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Full Length Research Paper

Rhizobacteria of pepper (*Piper nigrum*) and their antifungal activities

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The purpose of this study is to isolate and select indigenous soil *Bacillus* bacteria capable of developing multiple mechanisms of action related to the biocontrol of phytopathogenic fungi affecting pepper vines (*Piper nigrum*). The screening procedure consisted of antagonism tests against a panel of phytopathogenic fungi*, in vitro* detection of the antifungal products and root colonization assay. Four isolates, identified and designated as *Bacillus amyloliquefaciens* (WW6)*, Bacillus atrophaeus* (MPB), *Bacillus subtilis* (CBF) and *Bacillus vallismortis* (WW14) were selected for further studies. All bacterial isolates obtained where effective for the *in vitro* control of the growth of phytopathogenic fungi, where the control mechanisms used by the bacteria involved the secretion of protease and cellulase enzyme that are responsible for fungal cell wall hydrolysis. The bacteria also produced volatile as well as diffusible substances. Malformation of hyphae occurred in the presence of both bacteria. Hyphae were thickened, vacuolar and many swellings occurred in them or at the tips of hyphal strand. On the other hand, all bacteria grew well in conditions similar to those that can be found at the field level (considering pH, salinity, and temperature) and showed a good capacity of pepper root colonization. These results suggest that all the bacterial isolates studied have an excellent potential to be used as biocontrol agents for controlling phytopathogenic fungi greenhouses at the field level.

Key words: Biocontrol, *Bacillus* spp, *Piper nigrum, a*ntifungal.

INTRODUCTION

Piper nigrum L., generally known as pepper, is an important tropical spice and it is the most important spice traded, in terms of quality as well as value, and accounts for a significant portion of world trade in spices. In Malaysia, it is cultivated over an area of 14,735 ha with the production of 25,672 ton giving yield of 4.6 ton per hectare (Malaysian pepper Board Annual Report, 2010). This yield is quite low as compared to other developing countries like Vietnam, India, and Brazil. There are number of reasons for this low production of pepper in Malaysia, with pathogenic foliar and other soil-borne diseases constituting the most important limiting factors. In Malaysia, screening of pepper field showed that, the most frequently isolated and important fungal pathogens that infected pepper genotypes are *Fusarium solani, Phytophthora capsici, Colletotrichum capsici*, *Colletotrichum gloeosporioides*, S*eptobacidium* spp. and R*igidoporus ligosus*. These pathogens attack both the above and underground parts of pepper vine, leading to pre- and post-emergence death, damping-off, leaf blight, root rot, leaf and fruit spot, and root damage as reported by Fatimah et al. (2003). Different approaches have been used to prevent, mitigate or control plant diseases. Currently, although the uses of chemical are comparatively suitable to control the fungal diseases, but the continuous and abusive application of chemical products has lead to the apparition of environment and human health problems. Therefore, the development of biological control is one of the attractive and alternative to synthetic fungicide due to their ability to antagonize the pathogen by different mode of action. The mechanisms of biological control of plant pathogens by antagonistic bacteria and fungi have been the subjects of many studies in the past two decades (Janisiewicz et al. 2000). Among these rhizobacteria, several stains of *Bacillus subtilis* have been reported to be a promising candidate

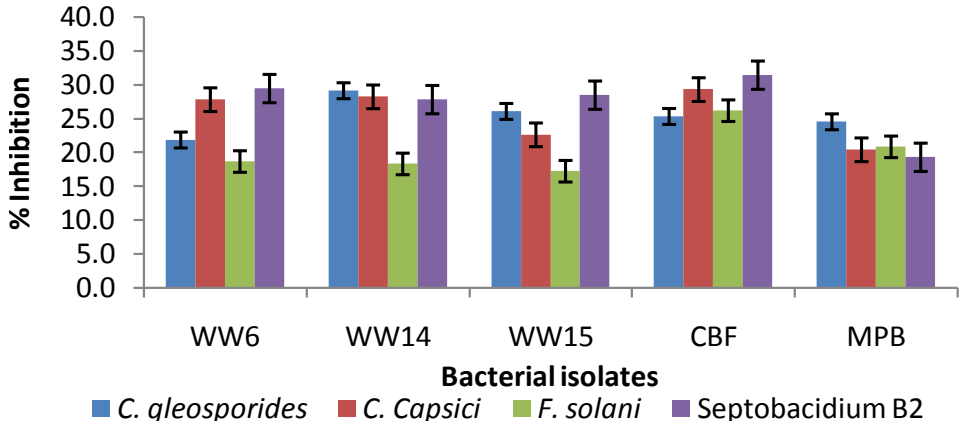

Figure 1. Effect of volatile antiboiotics of baterial isolates on the radial growth of pathogen fungus *in vivo.*

with proven excellent characteristics like effective root colonization, versatile activity against multiple pathogens and promising ability to sporulate (Kloepper et al., 2004; Romero et al., 2004; Hassan et al., 2010).

Currently, there is an increasing interest in the introduction of bacterial biocontrol agent for managing fungal infestation, partly as a response to public concern about non-target effects on synthetic fungicide. However, the development of biological control product is facing several challenges due to the lack of certain information either on crop or biological control agent itself. For example, Rahman et al., 2007 showed that Gram-negative bacteria (*Burrkholderia cepacia*) is an effective biocontrol agent against anthracnose on papaya but the pathogenicity of the bacteria strains to human is still unknown. Besides, the inconsistency of field results regarding microbial disease repression is also another factor that contributes to the failure of the development of biological control. On the other hand, there are numerous reports of rhizobacteria that exert a beneficial effect on plant growth (Korsten and Jager, 1995; Compant et al., 2005 and Janisiewicz et al., 2000) for example, the *Bacillus* spp are getting more attention due to their ability to produce different type of antibiotic (baculysin, iturin, mycosubtilin), UV light resistant spores, are able to induce growth and defense response in host plant (Shoda, 2000). This present study was undertaken to determine the antifungal activities of the isolated bacteria on some fungal phytopathogens and spoilage pathogens that affect the pepper.

MATERIALS AND METHODS

Isolation of pathogenic fungi

Pieces of pepper roots and crown obtained from pepper vine that showed symptoms of disease were submerged in 5% sodium hypochloride for five minutes. After this treatment, they were extensively washed with sterile distilled water and placed on Petri dishes containing potato-dextrose-agar (PDA, Difco) and incubated at 30°C for 48 h. The fungi isolates were identified and stored at 5°C in tubes containing PDA.

Sampling, isolation and selection of bacteria

Potential bioantagonistic bacteria were isolated either from rhizoplane or from healthy or diseased pepper vines as follows: a) root pieces from pepper vines were washed with tap water, placed inside tubes containing $MgSO_4$ and subjected to ultrasound (50 to 55 kHz during 5 min) (Pumarino, 1995). The bacterial suspension obtained was diluted to 10^{-3}, 10^{-5} y 10^{-7} for selection; b) 1 g soil was placed in a tube containing sterile distilled water, and dilutions similar to those earlier mentioned were done. For both types of samples, 0.1 ml of each dilution was placed in B King medium and PDA, incubated at 22°C until colony development was observed.

The obtained bacteria were pre-evaluated against the isolated *Colletotrichum gloesporioides*, the most prevalent pathogenic fungus in pepper vine. 20 µl of bacterial suspensions (5×10^9 cfu ml^{-1}) 24 h old, obtained from different samples, were placed on different 0.5 cm sterile paper disks. Disks were placed on a Petri dish containing PDA, surrounding a 10 mm diameter disk containing mycelium of a four-day culture of *C. gloesporioides*, placed in the center of the plate. It was incubated for 48 h at 22°C, and inhibition of mycelium growth was checked. Bacteria that did not inhibit fungal growth were discarded. Those with bioantagonistic activity were stored in tubes containing B King medium at 5°C, and in flasks containing tryptone soy broth (TSB) plus glycerol for storage at -21°C (Raupach and Kloepper, 1998).

Antagonism against pathogenic fungi

Bacterial isolates were tested against *F. solani, P. capsici, C. capsici*, *C. gloesporioides* and *Septobacidium* spp using dual cultures techniques. One 10 mm disk of a pure culture of pathogenic fungus was placed at the center of a Petri dish containing PDA. A circular line, made with a 6 cm diameter Petri dish dipped in a suspension of bioantagonistic bacteria (5×10^9-cfu ml^{-1}), was placed surrounding the fungal inoculum (Figure 1). Plates were cultured for 72 h at 22°C and growth diameter of the pathogen (fungal growth) was measured and compared to control growth where the bacterial suspension was replaced by sterile distilled water. Each experiment considering a single pathogen isolate was run in triplicate and was repeated at least three times.

Results obtained are expressed as means % inhibition + S.D. of the growth of the corresponding pathogen isolate in the presence of any of the bacterial isolates. Percent inhibition was calculated using the following formula:

% inhibition = [1 - (Fungal growth / Control growth)] × 100

Molecular identification of bacterial isolates

Total genomic DNA was prepared from LB cultures by using the Wizard genomic DNA purification kit (Promega Inc., Madison, WI, USA) and adjusted to 50 ng/µL. Bacterial isolates were analyzed by PCR method using Universal primer P1 (forward primer) and P6 (reverse primer) as described by Tan et al. (1997). Amplifications were performed in an MJ Research PTC-100 thermocycler using standard conditions. Amplified products were separated by electrophoresing on 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light. Desired product were eluted from gel using the gel extraction kit (Qiagen Inc) and sequenced direct on the sequencer ABI Prism 3100 Genetic analyser (Hitachi, Japan) using Big Dye Terminator v 1.1 Cycle sequencing kit at the First BASE Laboratories Sdn Bhd, Malaysia. The 16S rRNA gene sequences will be compared with those in GenBank using the BLASTN program and strains that were closely related were aligned using the CLUSTAL W program.

Production of diffusible antibiotics

PDA plates, covered with a cellophane membrane, were inoculated in the center with 100 µl of a bioantagonistic bacterial suspension (5×10^9 cfu ml^{-1}). After incubation for 72 h at 22°C, the membrane with the grown bacterial isolate was removed, and the plate was inoculated in the middle with a 10 mm disk of a pure culture of pathogenic fungus. Plates were further incubated at 22°C for 48 h and the growth of the pathogen was measured. Controls were run with mocked inoculated PDA containing plates on the cellophane membrane (replacing the bacterial suspension by sterile distilled water), and further inoculated with pathogenic fungus. Each experiment considering a single bacterial isolate was run in triplicates and was repeated at least three times. Results are expressed as means of % inhibition + S.D. of growth of pathogen in the presence and absence of any bioantagonistic bacterial isolate.

Production of volatile antibiotics

100 µl of a bioantagonistic bacterial suspension (5×10^9 cfu ml^{-1}) were placed at the center of one half Petri dish containing King B medium, and a 10 mm disk of four days old pure culture of pathogenic fungus was placed at the center of another Petri dish containing PDA. Both half plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension, and were sealed to isolate the inside atmosphere and to prevent loss of volatiles formed. Plates were incubated at 22°C for 48 h and the growth of the pathogen was measured and compared to controls developed in the absence of the bioantagonist (mocked inoculation with an 8 mm disk of PDA). Each experiment considering a single bacterial isolate was run in triplicate and was repeated at least three times. Results obtained are expressed as means of % inhibition + S.D. of the growth of pathogen in the presence and absence of any bacterial isolate.

Study on hyphal morphology

A 6 mm diameter mycelial plug from four days old PDA culture of *C. gloeosporioides* was transferred to the center of a Petri dish, containing 15 ml of PD broth and incubated at 28±2°C for 2 days. When mycelial growth on the surface of the liquid reached a diameter about 1.5 cm, 1 mm bacterial suspension (109 cfu ml^{-1}) was transferred to the plate and the culture was further incubated at 28±2°C for 3 days. Hyphal strands at the end of the fungal colony were removed and examined under a microscope for abnormalities (Sariah, 1994). Cultures of *C. gloeosporioides* in PD broth without bacterial inoculation served as control. The experiment was repeated for other pathogenic fungi.

Detection of hydrolytic enzymes

Chitinase activity was measured according to Chernin et al. (1995), protease activity according to Katekan et al. (2009), and cellulolytic activity on microcrystalline cellulose-containing plates as described by Kasana et al. (2008).

Effect of different salt concentrations, pH values and temperatures on development of antagonistic bacteria

The development of bioantagonistic bacteria was tested under NaCl concentrations of 0, 50, 100, 150, 200 and 250 mM; at pH values which ranged between 5.0 and 8.0 (with intervals each 0.5 pH units); and temperatures of incubation of 10, 15, 22, 28 and 37°C. 0.5 ml of a bacterial suspension (5×10^9 cfu ml^{-1}) from a 24 h old culture were placed in a tube containing 4.5 ml of peptone broth previously adjusted at the desired NaCl concentration and/or pH value. Tubes were incubated for 48 h at the temperatures mentioned earlier, and bacterial growth was estimated at 600 nm, after serial dilutions when necessary. The experimental unit was one tube for each experimental condition. Results correspond to the mean of all experiments, which were repeated at least four times. Data was analyzed using ANOVA and the Duncan multiple test was used to establish significant differences.

Colonization assay

This experiment was performed under aseptic conditions. Pepper cutting were surface sterilized as described by Misaghi (1990) and left for 1 h either in the water or in contact with a bacterial suspension. Pepper cutting were kept in the plant house with proper maintenance. Three replicate were used for each treatment. After five days, root pieces from pepper vines were washed with tap water and placed inside tubes containing $MgSO_4$ and subjected to ultrasound (50 to 55 KHz during 5 min) (Pumarino, 1995). The bacterial suspension obtained was diluted to 10^{-3}, 10^{-5} y 10^{-7} for bacterial CFU counting. Results correspond to the mean of all experiments, which were repeated at least 3 times. Data was analyzed using ANOVA test to establish significant differences.

RESULTS

Isolation and Identification

A primary selection was made from the antagonism test plates where the confluent growth of bacteria from the pepper rhizosphere inhibited the development of fungal mycelia. Pure bacterial cultures isolated from those plates were tested for fungal antagonism. This procedure resulted in 150 initial isolates that inhibited *C.*

Table 1. Antagonism of isolates against different pathogenic fungi using dual culture techniques.

Pathogenic fungus	% inhibition					
	WW6	WW14	WW15	SW15	CBF	MPB
Colletotrichum gleoporides	42.4±3.0	52.4±3.3	52.4±2.8	43.8±3.8	50.1±2.3	40.4±1.7
Colletotrichum capsici	53.1±2.2	43.0±1.5	42.6±1.3	33.0±2.9	43.3±1.7	44.7±2.2
Fusarium solani	43.0±1.3	36.4±1.6	43.6±1.3	NE	44.0±1.8	45.7±1.4
Septobacidium spp (B2)	45.9±1.8	37.8±3.3	49.3±2.8	32.3±1.5	46.3±1.6a	49.3±2.1
Phytophthora capsici	NE	NE	NE	NE	32.7±2.6	NE

NE- No inhibition effect on respective fungus.

gloeosporioides more than 20% with respect to the fungi growing alone. By applying additional selective criteria as described in Materials and Methods, the number of selected isolates was reduced to 50. Looking for bacteria with a wide range of antifungal action, six out of the 50 isolates were reselected after testing them against a panel of phytopathogenic fungi. These six strains were subjected to molecular identification. These six (6) bacterial isolates were identified as *Bacillus* group and Enhydrobacter group. The 16S rDNA sequence analyses showed that the strain WW14 and WW15 had identical pattern to those of the reference strains of *Bacillus vallismortis.* Other isolates, such as WW6 and CBF, and MPB were classified as belonging to *Bacillus amyloliquefaciens*, *B. subtilis* and *Bacillus atrophaeus*, respectively. These three isolates shared 99% sequence similarity with the reference strains of Bacillus species. In the case of strain SW15, 16S rRNA gene sequence similarity result showed that this strain was classified as *Enhydrobacter aerosaccus* with 99% sequence similarity.

Mechanism of antagonism

Dual culture assay

There was no physical contact between any of the antagonists and pathogen (Figure 1). An inhibitory halo was observed suggesting the presence of fungistatic metabolites secreted by the bacteria. Microscopic observation of fungal zone also showed the cytoplasmic leakage at the hyphal septum. Bacterial strains significantly reduced pathogen growth in comparison with the control. Table 1 summarized the antagonistic activity of bacterial isolates against various type of pathogen. From the data collected, Strain CBF, *B. subtilis* were found to be the most effective isolates that were able to suppress the growth of *C. gloeosporioides, C. capsici, F. solani, Septobacidium* spp (B2) and *P. capsici*. Among the strains tested against pathogenic fungi, all the strains were able to suppress more than 3 pathogenic fungi except for strain SW15. Efficient antagonist bacteria of WW6, WW14, WW15, CBF and MPB were selected for further studies on the basis of this test.

Production of volatile antibiotics

All antagonist isolates were showed to be significantly different from the control ($P < 0.01$). The growths of all pathogenic fungus were significantly inhibited by antagonistic bacterial. Strain CBF was the antagonistic bacteria isolate that showed the best inhibitory effect on the growth of all the pathogen tested, although all bacteria also showed inhibitory effect on pathogen tested.

Production of diffusible antibiotics

The effect of diffusible antibiotics was similar to that of volatile antibiotic. All the pathogen fungus was inhibited independently by each of the bacterial stains at varying level. Strain CBF was the antagonistic bacterial that show the best inhibitory activity with 36.9% inhibition against *C. gloeosporioides,* 33.7% for *C. capsici,* 35.5% for *F. solani* and 36% for *Septobasidium* spp (Figure 2). Strains WW6, WW14, WW15, and MPB were also able to significantly suppressed pathogen growth although they all showed minor inhibitory effect on pathogenic the fungus tested.

Study on hyphal morphology

Hyphal morphology study showed that there is a change in mycelia colour of pathogenic fungus after inoculated with antagonistic bacteria. Microscopic observation on the inhibition zone, allowed detecting cytoplasmic leakage that could be observed up to the hyphal septum, resulting in deformation of the mycelia hyphae. Hyphae tips of the fungus became malformed and hyphae were thickened and vacuolar compared with hyphae in the control plate. Many swellings occurred in the hyphae or at the tips of hyphal strands, whereas normal hyphal walls were smooth with no swellings or vacuolation (Figure 3).

Secretion of enzymes involved in bioantagonism

All five antagonists (WW6, WW14, WW15, CBF and MPB)

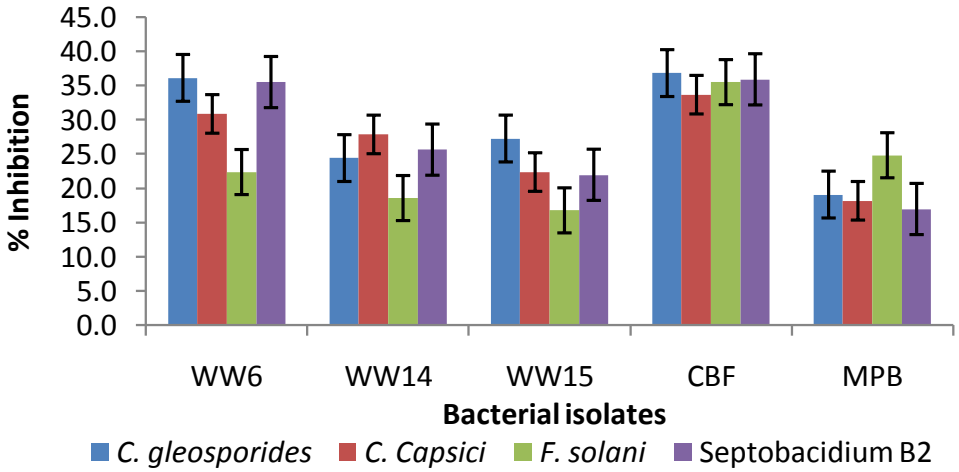

Figure 2. Effect of diffusible antiboiotic of baterial isolates on the radial growth of pathogen fungus *in vivo.*

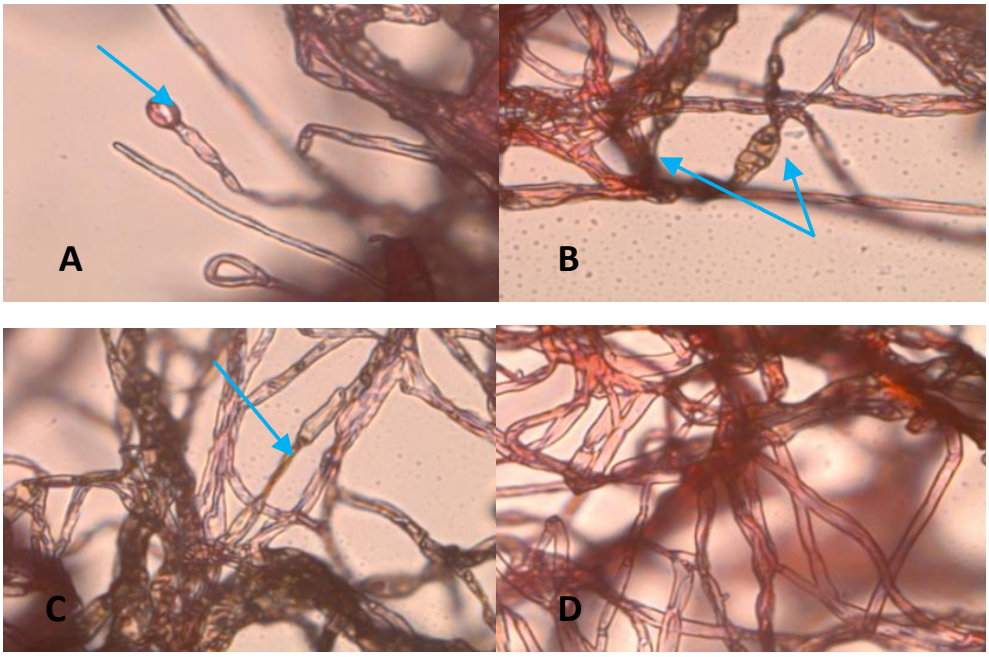

Figure 3. Hypha morphology of pathogenic fungus as effected by antagonists. Occurrence of bubbles and vacuole (A), thickened and swelled hyphae (B), slimming of hyphae (C) and (D), normal hyphae.

were found to produce cellulase enzyme by the formation of clearing zone in the culture medium. Regarding the protease activity, out the 5 tested strains, only strain MPB did not form any clearing zone in the skim milk agar medium. In term of chitinase activity, none of the bacteria isolates were able to grow in solid and liquid media that containing chitin as the sole carbon source. Thus, all tested strains were considered as negative for chitinase production.

Bacterial growth at different NaCl concentration

Figure 4 summarized the results of the growth of the different bacterial isolates under different concentrations of NaCl. The best growth of *Bacillus* strain WW14 and WW15 were observed at 150 mM NaCl, while the best growth of the remaining *Bacillus* strain WW6, CBF, and MPB were observed at the concentration of 200 mM of NaCl.

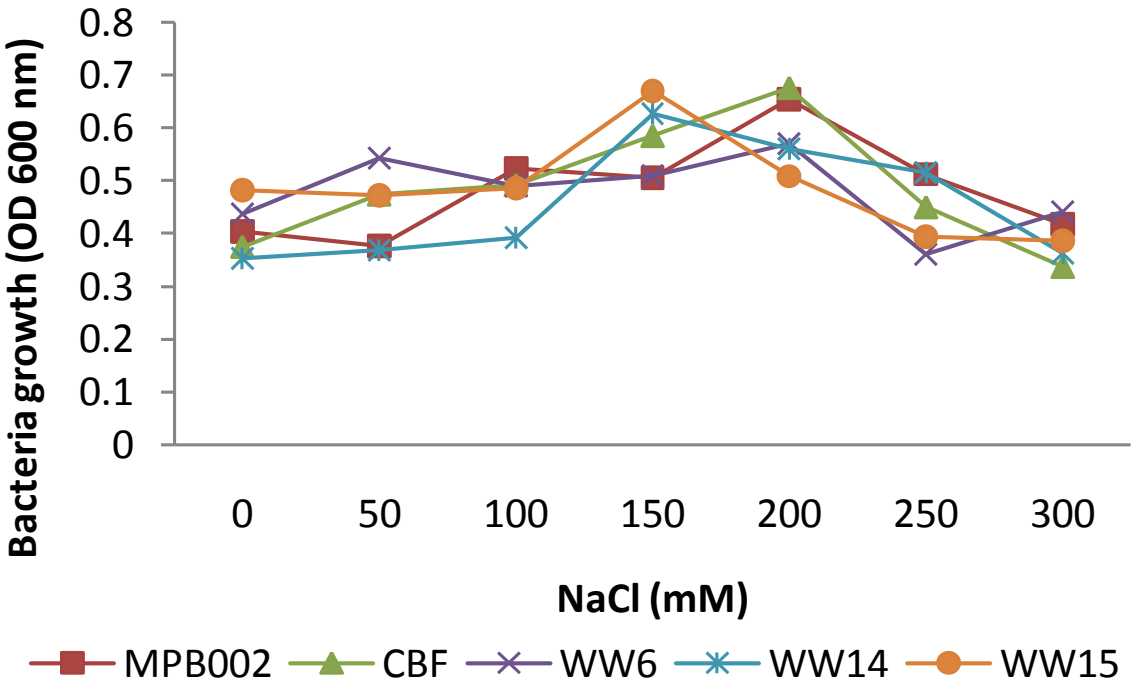

Figure 4. Growth of bioantagonistic bacteria at different NaCl concentrations.

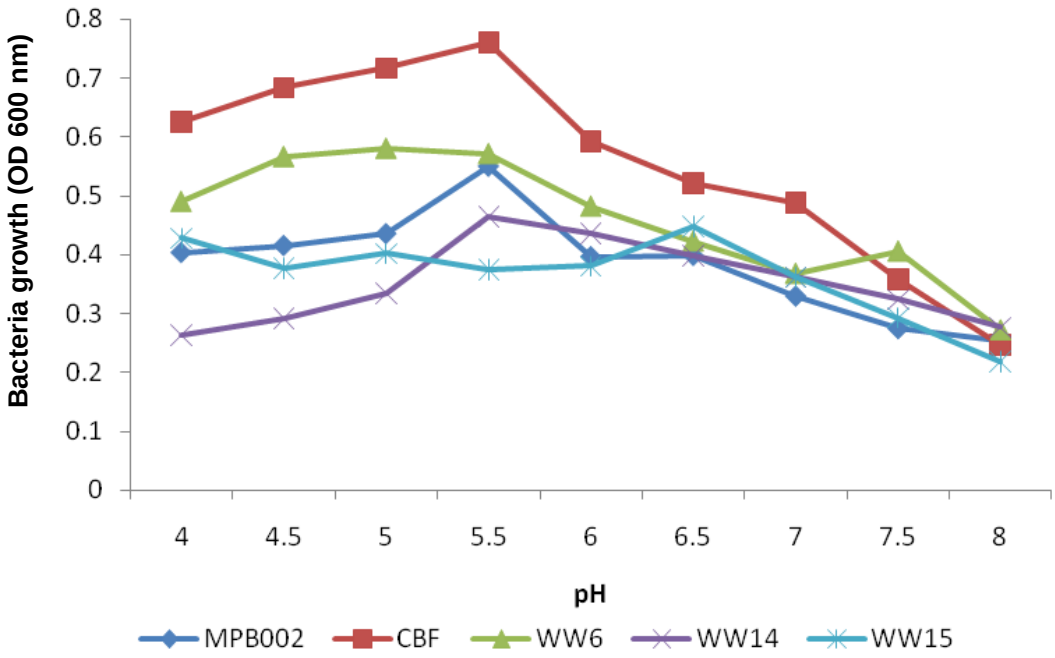

Figure 5. Growth of bioantagonistic bacteria at different pH values.

Bacterial growth at different pH

All the bacteria isolates grew best at pH between 5.0 and 5.5. *Bacillus* CBF showed its highest growth at pH 5.5 and all the five antagonistic bacteria did not grow at pH 8.0 (Figure 5).

Bacterial growth at different temperatures

Temperatureplay a key role on the bacteria growth and it was observed that the optimal growth temperature for all antagonistic isolates were at 30°C, except for bacterial isolates strain, MPB which having optimal growth

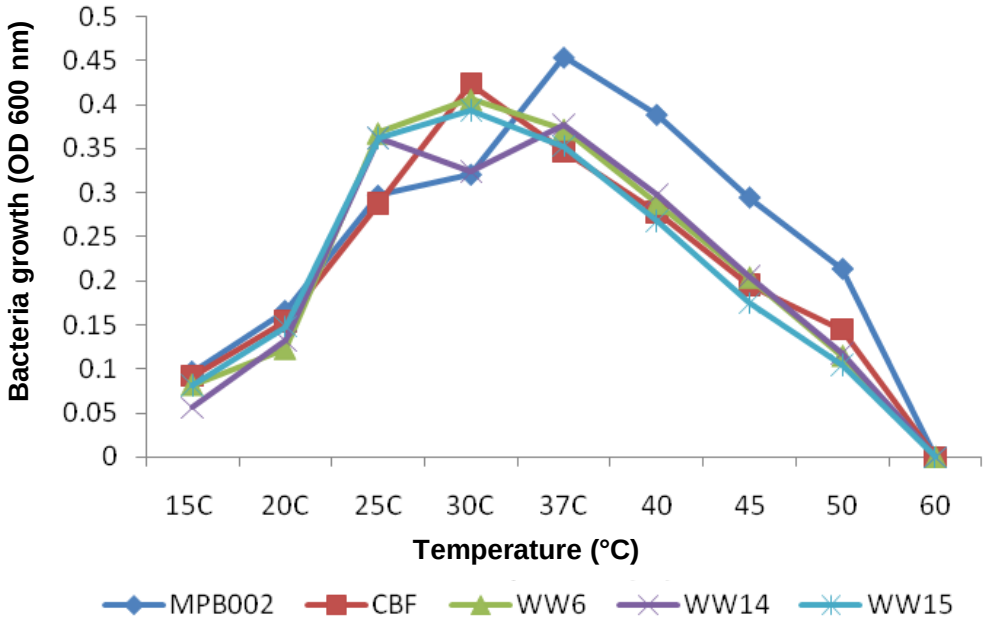

Figure 6. Growth of bioantagonistic bacteria at different temperature.

Table 2. Root colonization of bacterial isolates.

Bacterial Strain	Root colonization (log CFU/mg)
WW6	5.69[c]
WW14	4.62[b]
WW15	4.42[b]
CBF	6.61[d]
MPB	1.44[a]

temperature at 37°C. It was observed that as the temperature increased from 15 to 37°C, the microbial growth gradually increased and after reaching a maximum, it started to decline (Figure 6). At the temperature 60°C, no microbial growths were observed as this temperature was inhibitory for all bacterial isolates.

Colonization assay

The results obtained for root colonization is presented in Table 2. The quantification of bacteria adhering to the surface of root resulted in a concentration of CBF and WW6 higher than that of WW14, WW15 and MPB. The rank order of the bacteria that are able to colonize roots are CBF> WW6 > WW15 >WW14 > MPB. The analysis of this assay also showed that adherent of MPB strains to root surface were significantly lower than other bacterial isolates. This indicated that this strain has weak root colonization ability. These findings supported the agreement with previous reports that antagonistic bacteria with strong root colonization ability are having colonization values ranging from 4.0 to 6.0 log CFU/mg (Egamberdieva, 2010; León et al., 2009).

Mean values of five independent experiments. Means in a column with the different letters represents significant different at 0.05 level according to Tukey's multiple comparison test using the SPPSS software.

DISCUSSION

The screening strategy carried out in this paper consisted of the isolation of culturable bacteria strains that are able to inhibit the growth of pathogenic fungus that affect the pepper vine. In the present study, the bacterial isolates isolated from different locations were evaluated for their antagonism against pathogenic fungus that affecting pepper vine. The study resulted in the selection of five

antagonistic bacteria that are able to suppress the growth of *F. solani, C. capsici*, *C. gloeosporioides*, and S*eptobacidium* spp. based on 16S rDNA sequence analyses, all of this bioantagonistic bacterial isolates are belonging to *Bacillus* spp. These results are consistent with the early raised hypothesis that this group of microorganisms is responsible for this kind of phenomenon in the soil such as activity against *F. solani* (Jing et al., 2009); *C. gloeosporioides* (Havenga et al., 1999); *Meloidogyne incognit* (Ismail and Fadel, 1997), avocado post-harvest pathogens (Korsten and Jager, 1995) and peach brown rot (Pusey, 1988).

Dual culture assay showed that strains CBF was the most efficient bioantagonistic bacteria with 50.1% inhibition against *C. gloeosporioides*, 43.4% against *C. capsici*, 44% against *F. solani*, 46.3% against S*eptobasidium* spp and 32.7% against *P. capsici*. The strains WW6, WW14, WW15, SW15 and MPB do not show any inhibitory activity against *P. capsici* although all of these strain showed high inhibition percentage against other pathogens. This might be due to the presence of certain fungistatic metabolites secreted by bacteria CBF, *(B. subtilis)*. This finding is in agreement with Korsten and Jager (1995) who reported that *B. subtilis* was detected as antibiotic producer.

The result of the present study shown that the bacteria isolates did produce hydrolytic enzyme that inhibit the mycelia growth of the fungal. It was found out that the protease production seemed to be the mechanisms responsible for strains WW6, WW14, WW15, CBF antagonism except for strain MPB. This result is consistent with finding reported by Huang et al. (2004). The most compelling evidences to support microbial proteases as virulence factors derived from studying protease deficient mutants (Ahman et al., 2002; Tian et al., 2006). Besides protease production, the release of cellulase is also another mechanisms responsible antagonism for all the bacterial isolated. This enzyme are used by bacterial isolated to cellulosic the cell walls of pathogens (Brantlee et al., 2011)

It is well known that environmental parameters affect the growth of bacteria and their production of toxins (Weinberg, 1985; Arp, 1988). From the analysis, it was found out that the optimal level for bacterial isolates to enhance virulence to pathogenic fungus are salinity 150 to 200 mM. In fact, high concentrations of NaCl do not limit the growth of any bacterial isolates cause the electric conductivity of soils from where these bacterial were isolated between 0.9 to 10 mmhpcm^{-1} which correspond to NaCl concentration lower than 50 mM. Therefore, there is no possibility that NaCl could inhibit the growth of bacteria at the sampling site.

pH was considered as one of the master variable affecting the antagonism (Thayer et al., 1987). From the data collected, the optimum pH was ranged from pH 5 to 5.5. This finding agreed in part with those which described the optimum growth of *B. subtilis* was between 5.7 and 6.8 (Claus and Berkeley, 1986). In addition, this pH are coincident with the optimum pH for the growth of pepper vine which is between 5.0 to 5.5 (Fatimah et al., 2003).

Temperature is a highly significant factor that affects the bacterial growth because at certain temperatures, proteins and enzymes begin to break down and lose functionality. The data collected suggests that the optimal temperature for growth of these antagonistic bacteria were between 30 to 37°C. These values were found to be similar to those reported by other workers previously (Claus and Berkeley, 1986). These results were expected as most of the soil microorganisms are mesophilic (25 to 40°C) and optimum temperature for most mesophiles is 37°C.

The results of the colonization of the pepper vine rhizosphere confirm reports on the interactions of *Bacillus* strains, supporting their use as rhizosphere colonizers (Kang et al., 2006; Barea et al., 2005; Reva et al., 2004).

The screening procedure is demonstrated to be very effective. Although, we did not establish a direct relationship between the described mechanisms and the protection that these strains demonstrate towards damping-off, the main goal of this work was accomplished and it shows a promising beginning for the formulation of inoculants that include indigenous bacteria. From all these results it may be concluded that the *in vitro* results were complementary to *in vivo* effects described by Korsten (1993). It thus, seems reasonable to assume that more than one mode of action is involved in the inhibition of fungus growth by *Bacillus* spp and these mechanisms may act synergistically. This most probably include production of antibiotics, enzyme and metabolites. The practical significance of this type of studies acquires its real importance when considering the need to replace fumigants and other chemical control procedures for the treatment of soil and/or plant diseases. On the basis of aforementioned results, it is concluded that strains WW6 (*B. amyloliquefaciens),* WW14 and WW15 (*B. vallismortis)* and CBF (*B. subtilis)* can be used at field level to biocontrol the fungus disease in pepper vine.

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