View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by ePrints@Bangalore University

The Bioscan 6(2) : 239-244, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

SELECTION OF EFFECTIVE BIO - ANTAGONISTIC BACTERIA FOR BIOLOGICAL CONTROL OF TOMATO WILT CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

B. B. ASHA, S. CHANDRA NAYAKA[1], A. C. UDAYA SHANKAR[1], C. SRINIVAS* AND S. R. NIRANJANA
[1]Deaprtment of Microbiology and Biotechnology, Bangalore University, Bangalore - 560 056, Karnataka
Asian Seed Health Centre, Department of Studies in Biotechnology,
University of Mysore, Manasagangotri, Mysore - 560 056
E-mail: moonnayak@gmail.com

KEY WORDS
Pseudomonas fluorescens
Biological control
Tomato wilt

Received on
27.01.2011

Accepted on :
13.05.2011

***Corresponding author**

ABSTRACT

Bacteria from the rhizoplane soil and surrounding soil of healthy and *Fusarium oxysporum* diseased tomato plants of district regions of Karnataka were collected. The best bacterial strains, based on their ability to control development of *Fusarium oxysporum* isolate, were identified as BS1, BS5 and BS18. All bacterial isolates resulted effective for the in vitro control of growth of *Fusarium oxysporum*, where the control mechanisms used by the bacteria do not involve the secretion of fungal cell wall hydrolytic enzymes. On the other hand, all bacteria grew well in conditions similar to those that can be found at the field level (considering pH, salinity, $Fe^{3+}$and temperature) and showed a good capacity of tomato root colonization. These results suggest that *Pseudomonas fluorescens* isolates studied have an excellent potential to be used as biocontrol agents of *Fusarium oxysporum* in tomato greenhouses at the field level.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important horticultural crops belonging to the family Solanaceae. It is grown through out the world and is important commercially for fresh market and processing. Tomatoes are susceptible to a large number of diseases. Among the fungal diseases of tomato, Wilt of tomato caused by *Fusarium oxysporum* f.sp.*lycopersici* (sacc.) is one of the most destructive diseases all over the world (Beckman, 1987). This pathogen invades through wounds on roots. Infected plants become stunted, chlorotic and wilt (Jones *et al*., 1991). In India most of the common varieties are susceptible and fungicides are frequently used to control the disease (Chauhan, 1988; Kapoor, 1988). However, the fungicide applied at the time of sowing may not persist for the whole cropping season. In this context, biological control is an alternative strategy for disease management. Several microorganisms have been successfully used for the control of tomato wilt (Podile *et al.,* 1995; De Cal *et al.,* 1995; Padmodaya and Reddy, 1998). Among them PGPR (Plant Growth Promoting Rhizobacteria) are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Kleopper, 1994; Glick, 1995; Cleyet *et al*., 2001). In recent years, there has been much success in obtaining effective control of plant pathogens using beneficial biocontrol agents such as strains of *Pseudomonas* species have been used extensively for plant growth promotion and disease control because of many properties such as efficient colonization of underground plant organs, utilization of a large number of organic substrates commonly found in root exudates and production of a variety of secondary toxic to fungi and bacteria. Growth promotion results mainly from suppressing soil-borne pathogens and other deleterious micro-organism (Schippers *et al*., 1987). *Pseudomonads* and *Rhizobia* are also reported to direct plant growth promoting activities leading to yield promotion (Arora *et al*., 2001; Gupta *et al*., 2002; Deshwal *et al*., 2003).

The selection of bioantagonistic bacteria, to take into account the direct effect on pathogen development, must consider conditions where the bioantagonist should develop, *i.e.* salinity and pH of soils and different temperature, among others. The main objective of present study is the isolation of antagonistic bacteria that could control *Fusarium oxysporum*, both *in* vitro and *in* vivo, and their characterization in terms of antagonistic mechanisms used to.

MATERIALS AND METHODS

Isolation of *Fusarium oxyporum*

The seeds of tomato were placed on wet blotter disc following the standard blotter method, (ISTA, 2005 to analyze seed borne infection. The plates were incubated for 7 days at 22ºC under 12hr altering cycles of light and darkness. After incubation, fungi developed on each seed we examined under

different magnifications of stereomicroscope and identified. The identification of these fungi was based on the way they grow on seeds, "habit characters" and on the morphological characters of conidia observed under compound microscope. The pathogen *Fusarium oxysporum* was isolated and maintained on potato dextrose agar plates by regular sub culturing.

Isolation, selection, innoquity and identification of bacteria

The potential bioantagonistic bacteria were isolated from rhizoplane soil of healthy tomato plants. One gram soil was diluted to 10^{-3} to $10^{-7}$for selection and the diluted sample (0.1 mL) was placed in Kings B medium and incubated at 22ºC for two days.

The isolated bacteria were pre tested against the pathogen *Fusarium oxysporum*. An aliquot (20 μL) bacterial suspension ($5x10^9$cfumL^{-1}) 24hr old, obtained from different tomato fields were placed on different 0.5cm sterile paper disks. Disks were placed on a Petri dish containing PDA, surrounding a 10mm diameter disk containing mycelium of a four day culture of *Fusarium oxysporum*, placed in the centre of the plate. It was incubated for 48hr at 22ºC, and inhibition of fungal growth was checked. Those with bioantagonistic activity were selected and maintained by regular sub culturing.

***In vitro* antagonism test**

Dual cultures

Pure culture of *F.oxysporum* (10mm disk) was placed at the centre of a Petri dish with PDA medium.
A circular line made with a 6cm diameter Petri dish dipped in a suspension of bioantagonistic bacteria (5×10^9cfumL^{-1}) was placed surrounding the fungal inoculums (Fig. 1). Plates were incubated for 72hr at 22ºC and growth parameter of the pathogen (*F. oxysporum* growth) was measured and compared to control growth where the sterile distilled water was used instead of bacterial suspension. Each experiment was repeated for three times and results were expressed as means% inhibition $\pm$ S.D. of the growth of the corresponding *F.oxysporum* isolate in the presence of any of *P.fluorescens*. Percent inhibition was calculated using the following formula; %inhibition = 1-(Fungal growth/control growth) $\times$100 Student test was performed as $p\leq 0.05$.

Antagonistic mechanisms

Production of diffusible antibiotics

Plates with PDA media covered with a cellophane membrane, were inoculated in the centre with 100μL of a bioantagonistic bacterial suspension (5×10^9cfumL^{-1}). After incubation for 22hr at 22ºC, the membrane with the grown bacterial isolate was removed, and the plate was inoculated in the middle with a 10mm disk of a pure culture of *F.oxysporum*. Plates were further incubated at 22ºC for 48hr and then growth of the pathogen was measured. Control plates were kept with sterile distilled water and further inoculated with *F.oxysporum*. Each experiment was done in replicates and was repeated at thrice. Results were expressed as mentioned above.

Production of volatile antibiotics

Bioantagonistic bacterial suspension (100μL, 5×10^9 cfumL^{-1}) was placed at the centre of one half petridish containing king B medium, and a10-mm disk of four days old pure culture of *F.oxysporum* was placed at the centre of another petridish containing PDA. Both half plates were placed face to face preventing any 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 22ºC for 48hr and the growth of the pathogen was measured and compared to controls developed in the absence of the bioantagonist. Each experiment was run in triplicate and was repeated at least three times. Results were expressed as mentioned above.

Secretion of enzymes involved in bioantagonism

Bacteria were cultured either on plates with solid medium or in tubes with liquid medium after inoculation with 100μL of a bacterial suspension (5×10^9cfumL^{-1}). Media contained either chitin or yeast glucans as the sole carbon source. Development of bacteria was assessed through colony counting after incubation at 22ºC for 48hr of plates with solid media or through measurement of absorbance at 600nm after incubation of tubes with liquid medium at 22ºC for 48hr. Also concentrated supernatants from liquid cultures of each bacteria was analyzed for the presence of endochitinase, 1-3 glucanase and protease activity, as described by zaldivar *et al*., 2001 and Perez *et al*., 2002.

Effect of Fe^{+3} on antagonism level

This effect was tested according to Pumarino, (1995) using $FeCl_3\times6H_20$ at 0, 5, 10, 15 and 20 ppm added to the King B medium. Pure culture of *F.oxysporum* (10mm disk) was placed at the centre of a Petri dish with Kings B medium containing $FeCl_3$ in different concentrations as mentioned above. A circular line made with a 6cm diameter Petri dish dipped in a suspension of bioantagonistic bacteria (5×10^9cfumL^{-1}) was placed surrounding the *F.oxysporum*. Plates were incubated for 72hr at 22ºC and growth parameter of the pathogen (fungal growth) was measured and compared to control growth where the sterile distilled water was used instead of bacterial suspension. Each experiment was done in replicates and was repeated at thrice.

Effect of different salt concentrations on development of antagonistic bacteria

The growth of *P.fluorescens* isolates were tested under NaCl concentrations of 0, 50,100,150,200 and 250mM. An aliquot of 0.5mL of a bacterial suspension (1×10^8cfu mL^{-1}) from a 24 hr old culture were placed in a tube containing 4.5mL of peptone broth previously adjusted at the desired NaCl concentration. Tubes were incubated for 48hr at the temperatures mentioned above, and bacterial growth was estimated at 600nm, 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 three times.

Effect of different pH values on development of antagonistic bacteria

An aliquot of 0.5mL of a *P.fluorescens* suspension (5×10^9cfu mL^{-1}) from a 24 hr old culture were placed in a tube containing 4.5mL of peptone broth previously adjusted at the desired pH

values which ranged between 5.0 and 8.0 (with intervals each 1 pH units). Tubes were incubated for 48hr at the temperatures mentioned above, and bacterial growth was estimated at 600nm, 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 three times.

Effect of different temperatures on development of antagonistic bacteria

An aliquot of 0.5mL of a *P.fluorescens* suspension (5×10^9cfu mL^{-1}) from a 24 hr old culture were placed in a tube containing 4.5mL of peptone broth and kept under different temperatures of incubation of 5, 10, 15, 28 and 37ºC. Tubes were incubated for 48hr at the temperatures mentioned above, and bacterial growth was estimated at 600nm, 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 three times.

Bacterial ability for root colonization

Root colonization bio assay was carried out following standard procedure of Silva *et al.,* (2003). Surface sterilized tomato seeds were soaked in 25ml of *P. fluorescens* suspension of (1×10^8cfu mL^{-1}) for 24 hr and then transferred to sterile 0.6 percent water agar tubes. The seedlings were allowed to grow at room temperature. Periodic visual inspections were performed daily in order to detect bacterial growth around arising roots.

Data analysis

Data from laboratory and greenhouse studies were analyzed separately for each experiment and were subjected to arcsine transformation and ANOVA (SPSS Software version II). Treatment means were separated by Tukey's HSD test.

RESULTS

Bacterial antagonists

Twenty bacterial isolates were initially isolated and from all these isolates (Table1), three isolates which showed antagonistic properties against *Fusarium oxysporum* were selected for further study. These three isolates were identified as BS1, BS5 and BS18.

***In vitro* antagonism**

Direct antagonism through Dual cultures

The antagonism of three *P.fluorescens* isolates was tested on PDA plates. In dual cultures three *P.fluorescens* showed inhibition of *F.oxysporum* growth. Among them BS1 was found to be most effective isolate in suppressing the pathogen *F.oxysporum* growth (Table 2). The three *P.fluorescens* isolates used in the present study showed different degrees of mycelial growth inhibition suggesting genotypic variability as a consequence of the rhizospheric influence from which these isolates were recovered.

Antagonistic mechanisms

Diffusible antibiotics

Isolate BS1 was the antagonistic bacteria isolate that showed the best inhibitory effect on the growth of *F.oxyporum* isolates tested, although all bacteria showed inhibitory effect on *F. oxysporum* growth (Table 3).

Volatile antibiotics

Results similar to those obtained when testing the effect of diffusible antibiotics were obtained when the effect of volatiles was tested (Table 4). Isolate BS1 showed good inhibitory effect compared to other two.

Secretion of enzymes involved in bioantagonism

None of the three bacteria was able to grow in solid or in liquid media that contained chitin or yeast glucans as the sole carbon source. Moreover, endochitinase or 1-3 glucanase or protease activity was not detected in supernatants from liquid cultures suggesting that enzymes, if synthesized, are not secreted, or that these enzymes are not produced by the bacteria.

Effect of Fe^{3+}on antagonism

The three antagonistic bacteria showed similar behavior on *F.oxysporum* growth at the Fe^{3+}concentratons tested (Table5).

Bacterial growth at different NaCl concentrations

The growth of the different bacterial isolates tested as antagonists of *F.oxysporum* under different concentrations of NaCl (Fig. 1). The best growth of bacterial isolate BS1 was observed at 50,100,150 and 200mM NaCl. Less growth was observed at 250mM NaCl concentration inhibited. BS5 showed best growth at 50, 100, 150mM NaCl concentrations. Concentrations of 250mM NaCl inhibited the growth of BS1, BS5 and BS18 compared to controls run in the absence of

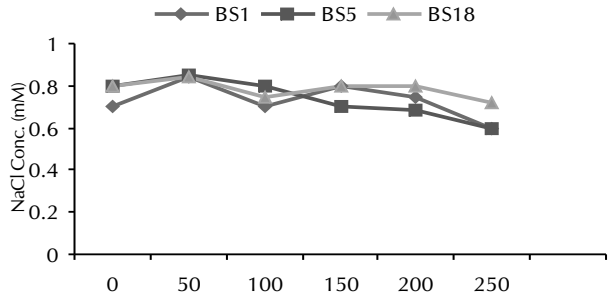

Figure 1: Bacterial growth at different NaCl concentrations

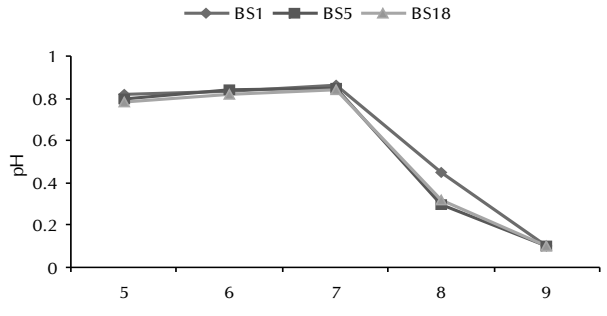

Figure 2: Bacterial growth at different pH

Table1: Pre evaluation of antagonistic properties of bacteria isolated from soil and rhizoplane of healthy tomato plants, collected at different tomato growing fields of Karnataka

Potential antagonistic bacterial isolates	*F.oxysporum* growth(mm)	%inhibition of *F.oxysporum* radial growth
control	3.5 ± 0.058^{a}	0.00 ± 1.65^{g}
BS1	2.2 ± 0.058^{g}	37.1 ± 1.65^{a}
BS2	3.1 ± 0.058^{bcd}	11.43 ± 1.64^{def}
BS3	3.5 ± 0.058^{a}	0.00 ± 1.65^{g}
BS4	2.8 ± 0.058^{def}	20.00 ± 1.65^{bcd}
BS5	2.7 ± 0.058^{ef}	22.86 ± 1.65^{cb}
BS6	3.2 ± 0.058^{abc}	8.58 ± 1.65^{efg}
BS7	2.8 ± 0.058^{def}	20.00 ± 1.65^{bcd}
BS8	3.5 ± 0.058^{a}	0.00 ± 1.65^{g}
BS9	3.0 ± 0.058^{bcde}	14.28 ± 1.65^{bde}
BS10	2.9 ± 0.058^{cdef}	17.15 ± 1.65^{cdef}
BS11	2.9 ± 0.058^{cdef}	17.15 ± 1.65^{cdef}
BS12	3.3 ± 0.058^{ab}	5.72 ± 1.65^{gf}
BS13	3.0 ± 0.058^{bcde}	14.28 ± 1.65^{cdef}
BS14	2.8 ± 0.058^{def}	20.00 ± 1.65^{bcd}
BS15	3.1 ± 0.058^{bcd}	11.43 ± 1.65^{def}
BS16	2.9 ± 0.058^{cdef}	17.15 ± 1.65^{bcde}
BS17	3.5 ± 0.058^{a}	0.00 ± 1.65^{g}
BS18	2.6 ± 0.058^{f}	25.72 ± 1.65^{b}
BS19	2.9 ± 0.058^{cdef}	17.15 ± 1.65^{bce}
BS20	3.2 ± 0.058^{abc}	8.58 ± 1.65^{gf}

Growth of *F.oxysporum* was evaluated after 72hr at 22ºC in PDA medium; The Experiment was performed three times and values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at p≤0.05

Table 2: Growth inhibition of *F.oxysporum* in dual culures against *P.fluorescens* isolates

Isolates	Fungal growth(mm)	%inhibition
BS1	2.2 ± 0.5^{b}	37.14 ± 1.6^{a}
BS5	2.5 ± 0.5^{a}	28.57 ± 1.6^{b}
BS18	2.3 ± 0.5^{ab}	34.28 ± 1.6^{ab}

Growth of *F.oxysporum* was evaluated after 72hr at 22ºC in PDA medium; The Experiment was performed three times and values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at p≤0.05

Table 4: Effect of volatile antibiotics secreted by *P.fluorescens* on radial growth of *F.oxysporum*

Isolates	Fungal growth (mm)	%inhibition
BS1	2.4 ± 0.05^{b}	31.42 ± 1.64^{a}
BS5	3.0 ± 0.05^{a}	15.24 ± 2.51^{b}
BS18	2.7 ± 0.05^{b}	22.6 ± 4.29^{ab}

Growth of *F.oxysporum* was evaluated after 72 hr at 22ºC in PDA medium; The Experiment was performed three times and values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at p≤0.05

NaCl. BS18 showed best growth at 50,100,150mM NaCl concentrations (Fig. 1).

Bacterial growth at different pH values

Isolates BS5 and BS18 grew best at pH values5, 6 and 7 and showed its highest growth at pH 6 and 7. BS1 showed its highest growth at pH 6 and the three antagonistic bacteria almost did not grow at pH 9.0 (Fig. 2).

Table 3: Effect of diffusible antibiotics secreted by *P.fluorescens* isolates on radial growth of *F.oxysporum*

Isolates	Fungal growth(mm)	%inhibition
BS1	2.8 ± 0.05^{a}	20.71 ± 2.3^{b}
BS5	3.1 ± 0.05^{b}	31.42 ± 1.6^{a}
BS18	3.0 ± 0.05^{b}	28.56 ± 1.6^{ab}

Growth of *F.oxysporum* was evaluated after 72 hr at 22ºC in PDA medium; The Experiment was performed three times and values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at p≤0.05

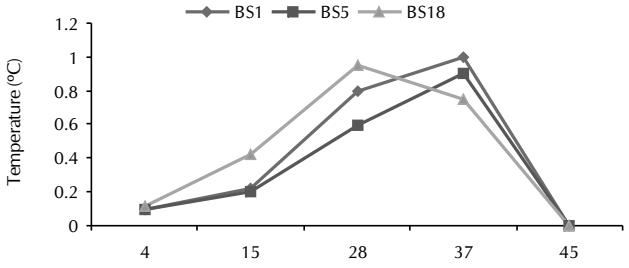

Figure 3: Bacterial growth at different temperature (ºC)

Bacterial growth at different temperatures

The poor growth of all the three bacteria was observed at

Table 5: Efeect of different concentrations of Fe^{3+} on the radial growth of *F.oxysporum* in the presence of *P.fluorescens*

Fe^{3+} as $FeCl_3$(μM)	BS1 Growth of *F.oxysporum* (mm)	BS5 %inhibition	BS18 Growth of *F.oxysporum* (mm)	%inhibition	Growth of *F.oxysporum* (mm)	%inhibition
00	1.9 ± 0.05^{bc}	45 ± 1.73^{ab}	2.0 ± 0.05^{b}	42.3 ± 1.45^{a}	2.1 ± 0.05^{b}	40 ± 1.4^{a}
05	2.1 ± 0.05^{ab}	40 ± 1.45^{bc}	2.0 ± 0.05^{b}	42.3 ± 1.45^{a}	1.9 ± 0.05^{b}.	45 ± 1.7^{a}
10	2.2 ± 0.05^{a}	37 ± 1.73^{c}	2.4 ± 0.05^{a}	30.6 ± 1.76^{b}	2.6 ± 0.05^{a}	26 ± 1.1^{b}
15	1.8 ± 0.05^{c}	48 ± 1.73^{a}	1.9 ± 0.05^{b}	45 ± 1.73^{a}	2.1 ± 0.05^{b}	40 ± 1.4^{a}
20	2.3 ± 0.05^{a}	34 ± 1.73^{c}	2.1 ± 0.05^{b}	39.6 ± 1.45^{a}	2.4 ± 0.05^{a}	30 ± 1.7^{b}

Growth of *F.oxysporum* was evaluated after 72 hr at 22ºC in PDA medium; The Experiment was performed three times and values are the mean with in the column sharing the same letters are not significantly different according to Tukey's HSD at p≤0.05

5ºC and 10ºC and little growth observed at 15ºC and isolate BS1shows better growth at 28ºC and no growth at 45ºC .while isolate BS5 and BS18 grew well at 37ºC (Fig. 3).

Bacterial capacity to colonize tomato roots

The analysis of results from this assay showed that all the three isolates of *Pseudomonas fluorescens* (*P. fluorescens* BS1, BS5 and BS18) showed positive for root colonization.

DISCUSSION

Pseudomonas possesses diverse mechanisms by which they can exert inhibitory activity towards phytopathogens and thereby mediate crop protection. They have a high growth rate relative to many other rhizosphere bacteria, are easy to grow in vitro, and can subsequently be reintroduced into the rhizosphere by seed bacterization. In dual cultures all bacterial isolates showed variations in inhibition of mycelial growth *F.oxysporum*. Several workers also reported similar effects where numerous fungi including *Fusarium* species (Sivamani and Gnanamanickam, 1988; Khan and Zaidi, 2002). *Fluorescent Pseudomonads* and other plant growth promoting antagonistic rhizobacteria represent a diverse but dominant bacterial group in soil, and their influence on plants varies from beneficial to deleterious although some behave neutral behavior. The three *P.fluorescens* isolates used in the present study showed different degrees of mycelial growth inhibition suggesting genotypic variability as a consequence of the rhizospheric influence from which these isolates were recovered.

One of the most effective mechanisms that antagonist employ to prevent proliferation of phytopathogens is the synthesis of antibiotics. Fluorescent *Pseudomonas* spp. has been reported to produce 2, 4-diacetylfluoroglucinol (2, 4-DAPG) by which they provide biological control of soil-borne pathogens on a wide range of crops and have a key role in the suppressiveness of some plant pathogens (Raaijmakers and Weller, 2001; Weller *et al*., 2002). The volatiles released by the *P.fluorescens* isolates had antagonistic influence on the growth of the pathogen *F.oxysporum* isolates. The growth of *F.oxysporum* was inhibited in dual plate inverted chamber suggesting the involvement of gaseous molecules.

None of the three bacteria was able to grow in solid or in liquid media that contained chitin or yeast glucans as the sole carbon source. Moreover, endochitinase or 1-3 glucanase or protease activity was not detected in supernatants from liquid cultures suggesting that enzymes, if synthesized, are not secreted, or that these enzymes are not produced by the bacteria. If we consider that none of three bacteria was able to secrete any of the enzymes involved in biocontrol, and that all had the ability to control *F.oxysporum* through secretion of diffusible and volatile metabolites, it may be concluded that they use these two latter mechanisms of biocontrol as opposite to some fungal biocontrol microorganisms that also use fungal cell wall hydrolyzing enzymes within their biocontrol mechanisms (Perez *et al.,* 2002).

Iron is a fundamental element for respiration of several aerobic and facultative anaerobic microorganisms, and therefore, its availability in soil is essential (Leong, 1986). On the other hand siderophores are low weight compounds with high affinity for Fe^{3+} (Neilands, 1981) which are produced under limiting concentrations of iron. These compounds are able to transport this element inside the cell for metabolic functions (Press *et al.,* 2001), and microorganisms which are able to produce siderophores show competitive advantages as compared to those that do not produce them. From this point of view, the competence for iron increases in conditions where this element is limiting but this condition is reverted when iron is added to the culture medium (Elad and Baker, 1985). In the present study the three antagonistic bacteria showed similar behavior on *F.oxysporum* growth at the different Fe^{3+}concentratons tested. If it is considered that iron available in soils fluctuate between 1.8 and 27ppm it may be concluded that this element is not limiting for the antagonistic activity of these bacteria on *F.oxysporum*.

The results obtained from the present study confirm that high concentrations of NaCl are not limiting the growth of any of the bacteria.

All the bacteria showed highest growth at pH 6 and 7and almost did not grow at pH 9.0. These results agree in part with those which describe the optimum growth of *Pseudomonas* is between 5.7 and 6.8 (Claus and Berkely, 1986). In addition, it is important to mention that pH values where the highest growth of these bacteria was obtained are coincident with the optimum pH for tomato development which is between pH 5.5 and 7.0(Nuez, 1995). On the other hand, soils from where bacteria were obtained showed a pH value between 6.2 and 7.2 when suspended in water suggesting that soil pH does not affect their growth.

The poor growth of all the three bacteria was observed at 5ºC and 10ºC and little growth observed at 15ºC, shows better growth at 28ºC and 37ºC, no growth at 45ºC. When considering that soil temperatures are closed to 30ºC at the production season (Gonzalez, 2001), bacterial growth will not be affected by this condition. This fact, along with results obtained for growth at different Fe^{3+}or NaCl concentrations and different pH values, suggest that development of three bacteria should not be affected on the field conditions.

For any disease suppressive mechanism to be effective it is important that the antagonist first be able to efficiently establish itself in the rhizospere of that particular plant (Kleopper *et al*., 1980). The use of bacteria to exert an appropriate biological control of *Fusarium oxysporum* and other soil borne fungi relies on their ability to colonize roots efficiently. The ability to colonize roots is highly variable between rhizobacteria, being this characteristic reflection for their ability to compete for ecological niches in the rhizosphere (Misaghi, 1990). The analysis of results from this assay showed that all the three isolates of *Pseudomonas fluorescens* (*P. fluorescens* BS1, BS5 and BS18) showed positive for root colonization.

From all these results it can be concluded that the biocontrol effect of antagonistic bacteria (*P. fluorescens* BS1, BS5 and BS18) isolated from soils against *Fusarium oxysporum* are adequate for their use at the field level, especially BS1 isolate. Within the mechanisms used by these bacteria are the secretions of volatile and diffusible metabolites but not of fungal cell wall hydrolytic enzymes. In addition, they show growth characteristics, which are compatible to the conditions of salinity, pH, Fe^{3+} and temperature that can be found in soils

where tomato plant is commonly cultivated. Therefore these bacteria could be used at the field level to biocontrol the *Fusarium oxysporum* f. sp. *lycopersici*, the causative agent of wilt of tomato.

REFERENCES

Arora, N. K., Kang, S. C. and Maheshwari, D. K.2001. Isolation of siderophore - producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of ground nut. *Curr. Sci.* 673 – 677.

Beckman, C. H. 1987. The nature of wilt diseases of plants. The American Phytopathological Society. St.Paul. p. 174.

Chauhan, M. S. 1988. Reaction of varieties/lines of tomato to wilt. *Indian J.Mycol.Pl.Path.***18:**72-73.

Claus, D. and Berkely, R. 1986. Genus Bacillus Cohn.**,** 1872. In: Bergey's manual of systematic bacteriology. *Williams and Williams, Baltimore, MD, USA.* **2:** 1105-1139.

Cleyet, M. J. C., Larchere, M., Bertrand, H., Rapior, S. and Pinochet, X. 2001. Plant growth enhancement by bacteria. In: Morotogaudry, J. F. (Ed.), Nitrogen Assimilation by plants: Physiological, Biochemical, and Molecular Aspects. Science Publishers Inc., Plymouth, UK, pp. 185-187.

De Cal, A., Pascual, S., Harena, I. and Malgarejo, P. 1995. Biological control of *Fusarium oxysporum* f.sp. *lycopersici. Pl. Path.* **44:** 909-917.

Deshwal, V. K., Dubey, R. C. and Maheshwari, D. K. 2003. Isolation of plant growth-promoting strains of *Bradyrhizobium* (*Arachis*) sp. with biocontrol potential against *Macrophomona phaseolina* causing charcoal rot of peanut. *Curr. Sci.* **83(3):** 443-448.

Elad, Y. and Baker, R. 1985. Influence of trace amounts of cations and siderophore-producing *pseudomonads* on chlamydospore germination of *Fusarium oxysporum*. *Phytopathology*. **75:** 1047-1052.

Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. *Canadian J.Microbiology*. **41:**109-117.

Gonzalez, R. 2001. Control biologico de *Fusarium solani* (Mart) Sacc. *en Lycopersicon esculentum* Mill mediante bacterias y *Trichoderma* spp. Tesis (Memoria de Titulo Ingenieria Agraria). Santiago, Chile, Universidad de Chile, Facultad de Ciencias Agrariasy Forestales. p. 70.

Gupta, C. P., Dubey, R. C. and Maheshwari, D. K. 2002. Plant growth enhancement, suppression of *Macrophomina phaseolina* causing charcoal rot of pea nut by fluorescent *Pseudomonas*. *Biol. Fert. Soils.* 35: 295 - 301.

International Seed Testing Association (ISTA). 2005. Proceedings of the International Seed Testing Association. International rules of seed testing. *Seed Science and Technology*. **15A:** 1-9.

Jones, J. B., Jones, J. P., Stall, R. E. and Zitter, T. A. 1991. Compendium of Tomato Diseases. St. Paul, MN, USA, APS Press.

Kapoor, I. J. 1988. Fungi involved in tomato wilt syndrome in Delhi, Maharashtra and Tamil Nadu. *Indian Phytopath.* **41:** 208-213.

Khan, M. S. and Zaidi, A. 2002. Plant growth promoting rhizobacteria from rhizosphere of wheat and chickpea. *Ann. Protec. Sci.* **10(2):** 265-271.

King, E. O., Ward, M. K. and Raney D. E. 1954. Two simple media for the demonstration of pyrocyanin and fluorescin. *J. Lab. Clin. Med.* **44:**301-307.

Kleopper, J. W., Leong J., Teintze, M. and Schroth, M. N. 1980. Enhanced plant growth by siderophores produced plant growth promoting rhizobacteria. *Nature.* **286:** 885-886.

Kleopper, J. W. 1994. Plant growth promoting rhizobacteria. In: Okon, Y. (Ed.), Azospirillum/Plant associations, CRC Press, Boca Raton, FL, pp. 137-166.

Leong, J. 1986. Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. *Annual Review of Phytopathology*. **24:**187-209.

Misaghi, J. 1990. Screening bacteria for root colonizing ability by a rapid method. *Soil Biology Biochemistry*. **22:**1085-1088.

Neilands, J. B. 1981. Microbial iron compounds. *Annual Review of Biochemistry*. **50:** 715-731.

Nuez, F. 1995. El cultivo del tomate. Ed.Mundi-Prensa. Madrid, Espana. 793 pp. ISBN 8471145499.

Padmodaya, B. and Reddy, H. R. 1998. Screening of antagonists against *Fusarium oxysporum* f.sp. *lycopercisi* causing seedling disease and wilt in tomato. *Indian J. Mycol. Pl. Pathol.* **28:**339-341.

Podile, A. R., Prasad, G. S. and Dube, H. C. 1995. *Bacillus subtilis* as antagonist to vascular wilt pathogens. *Current Sci.* **54:** 864-865.

Press, C. M., Loper, J. P. and Kleopper, J. W. 2001. Role of iron in Rhizobacteria- mediated induced systemic resistance of cucumber. Phytopathology. **91(6):** 593-598.

Perez, L. M., Besoain, X., Reyes, M., Pardo, G. and Montealegre, J. 2002. The expression of extra cellular fungal cell wall hydrolytic enzymes in different *Trichoderma harzianum* isolates correlate with their ability to control *Pyrenochaeta lycopersici*. *Biological Research*. **35:** 401-410.

Pumarino A. 1995. Evaluation *in vitro* del control biologico de la Fusariosis del frejol. Tesis (Memoria de Titulo Ingenieria Agraria). Santiago, Chile, Universidad de Chile, Facultad de Ciencias Agarias y Forestales. p. 70.

Raaijmakers, J. M. and Weller, D. M. 2001. Exploiting genotypic diversity of 2, 4-diacetylphloroglucinol-producing *Pseudomonas* spp. Characterization of superior root colonizing *P.fluorescens* strain Q8r1-96. *Appl. Environ. Microbiol.* **67:** 2545-2554

Schippers, B., Bakker, A. W. and Bakker, P. A. H. M. 1987. Interaction of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annual Reviews of Phytopathology*. **25:** 339-358.

Silva, H. S. A., Romeiro, R. D. S. and Mounteer, A. 2003. Development of root colonization bioassay for rapid screening of rhizobacteria for potential biocontrol agents. *J. Phytopathology*. **150:** 500-506.

Sivamani, E. and Gnanamanickam, S. S. 1988. Biological counts of *F.oxysporum*.f.sp.*Cubence* in banana by inoculation with *Pseudomonas fluorescens*. *Plant and soil.* **107:** 3-9.

Weller, D. M., Raajmakers, J. M., McSpadden Gardener, B. B. and Thomashow, L. S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* **40:** 309-348.

Zaldivar, M., Velasquez, J. C., Contreras, I. and Perez, L. M. 2001. *Trichoderma aureoviride* 7-121, a mutant with enhanced production of lytic enzymes: its potential use in waste cellulose degradation and/ or biocontrol. *Electronic J. Biotechnology [online].* **4(3):**