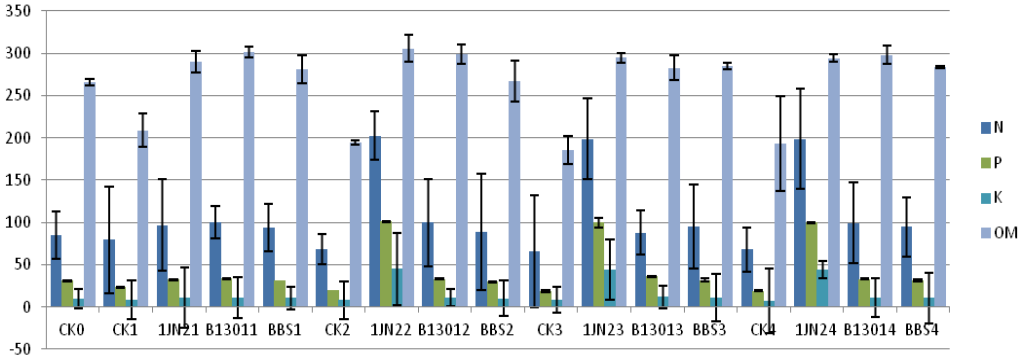

Figure 2. Concentration of available N, P, K and organic matter after treated by the three BCAs. B1301, *Bacillus megaterium*; 1JN2, *Bacillus subtilis*; BBS, AR156 and SM21 combination with volume 1:1 as earlier described. 1, 2, 3 and 4 represent samples collected 1, 3, 8 and 14 weeks after treatment. N, Available N; P, available P; K, available K; OM, organic matter.

B1301 was the most powerful BCA not only in single treatment but also in combination with the two fertilizers whose biocontrol efficacies reached 68 and 73%. The aqueous solution of *B. subtilis* and *B. cereus* which showed high activity against Ralstonia wilt on tomato and pepper in our previous study also showed excellent biocontrol ability on ginger. *B. subtilis* strain 1JN2 was isolated from the stem of ginger in previous work and its biocontrol efficacy against ginger wilt reached 69%. However, it reduced to 55% in this study and amendment with maize powder and soybean residue complemented its ability partially or even higher.

Soil sample content analysis

Available N, P and K in the rhizosphere soil were equal one week after treatment with maize powder, soybean residue and the biocontrol agent 1JN2, but the content were significantly increased after three weeks treated (Figure 1). The concentration was maintained at the high level during all the growth period compared with the blank control. Among the three biocontrol strains, only 1JN2 has the ability to improve the nutrient (Figure 2). When considering the organic matter we can see that all the treatments had the ability to increase the concentration of organic matter. The concentration of organic matter one week after treatment by biocontrol agents and fertilizers were equal to that in the blank control. However, the blank control decreased significantly with the ginger growth while the other treatments maintained the high level of organic matter during the whole growing period.

Colonization of BCAs and their impacts on the bacterial community structure

Denaturing gradient gel electrophoresis (DGGE) was

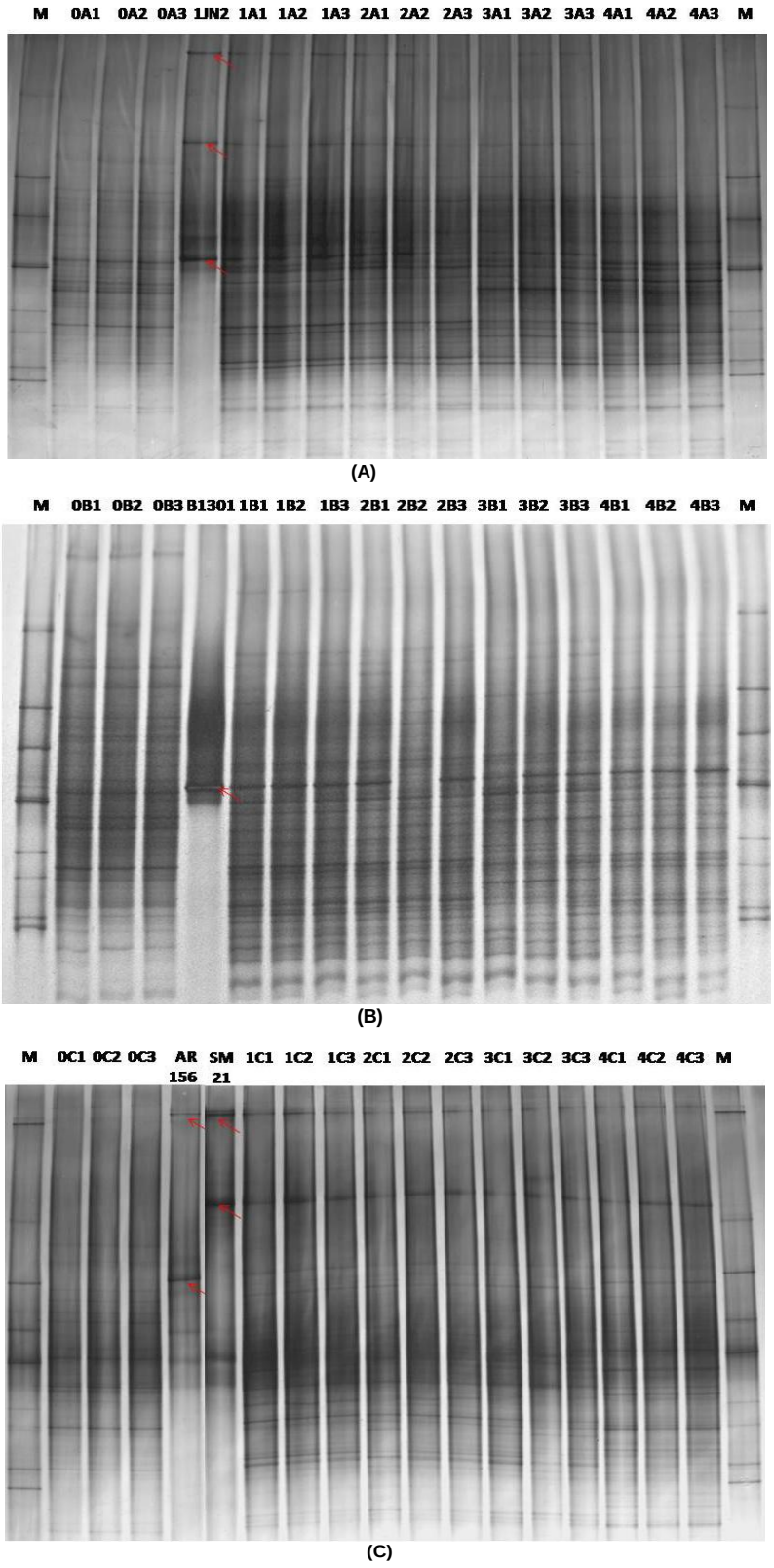

Figure 3. DGGE fingerprint of the rhizosphere soil samples after treated by the three BCAs. A, 1JN2; B, B1301; C, BBS combination. 1, 2, 3 and 4 means samples collected 1, 3, 8 and 14 weeks after treated. 0 means soil samples collected before each treated. Arrow directed bands means the inoculated BCAs.

done to detect the colonization ability of the applied BCAs. According to the DGGE fingerprint we observed that the colonization ability of the three BCAs were different from each other. For 1JN2, it could be detected on 1, 3 and 8 weeks, but there are no distinguishable bands after 14 weeks treatment (Figure 3A). For B1301, AR156 and SM21 (AR156 and SM21 are the components of the aqueous solution of *B. subtilis* and *B. cereus*), they could be detected 14 weeks after being treated (Figure 3B and C), which meant that it covers the whole growth period of the ginger plant.

Based on the DGGE fingerprint, we also observed that the dominant bands had no change after being treated by the BCAs. The diversity of the rhizobacteria community increased compared to the fingerprint before treatment, and much more weak bands appeared with ginger growth.

DISCUSSION

PGPR is a wide variety of soil bacteria which when grown in association with a host plant, results in stimulation of growth of their hosts. These rhizosphere bacteria enhance plant growth and yield either directly or indirectly (Vessey, 2003). The direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment (Glick et al., 1999). The indirect promotion of plant growth occurs when PGPR lessens or prevents the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host (Cartieaux et al., 2003). The direct growth promoting mechanisms are as follows: (i) nitrogen fixation; (ii) solubilization of phosphorus; (iii) sequestering of iron by production of siderophores; (iv) production of phytohormones such as auxins (indole acetic acid, IAA), cytokinins, gibberellins and (v) lowering of ethylene concentration (Kloepper et al., 1989; Glick et al., 1999). The indirect mechanisms of plant growth promotion by PGPR include (i) antibiotic production; (ii) depletion of iron from the rhizosphere; (iii) synthesis of antifungal metabolites; (iv) production of fungal cell wall lysing enzymes; (v) competition for sites on roots and (vi) inducing systemic resistance (Glick et al., 1999).

The mechanism of biocontrol agent 1JN2 showed in this study was thought to increase the nutrient for the host plant growth promotion including available N, P, K and organic matter. This *Bacillus* strain was isolated from ginger stem in our previous study and showed significant biocontrol efficacy. The other biocontrol agent, the aqueous solution of *B. subtilis* and *B. cereus* which showed high biocontrol ability on other host such as tomato and pepper, also had good biocontrol ability on ginger although there was a little decrease. The colonization ability of the aqueous solution of *B. subtilis* and *B. cereus* is better than 1JN2, which could cover all the plantation period. The *B. megaterium* B1301 also showed high colonization ability in this study. They are supposed to get their high biocontrol activity by competition against pathogenic bacteria. In addition, all these strains could produce plant phytohormone IAA (data not shown), which can directly promote plant growth.

Organic fertilizers also inhibit plant disease and plant growth promotion (Bardin et al., 2004; Trillas et al., 2006; Ling et al., 2010). Some of them can improve the content of chlorophyll (Amujoyegbe et al., 2007) and others can help to convert the nitrate into volatile ammonia (Bardin et al., 2004). Thus, in this study, we used two organic fertilizers in combination with BCAs to promote their activity in inhibiting plant pathogen. Soybean residue is one of the most common organic fertilizers used in China. Maize powder is recommended by the farmers who grow ginger in Shandong province which is the biggest ginger productive province. The exactly mechanism is unknown, but it showed high ability in inhibiting disease and promoting plant growth in practice. Although single organic fertilizer showed poor biocontrol activity, however, when used together with BCAs they can improve the efficacy significantly. This may be as a result of the organic matter enabling the biocontrol agents to survive and to colonize the root, since both two fertilizers could improve the concentration of organic matter significantly.

External biocontrol agents were thought to have some impacts on the resident microorganism community structure. But the answer is: it depends. This dependence may stem from interactions within and between indigenous populations. In relation to the soil–plant–environment background, certain groups may be enhanced, while others may be inhibited, or the introduced PGPR may not affect population structure (Nacamulli et al., 1997; Schwieger and Tebbe, 2000; Bacilio-Jimenez et al., 2001; Dobbelaere et al., 2003). In this study, little influence was seen when treated by the different BCAs. The dominant bands did not change after inoculation, but the diversity of the rhizosphere bacteria community increased. Much more weak bands emerged after treatment, especially in the soil samples which collected on 60 and 120 days after treatment. Based on these results, we can confirm the conclusion that the stage of plant development plays an important role on the rhizosphere bacterial community structure more than exogenous inoculations (Castro-Sowinski et al., 2007).

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REFERENCES

Amujoyegbe BJ, Opabode JT, Olayinka A (2007). Effect of organic and inorganic fertilizer on yield and chlorophyll content of maize (*Zea mays* L.) and sorghum *Sorghum bicolour* (L. Moench). Afr. J. Biotechnol., 6(16): 1869-1873.

Bacilio-Jimenez M, Aguilar-Flores S, del Valle MV, Prez A, Zepeda A, Zenteno E (2001). Endophytic bacteria in rice seeds inhibit early colonization of roots by Azospirillum brasilense. Soil Biol. Biochem., 33: 167–172.

Bardin SD, Huang HC, Moyer JR (2004). Control of *pythium* damping-off of sugar beet by seed treatment with crop straw powders and a biocontrol agent. Biol. Control, 29: 453-460.

Borrero C, Trillas MI, Ordovás J, Tello J, Avilés M (2004). Predictive factors for the suppression of Fusarium wilt of tomato in plant growth media. Phytopathology, 94: 1094–1101.

Cartieaux FP, Nussaume L, Robaglia C (2003). Tales from the underground: Molecular plant-rhizobacteria interactions. Plant Cell Environ., 26: 189–199.

Castro-Sowinski S, Herschkovitz Y, Okon Y, Jurkevitch E (2007). Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. FEMS Microbiol. Lett., 276: 1-11.

Cotxarrera L, Trillas-Gay MI, Steinberg C, Alabouvette C (2002). Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress Fusarium wilt of tomato. Soil Biol. Biochem., 34: 467–476.

Dobbelaere S, Vanderleyden J, Okon Y (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant Sci., 22: 107–149.

El Albyad MS, EL Sayed MA and EL Shanshoury AR (1996). Effect of culture conditions on the antimicrobial activities of UV-mutants of *Streptomyces corchorusii* and *S. spiroverticillatus* against bean and banana wilt pathogens. Microbiol. Res., 151: 201–211.

Glick BR, Patten CL, Holguin G, Penrose DM (1999). Biochemical and genetic mechanisms used by plant growth promoting bacteria. London, UK: Imperial College Press, pp. 267.

Götz M, Gomes NCM, Dratwinski A, Costa R, Berg G, Peixoto R, Mendonca-Hagler H, Smalla K (2006). Survival of *gfp*-tagged antagonistic bacteria in the rhizosphere of tomato plants and their effects on the indigenous bacterial community. FEMS Microbiol. Ecol., 56: 207–218.

Guo JH, Guo YH, Zhang LX, Qi HY, Fang ZD (2001). Screening for biocontrol agents against cayenne pepper bacterial wilt. China J. Biol. Control, 17: 101–106.

Hayward AC (2000). *Ralstonia solanacearum*. Encycl. Microbiol., 4: 32–42.

Heuer H, Smalla K (1997). Application of denaturing gradient gel electrophoresis and temperature gradient gel electrophoresis for studying soil microbial communities. 1997, pp. 353-373. Mod Soil Microbiol (van Elsas JD, Wellington EMH, Trevors JT, ed.), Marcel Dekker, Inc., New York.

Heuer H, Wieland J, Sch¨onfeld J, Sch¨onw¨alder A, Gomes NCM, Smalla K (2001). Bacterial community profiling using DGGE or TGGE analysis. Environ Mol Microbiol: Protocols and Applications (Rouchelle P, ed), pp. 177–190. Horizon Scientific Press, Wymondham, UK.

Hoitink HAJ, Boehm MJ (1999). Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu. Rev. Phytopathol., 37: 427-446.

Hoitink HAJ, Fahy PC (1986). Basis for the control of soil-borne plant pathogens with composts. Annu. Rev. Phytopathol., 24: 93-114.

Hoitink HAJ, Schmitthener AF, Herr LJ (1975). Composted bark for control of root rot in ornamentals. Ohio Rep., 60: 25-26.

Kang Y, Mao G, Lu C, He L (1995). Biological control of bacterial wilt of tomato by extracellular protein defective mutant of *Pseudomonas solanacearum*. Acta Phytopathol. Sin., 22: 287-288.

Kempe J, Sequeira L (1983). Biological control of bacterial wilt of potatoes: Attempts to induce resistance by treating tubes with bacteria. Plant Dis., 67(5): 499–501.

Kloepper JW, Lifshitz R, Zablotowicz RM (1989). Free living bacterial inocula for enhancing crop productivity. Trends Biotechnol., 7: 39–44.

Ling N, Xue C, Huang QW, Yang XM, Xu YC, Shen QR (2010). Developments of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy to Fusarium wilt. Biocontrol., 55: 673-683.

Litterick AM, Harrier L, Wallace P, Watson CA, Wood M (2004). The role of uncomposted materials, composts, manures and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production—a review. Crit. Rev. Plant Sci., 23: 453–479.

Masaya N, Yoshitaka S, Sea S (1999). Suppression of growth of *Ralstonia solanacearum*, tomato bacterial wilt agent, on/in tomato seedings cultivated in a suppressive soil. Soil Sci. Plant Nutr., 45(1): 79-87.

Nacamulli C, Bevivino A, Dalmastri C, Tabacchioni S and Chiarini L (1997). Perturbation of maize rhizosphere following seed bacterization with Burkholderia cepacia MCI 7. FEMS Microbiol. Ecol., 23: 183-193.

Professional Committee of Agricultural Chemistry of Chinese Society for Soil Science (1989). Routine Analysis Method for Soil Agricultural Chemistry. Science Press, Beijing (In Chinese), pp. 67-119.

Raupach GS, Kloepper JW (1998). Mixtures of plant growth-promoting rhizobacteria enhance biological control of soilborne diseases and potential extension to systemic and foliar diseases. Phytopathol*.,* 88: 1158-1164.

Ren HC, Fang CT (1981). Identification of the causal organism of the bacterial wilt of Ginger. Acta Phytopathol. Sin. 0412-0914.0.1981-01-010.

Ritchie NJ, Schutter ME, Dick RP, Myrold DD (2000). Use of length heterogeneity PCR and Fatty Acid Methyl Ester profiles to characterize microbial communities in Soil. Appl. Environ. Microbiol., 66: 1668-1675.

Saravanan T, Muthusamy M, Marimuthu T (2003). Development of integrated approach to manage the fusarial wilt of banana. Crop Prot., 22: 1117-1123.

Schwieger F, Tebbe CC (2000). Effect of field inoculation with Sinorhizobium meliloti L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*) – Linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. Appl. Environ. Microbiol*.,* 66: 3556–3565.

Silveira EB, Da R, Mariano de LR, Michereff SJ (1995). Antagonism of *Bacillus* spp. Against *Pseudomonas solanacearum* and effect on tomato seedling growth. Fitopathol Bras., 20: 605-612.

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. App. Environ. Microbiol*.,* 67: 4742-4751.

Smith JJ, Offord LC, Holderness M, Saddler GS (1995). Genetic Diversity of *Burkholderia solanacearum* (synonym *Pseudomonas solanacearum*) race3 in Kenya. Appl. Environ. Microbiol., 61: 4263-4268.

Trillas MI, Casanova E, Cotxarrera L, Ordovas J, Borrero C, Aviles M (2006). Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedings. Biol. Control., 39: 32-38.

Van Overbeek M, Cassidy M, Kozdroj J, Trevors JT, Van Elsas JD (2002). A polyphasic approach for studying the interaction between *Ralstonia solanacearum* and potential control agents in the tomato phytosphere. J. Microbiol. Methods, 48: 69-86.

Vessey JK (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571–586.

Xiao T, Xiao CG, Zou Y, Yuan XL (2008). A preliminary study on the control of tobacco bacterial wilt by avirulent strains of *Ralstonia solanacearum*. Plant Prot., 34(4): 79-82.

Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y (1995). Transfer of two Burkholderia and Alkanigens species to Ralstonia genus. Nov.; Proposal of Ralstonia pickettii (Ralston, Palleroni and Doudoroff,

1973) Comb. Nov. and Ralstonia eutropha (Davis, 1969) comb. Microbiol. Immunol., 39: 897-904.
Yoshitaka S, Masaya N, Tomoko O, Takuya M (1999). Comparison of bacterial community structures in the rhizoplane of tomato plants grown in soils suppressive and conducive towards bacterial wilt. Appl. Environ. Microbiol., 65: 3996-4001.
Zhang GM, Fan GQ, Zhu HC (2001). Study on the pathogen of the ginger wilt disease in Shandong. J. Shandong Agric. Univ., 32(4): 418-422.
Zhu HH, Yao Q, Li HH, Yang SZ (2004). Inhibition of *Ralstonia solanacearum* by AM fungus *Glomus versiforme* and their effect on phenols in root. J. Microbiol., 31: 1-5.