Evaluation of an Antagonistic *Bacillus* sp. Isolated from Newly Cultivated Soil as a Biocontrol Agent Against Some Plant Pathogenic Fungi

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Abstract: *The major aim of the present study was to investigate the mode of action of an antagonistic Bacillus sp. isolated from a newly cultivated soil in controlling some plant pathogenic fungi. The isolation sites were cultivated with different vegetable crops whereby different bacterial species were present. Screening of the isolated bacteria resulted in a selection of gram positive and endospore forming bacteria called TB281. The isolated bacterium has a capability to inhibit Sclerotinia sclerotiorum. The antagonizing ability of TB281 towards Fusarium oxysporum f. sp. lycopersici, Fusarium oxysporium f. sp. fabae , Botrytis cinerea, Drechslera sp., and Rhizoctonia solani may put it up as a potential biocontrol agent. The mechanisms by which this isolate exerts its inhibitory effect on certain pathogens involve the production of diffusible inhibitory compounds and HCN in the medium. Glucanase and cellulase, lytic enzymes which degrade fungal walls, also are involved in the biocontrol of the phytopathogens. However, this isolate failed to produce siderophore and chitinolytic activity as modes of its biocontrol action. Thus, the present results suggest that the modes of action by which TB281 can control phytopathogens in vitro are thr capabilities to produce diffusible inhibitory factors, cell wall lytic enzymes as well as hydrogen cyanide. The results also indicate that this isolate is able to protect tomato plants cultivated in soil invested with F. oxysporum f. sp. lycopersici when it was applied at specified time. It was concluded that TB281, may be a good candidate as a potential biocontrol agent against some plant pathogenic fungi.*

Keywords: *Antagonistic activities of lytic enzymes; Bacillus sp.; Biocontrol; Modes of action*

INTRODUCTION

Soil microorganisms are the most valuable sources of natural compounds, providing agricultural and industrial antimicrobials and biocatalysts. The microorganisms can be found as single cells or microcolonies in association with the organic materials. Their metabolism and interaction with other organisms are dependent on environmental soil conditions which often differ between microhabitats (Whipps, 2001). Several uncontrollable environmental factors such as soil composition and pH (Ownley *et al.*, 2003), minerals and carbon sources (Shaukat and Siddiqui, 2003), water availability (Hase *et al.*, 2001) and potential competitive interaction with native microflora (Brimecombe *et al.*, 2000) play a major role in the biocontrol agent efficacy.

Nowadays, great urgency of need to obtain a domestic biocontrol agent is increasing due to the appearance of pathogens resistant to pesticides (Spotts and Cervantes, 1986) that including deployment of biocontrol agents with multiple mode of action such as production of antibiotics and lytic enzymes. Although it seems unlikely that the biocontrol would replace entirely pesticides, the development of biocontrol agents may be useful in an integrated approach to disease management (Budge and Whipps, 2001).

Bacillus spp. are common inhabitants of agriculture soil and have been isolated continuously as a biocontrol agent (Danby *et al.*, 1994). *Bacillus* strains are able to suppress plant pathogens through a number of modes of action including hydrolytic enzymes (Helistö *et al.*, 2001), antibiotics (Danby *et al.*, 1994, Kim and Chung, 2004), and siderophores (Hoffmann *et al.*, 2002). There are several reports of antibiotic production by members of the genus *Bacillus* that is belonging to lantibiotic, subtilin (Corvey *et al.*, 2003). There are two types of subtilin, type A like ericin from *B. subtilis* A 1/3 (Stein *et al.*, 2002) and type B that exhibit globular structures such as cinnamycin (Kessler *et al.*, 1992). These antibiotics may inhibit other bacterial growth by binding to one or more of the cell proteins or other essential components of the cellular metabolic pathway. More specifically, type A subtilin cause pore formation into the plasma membrane of gram positive bacteria (Driessen *et al.*, 1995). Understanding the mode(s) of action of a new isolate is an important prerequisite for both improving its performance and using it effectively to management plant diseases (Castoria *et al.* 1997).

The objectives of this study were to: (1) select a promising new antagonist from the roots of field-grown plants, (2) isolate and screen a wide range of isolates for their biocontrol capabilities, and (3) understand the mode of action of the selected

microorganisms as a first step toward development of an effective biological control.

MATERIALS AND METHODS

Media used

The following media were used for isolation: *Bacillus* sporulation agar (BSA) contained 8.0 g nutrient broth (Difco), 1.0 g yeast extract (Difco), 50 mg $MnSO_4$, and 15 g agar/ L . King's medium B (KMB) contained 20.0 g proteose peptone #3 (Difco), 2.5 g $K_2HPO_4.3H_2O$, 6.0 g $MgSO_4.7H_2O$, 15.0 g agar, 15 ml glycerol, 1 liter of distilled water) (King *et al.*, 1954), Nutrient agar (NA) (23 g/L. Difco) and Potato Dextrose Agar (PDA) (24 g/ L. Difco).

Isolation of bacteria

Isolation was carried out using plant materials collected from Agricultural Experimental Station, Suez Canal University. Sandy soil plots planted with eggplant, tomato, pepper, and onion were used for isolation. Roots of healthy plants and diseased plants were selected to search about antagonistic bacteria. Each plant root was shaken to get rid of the rhizosphere soil and placed in a tube containing 10 ml sterilized water. Sample tubes were shaken vigorously for 4-5 min. Serial dilutions were made and a sab was streaked on a Petri dish. The plates were incubated at 28°C and observed for colony growth. Pure cultures were obtained through repeated subcultures of a single colony, then, single colonies were transferred to NA slants for further studies.

Isolation and identification of fungi

Fungi were isolated from the diseased plants. The plant samples were surface sterilized, washed in sterilized water and blotted to dry, then transferred onto PDA plates. Fungal identification was performed on the basis of mycological characteristics described by Domsch *et al.* (1980), Nelson *et al.* (1983), and Alexopoulos *et al.* (1996). Purified cultures were kept at 40° C until use.

Pathogenicity tests

Pathogenicity tests were conducted on plants grown in a sterilized soil-sand mixture (4:1) in 20-cm pots with different pathogens isolated according to a modified method described by Haglund (1989)

In vitro screening and inhibition assay

The used fungal isolates were *F. oxysporium* f. sp. *lycopersici* (tomato), *F. oxysporium* f. sp. *fabae* (*fabae* bean), B. cinerea (strawberry), Drechslera sp. (barley), *S. sclerotiorum* (bean), *P. infestans* (potato) and *R. solani* (tomato).

The bacterial isolates were streaked individually on NA or PDA on the middle of the plates and a disk of fungal growth was placed on each side. Fungal disks were placed at a distance of 2 cm from the streaked bacteria after 24 h. The bacterial isolates showing an inhibitory ability were further screened against all the fungal isolates used in the experiment. The chosen bacteria were examined for gram stain reaction, shape, endospore forming, mobility, growth at 7% NaCl and growth at 45° C, as described by Leary and Chun (1988).

In vitro biocontrol activity of TB281

Production of diffusible inhibitory factors. Ten ml of 72 h-old culture of bacterial isolates was centrifuged at 10,000 rpm for 30 min. The supernatant was sterilized by filtration through a 0.45 and 0.22 µm pore-size membrane filters. The filtrate was boiled in a water bath for 15 min and 2,0 ml of the boiled filtrate was added to each PDA plate. Agar disks taken from the edge of 4 days-old fungal culture were placed in the center of the plates and incubated at 25° C until the growth in the control plates was covered by mycelium. The diameter of the fungal mycelium in the treated and untreated plates was determined.

Production of siderophores. To examine if the growth inhibition of the plant pathogenic fungi are due to the competition for Fe, test for antagonistic activity were carried out on plates containing one of the following media: NA, PDA, or casein agar glucose (CAG) that composed of 10.0 g casein hydrolysate, 5.0 g yeast (Difco), 5.0 g glucose, 4.0 g K_2HPO_4 and agar 17.0 g/ L. The treated plates were supplemented with 100 µM $FeCl_3$. Fungal growth inhibition was estimated after incubation for 2 days at 30°C, based on the width of clear inhibition zones.

Production of HCN. The determination was done as described by Bakker and Schippers (1987) on plates of PDA and NA media containing 4.4 g/ L glycine and 100 µM $FeCl_3$. In this method, the production of HCN is detected by a color change from yellow to brown-orange in a filter paper impregnated with solution of 5% picric acid and 2.0% sodium carbonate and attached to the lid of the Petri dish. Plates without bacteria were used as negative controls.

Detection of enzymatic activities. Bacteria were grown on 50 ml liquid basal medium consists of 6 g Na_2HPO_4, 4 g KH_2PO_4, 2 g $(NH_4)2SO_4$, and 0.2 g $MgSO_4.7H_2O$/ L, and incubated for five days on a rotary shaker. Each flask was amended with either a substrate or dried fungal tissues as inducers for enzyme expression. Fungal mycelium and substrates were added into the medium of the rate of 0.2% and 0.05%, respectively, according to Skujins *et al.* (1965). At the end of incubation period, the cultures were centrifuged under cooling at 10 000 rpm for 30 min and the supernatants were used for detection of enzymatic activities.

Cellulase activity was detected by measuring the released free glucose from cellulose spectrophotometrically by a modified method of Worthington (1988) in a reaction mixture (5 ml) consisting of 5% cellulose (Sigmacell type 20, Sigma) solution in 50 mM sodium acetate buffer, pH 5.0, at 37°C and one ml crude enzyme extract. The reaction mixture was incubated at 37 C for 2 h with moderate shaking. After incubation, immediately transferred into an ice bath for 5 min and centrifuged for another 5 min to clarify the supernatant. Into a cuvette, 3 ml infinity glucose reagent (Sigma) was added and equilibrated to 25°C, then 0.2 ml of the supernatant was supplemented and immediately mixed by inversion and the maximal increase was recorded in absorbance at 340 nm for 5 min indicating cellulase activity.

Glucanase activity was detected by a modified method of Lima *et al.* (1997) using Azurine-crosslinked pachyman (AZCL-pachyman, Megazyme) as substrate. The reaction mixture contained 0.4 ml potassium acetate buffer 10 mM, pH 5.0 and 0.1 ml crude enzyme extract. After equilibration for 3 min at room temperature, 100 µl of AZCL-pachyman (100 mg/ 3ml 10mM potassium acetate buffer pH 5.) were added, and the mixture was incubated at room temperature with moderate shaking for 30 min. The reaction was stopped by adding 700 µl of Tris (20% w/v), incubated for 3 min at room temperature and then briefly centrifuged and the amount of blue soluble dyed fragments released from AZCL-pachyman was determined spectrophotometrically at 595 nm by a spectrophotometer indicating glucanase activity.

Chitinase activity was detected on plates as described by Bargabus *et al.* (2002). Fifteen ml of 1% agarose and 0.1% chitin (Sigma C-7170) in 10 mM sodium phosphate buffer pH 5.0 were added to each Petri dish. Bacteria were added on four previously determined dots. The plates were incubated for 24 h. at 37° C. After incubation, 50 ml of 0.01% fluorescent brightener 28 (Sigma F-3543) dissolved in 500 mM Tris-HCl pH.8.9 was added to the plates and incubated for 10 min. The plates were washed three times with distilled water, then distained over night. The plates were examined under UV light for clear zones on a fluorescent background. Assays of enzyme activities were performed immediately after obtaining crude enzyme preparations.

Effect of TB281 on controlling *Fusarium* wilt of tomato

Tomato seeds were planted in sandy soil infested with TB281 when applicable, and grown in seedling plug trays (50 plugs/ tray). TB281 was grown on nutrient broth for 5 days and 3 ml of approximately 10^7-10^9 bacterial cell/ ml were added per plug. Five tomato seeds were planted in each cell. When the seedlings had 2-4 leaves, they were transplanted into pots (15x15 cm) containing non-sterile field soil artificially infested with *F. oxysporum* f. sp. *lycopersici* inoculum. Pathogen inoculum was grown for 15 days on PDB on a rotary shaker and added to soil at rate of 10^4 colony-forming units/g soil. Four plants per pot and five pots per treatment were used. Disease incidence was monitored for 5 weeks and assayed as percentage of seedlings showing wilt symptoms. TB281 was examined at different times.

Statistical analysis

All experiments were repeated at least twice. Experiments were analyzed using standard analysis of variance. Significance was evaluated at $P = 0.05$ according to least significant difference (LSD). Since repeated tests yielded similar results, data from a single representative experiment are presented.

RESULTS

Isolation of bacteria and screening *in vitro* for antagonists

Of 563 bacterial isolates obtained from different areas planted with different crops, only 10% showed antagonistic activities in vitro by slowing or stopping fungal growth on NA, PDA, or KB media. Bacterial isolates obtained on KB, representing 5.7% of the antagonistic isolates, showed little antagonistic activities, whereas *Bacillus* isolates, representing 4.3% of the antagonistic isolates, showed more inhibitory effect on inhibiting the plant pathogenic fungi used in this study.

Data in Table 1 show that four *Bacillus* isolates, from tomato, peppers and eggplants roots, exhibited antagonistic activities against two members of the genus *Fusarium* , B. cinerea, S. sclerotiorum, Drechlera sp, and R. solani. When the four isolates were tested against one of the Oomycetes member, *P. infestans*, only one of them showed antagonistic activity as well as its capability to shut down *S. sclerotiorum*. According to its antagonistic effect, isolate TB281was chosen as a potential biocontrol agent and was used for further studies.

As shown in Table 2, TB281 was tested against some plant pathogenic fungi on PDA medium and area of fungal growth was measured. The percentage of inhibition was differed considerably between pathogens. *S. sclerotiorum* was the most affected and was not able to grow whereas this isolate was able to inhibit *F. oxysporium* f.sp. *lycopersici* by 30.3%.

Table 1. The antagonistic activity of four *Bacillus* isolates against different plant pathogens.

Bacillus Isolates	TB281	TB365	PB139	EB216
B. cinerea	+	-	+	+
S. sclerotiorum	+	+	-	+
Drechlera sp.	+	+	+	+
R. solani	+	-	+	+
P. infestans	+	-	-	-
F. oxysporum f. sp. lycopersici	+	+	+	-

Table2. Percentage of growth inhibition caused by TB281 against fungal pathogens.

Fungal pathogens	Inhibition %
S. sclerotiorum	100
B. cinerea	90
Drechlera sp.	89.4
F. oxysporum f.sp. *lycopersici*	30.3
F. oxysporum f.sp. *fabae*	42.9
R. solani	63.3
P. infestans	73.8

Strain Characterization

Data in Table 3 show that TB281 is a gram positive, rod shaped and endospore forming bacteria. Examination of the bacterial mobility was positive. TB281 was able to grow on Casein medium plus glucose at 45° C.

Table 3. TB281 strain characterization

Test	Strain character
Gram stain	+
Shape	rod
Mobility	+
Spore forming	endospore
Growth at 45°C	+
Growth at 7% NaCl	+
Anaerobic growth	-

***In vitro* biocontrol activity of TB281**

Production of Inhibitory Factors. TB281 produced diffusible compounds in the medium which inhibited fungal growth of *F. oxysporum* on PDA plates, as shown in Figure 1.

Production of siderophores and HCN. Data in Table 4 indicate that plates supplemented with 100 µM $FeCl_3$ retained its ability to inhibit fungal growth, indicating that TB281 is not a siderophore

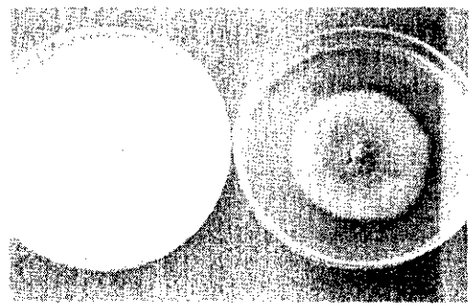

Fig.1. PDA plates supplemented with either boiled filtrate (right) or unboiled one (left).

producer under used experimental condition. TB281 turned picric acid indicator paper to brown-orange, indicating that it produces HCN. In addition, data in Table 4 also show that cellulase and glucanase activities were detected in solution assays. However, no chitinolytic activity was obderved for this isolate under the conditions of the present experiment (Table 4).

Table 4. Biocontrol activities of TB281

Tests	Biocontrol activity
Siderophores	-
HCN	+
Enzymatic activities	
Cellulase	+
Glucanase	+
Chitinase	-

(+) means production and (-) means lack of production

In vivo biocontrol activity of TB281

Application time of TB281 bacteria showed their different effects on protecting tomato plants. The best protection was obtained when TB281 was applied 7 days before seedling transplanting in the pots and also when it was applied at the cotyledons stage without significant differences (Table 5).

Table5: Development of *Fusarium* wilt of tomato as affected by TB281

Treatment	% wilted plants	% reduction
Soil infested with Pathogen only	73.3	0.0
Addition of bio-agent 7 days before sowing	66.7	9.0
Addition of bio-agent with seed during sowing	63.3	13.6
Addition of bio-agent at cotyledons stage	36.6*	50.0
Addition of bio-agent 7 days before transplanting	33.3*	54.5
Addition of bio-agent with transplanting	53.3	27.2

Values followed by an asterisk are significantly lower ($P < 0.05$) than the pathogen-only control treatment according to LSD test.

Discussion

The relatively reclaimed sandy soil of the Experimental Station of Faculty of Agriculture, Suez Canal University is not a prospective source of biocontrol agents. Shipton (1975) and Weller (1988) suggested that the time period of establishing a biocontrol agent in a given soil ranges from 2 to more than 10 years with continuously monoculture practice. The isolation area in the present study has been planted for about four seasons only. The lack of continuously farming in the isolating site, even with different crops, would not encourage establishing a big population of antagonistic microorganisms. Consequently, only10 % of the total isolates which are capable to inhibit plant pathogens was obtained.

The 10 percent of the total isolates (563) are differed in their nature. Some isolates produced fluorescence on KB medium whereas others were spore forming which suggests present of a wide rang of organisms at this stage of this newly cultivated soil. According to Weller (1988), biocontrol agents would be under selection in accordance to the kind of cultivated crops during the next few years. The monoculture would encourage some organisms different from the multiculture. Since monoculture is not a strategy adopted in Egypt, our research should study the effect of cultivating different crops on this new land.

Isolated strain, TB281, was chosen as a potential biocontrol agent according to its antagonistic effect in controlling some plant pathogenic fungi. This strain inhibited some phytopathogens belonging to different genera of fungi. In addition, it was able to completely prevent the growth of *S. sclerotiorum* which is a serious phytopathogen. TB281 capability to shut down *S. sclerotiorum* indicates the defection of the fungal genome to overcome the bacterial effect. Another significantly inhibited fungus was *B. cinerea* which was inhibited by 90.4% relative to the control. Although the highly achievement against the previously mentioned microrganisms, TB281 was not able to inhibit *R. solani* with more than 63%. Interestingly, TB281 showed different values of antagonistic ability against two species of the genus *Fusarium* . *F. oxysporum* f. sp. *lycopersici* was inhibited by 30.3% only whereas *F. oxysporum* f. sp. *fabae* was inhibited by 42.9%. Although the differences between the inhibition values against the two microorganisms were not significant, it may open a question of inconsistent performance of a biocontrol agent in the field application. In a study by Tehrani and Ramezani (2003) using 4 *Bacillus* isolates to control *F. oxysporum* on onion, they found that not all isolates were able to reduce the disease with consistent achievement. Studying the genetic bases and physiological responses of the two species of *F. oxysporum* to TB281may explain the lack of its consistency in the fields.

Preliminary identification trials showed that TB281 is a gram positive bacterium, spore forming and tolerating high temperature. Motility, growth at pH 5.7, and growth in 7% NaCl were all positive that may place it within the genus *Bacillus* . Although *B. subtilis* is present abundantly in the Egyptian soils, different species may present as a biocontrol agents in soil as reported by Danby *et al.* (1994) and Kotchoni *et al.* (2003). Identification of the species using 16S ribosomal DNA is needed.

A better understanding of TB281 mode of action against *F. oxysporium* will help comprehend how this antagonist acts against the plant pathogens in the soil. This new strategy can speed the development of a commercial biocontrol agent. One important mode of action in disease control is to act through utilization of the nutrient iron by producing siderophore. The TB281 strain retained its ability of inhibition in the presence of iron in the media, indicating that siderophore is not a mechanism of TB281 in disease control. *B. subtilis* was reported as one of the biocontrol agents that use siderophore as one of its mechanisms to inhibit other organisms. Hoffmann *et al.* (2002) and Berka *et al.* (2003) linked high-salinity with induction of iron limitation in *B. subtilis* and the bacterial genome. In addition, May *et al.* (2001) identified *dhb* operon that encodes the catecholic siderophore itoic acid (2, 3-dihydroxybenzoate (DHB)-glycine). HCN production is also considered as one mode of action for some biocontrol agents. In the present study, a weak orange pigmentation of the indicator paper was obtained which suggests that cyanide production may be considered as a factor in the inhibition of fungal growth by TB281 (Table 5).

Microorganisms producing a complex of lytic enzymes are considered a potential biocontrol candidates (Helistö *et al.* 2001; Kotchoni and Shonukan, 2002). It is well known that *Bacillus* strains are able to secrete a vast number of hydrolytic enzymes such as chitinases, laminarinases, and cellulases (Tang *et al.* 2004; Virupakshi *et al.* 2005,). *Bacillus* sp. X-b is a biocontrol agent that is able to produce a complex of hydrolases including chitinase, chitosanase, laminarinase, lipase and protease (Helistö *et al.*, 2001). *Bacillus* sp. X-b represents a potent member of bacilli family as a biocontrol agent. The main function of bacterial extracellular hydrolases is the release of nutrients from different substrates for the need of a bacterium. In addition, excreted enzymes along with other compounds like antibiotics may be used by bacteria for competition with other

microbial species. More specifically, it is often supposed that chitinase and glucanase are important for antagonistic activities which putatively act as depolymerases on fungal pathogen cell walls. Some of these enzymes are known to possess antifungal activity and to be involved in mycoparasitism (Castoria *et al.*, 1997). In this study, the isolated strain is able to produce cellulase and glucanase in vitro. Its potential to use this character under greenhouse or field conditions has to be tested.

A major consideration for successful biocontrol agent is the application of effective antagonist to the appropriate ecological niche at the proper time (Lewis *et al.*1995). For field application, seed treatment is one of the most suitable methods for biocontrol of soilborne pathogens (Harman 1992). As previously stated TB281 was isolated from a relatively new cultivated soil and expressed its potential over a number of other isolates. Examination of the inhibitory ability of TB281 against *F. oxysporium* f. sp. *lycopersici* in pots revealed that it may be used as a biocontrol agent in an integrated system. It is also interesting to observe that its performance in soil against *F. oxysporium* f. sp. *lycopersici* was better than in vitro. This result opens a new question about the bacterial ability to express a stronger activity in the complicated environment presented in soil.

In conclusion, the present results indicate that TB281 uses several modes of action in controlling plant pathogens which makes it a potential candidate for developing a potential biocontrol agent, and its present in the newly cultivated soil may offer a good opportunity to study its behavior in soil.

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REFERENCES

Alexopoulos C., Mims C., and Blackwell M. 1996. Introductory Mycology. John Wiley and Sons, INC. NY, USA.

Bakker A. and Schippers B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biology Biochem. 19:451-457.

Bargabus R., Zidack N., Sherwood J., and Jacobsen, B. 2002. Characterisation of systemic resistance in sugar beet elicited by a non-pathogenic phyllosphere-colonizing *Bacillus mycoides*, biological control agent. Physiology and Molecular Plant Pathol. 61: 289-298.

Berka R., Cui X., and Yanofsky C. 2003. Genome wide transcriptional changes associated with genetic alterations and nutritional supplementation affecting tryptophan metabolism in *Bacillus subtilis*. PNAS. 100: 5682-5687.

Brimecombe M. J., DeLeij F. A., and Lynch J. M. 2000. Effect of introduced *Pseudomonas fluorescens* strains of soil nematode and protozoan populations in the rhizosphere of wheat and pea. Microbial Ecol. 38: 387-397.

Budge S. P., and Whipps J. M. 2001. Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using Coniothyrium minitans and reduced fungicide application. Phytopathol. 91: 221-227.

Castoria R., De Curtis F., Lima G., and De Cicco V. 1997. ß-1,3-glucanase activity of two saprophytic yeasts and possible mode of action as biocontrol agents against post harvest diseases. Post harvest Biol. and Technol. 12: 293-300.

Corvey C., Stein T., Düsterhus S., Karas M., and Entian K. 2003. Activation of subtilin precursors by *Bacillus subtilis* extracellular serine proteases subtilin (AprE), WprA, and Vpr. Biochemical and Biophysical Resear. Communi. 304: 48-54.

Danby S., Hampson S.P., Joshi S., Sigee D. S. Epton H. A., and Leifert C. 1994. Activity of antibiotics produced by *Bacillus subtilis* and *Bacillus pumilus* against common fungal contaminants of plant tissue cultures. Physiology Growth and Development of Plants in Culture. Kluwer Academic Publishers, London: 404-408.

Domsch D. H., Gams W., and Andersson T. H. 1980. Copendium of Soil Fungi. Vol. 1. Academic Press, London.

Driessen A., Van den Hoover H., Kuiper W., Van de Kamp M., Sahl H., Konings R., and Konings W. 1995. Mechanistic studies of lantibiotic-induced permeabilization of phospholipids vesicles. Biochemistry 34: 1606-1614.

Haglund W. A. 1989. A rapid method for inoculating pea seedlings with *Fusarium oxysporum* f. sp. pisi. Plant DisseSE 73: 457-458.

Harman G. 1992. Development and benefits of rhizosphere competent fungi for biological control of plant pathogens. J. Plant Nutr. 15:835-843.

Hase C., Nievergelt J., Monne-Loccoz Y., and Défago G. 2001. Survival of biocontrol *Pseudomonas fluorescens* CHA0 in lysimeter effluent water depends on time of the year and type. J. Applied Microbiol. 90: 567-577.

Helistö P., Aktuganov G., Galimzianova N., Melentjev A., and Korpela T. 2001. Lytic enzyme complex of an antagonistic *Bacillus* sp. X-b: isolation and purification of components. J. Chromatography B. 758:197-205.

Hoffmann T., Schutz A., Brosius M., Volker A., Volker U., Bremer E. 2002. High-salinity-induced iron limitation in *Bacillus subtilis*. J. Bacteriology184:718-727.

Kessler H., Mierke D., Saulitis J., Seip S., Steuernagel S., Wein, T., and Will M. 1992. The structure of Ro 09-0198 in different environments. Biopolymers 32: 427-433.

Kim P. I. and Chung K. C. 2004. Production of an antifungal protein for control of *Colletotrichum lagenarium* by *Bacillus amyloliquefaciens* MET0908. FEMS Microbial Letter 1:177-183.

King E. O., Ward M. N., and Raney D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301-307

Kotchoni O. S., Shonukan O. O., and Gachomo W. E. 2003. *Bacillus pumilus* BpCR1 6, a promising candidate for cellulase production under conditions of catabolite repression. African J. Biotech. 2:140-146.

Kotchoni O.S., and Shonukan O.O. 2002. Regulatory mutations affecting the synthesis of cellulose in *Bacillus pumilus*. World J. Microbiol. Biotechnol. 18: 487-491.

Leary J.V. and Chun W.C. 1988. *Bacillus* . In: Laboratory Guide for Identification of Plant Pathogenic Bacteria. Pp.120-128, 2 nd Edition. APS Press.

Lewis J., Fravel D., Lumsden R. and Shasha B. 1995. Application of biocontrol fungi in granular formulations of pre-gelatinized starch-flour to control damping-off diseases caused by *Rhizoctonia solani*. Biological Cont. 5:397-404.

Lima L. H., Ulhoa C. J., Fernandes A. P., and Felix C. R. 1997. Purification of a chitinase from *Trichoderma* sp. and its action on Sclerotinia rolfsii and *Rhizoctonia solani* cell walls. J.General Appli. Microbiol. 43: 31-37.

May J. J., Wendrich T. M., and Marahiel M. A. 2001. The dhb operon of *Bacillus subtilis* encodes the biosynthetic template for the catecholic siderophore 2,3-dihydroxybenzoate-glycin-threonine trimeric ester bacillibactin. J. Biological Chem. 276: 7209-7217.

Nelson P. E., Toussoum T. A., and Marassas W. F. O. 1983. *Fusarium* species: An illustrated manual for identification. The Pennsylvania State University Press, University Park: 142-145.

Ownley B. H., Duffy B. K., and Weller D. M. 2003. Identification and manipulation of soil properties to improve the biological control performance of phenazine producing *Pseudomonas fluorescens*. Applied Environ. Microbiol. 69: 3333-3343.

Shaukat S. S., and Siddiqui I. A. 2003. The influence of mineral and carbon sources on biological control of charcoal rot fungus, *Macrophomina phaseolina* by fluorescent pseudomonads in tomato. Letter Applied Microbiol. 36: 392-398.

Shipton P. 1975. Take-all decline during cereal monoculture. In Biology and Control of Soil-borne Plant Pathogens, G. W. Bruehl, ed.), pp. 137-144. American Phytopathological Society, Press, St Paul.

Skujins J., Potgieter H., and Alexander M. 1965. Dissolution of fungal cell walls by a streptomycete chitinase and ß (1-3) glucanase. Archives Biochemistry and Biophysics 111: 358-364.

Spotts R. and Cervantes L. 1986. Populations, pathogenicity and benomyl resistance of Botrytis spp., *Penicillium* spp. and *Mucor piriformis* in packinghouses. Plant Disease 70: 106-108.

Stein T., Borchert S., Conrad B., Feesche J., Hofemeister B., Hofemeister J., and Entian K-D. 2002. Two different lantibiotic-like peptides originate from the ericin gene cluster of *Bacillus subtilis* A 1/3. J. Bacteriol. 184: 1703-1711.

Tang X., He G., Chen O., Zhang X., and Ali M. 2004. Medium optimization for the production of thermal stable ß-glucanase by *Bacillus subtilis* ZJF-lA5 using response surface methodology. Bioresource Technol. 93:175-181.

Tehrani S., and Ramezani M. 2003. Biological control of *Fusarium oxysporum*, the causal of onion wilt by antagonistic bacteria. Common Agricultural Appl. Biol. Sci. 68:543-547.

Virupakshi S., Gireesh K., Satish R., Gaikwad G. and Naik R. 2005. Production of a xylanolytic enzyme by a thermoalkaliphilic *Bacillus* sp. JB-99 in solid state fermentation. Process Biochemistry 40: 431-435.

Weller D. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathol. 26:379-407.

Whipps J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany 52: 487-511.

Worthington C. 1988. Worthington Enzyme Manual. pp. 76-79, Worthington Biochemical Corporation, Freehold, NJ.

تقييم أحد أنواع بكتيريا جنس باسيلس معزولة من أرض منزرعة حديثا كعامل مقاومة حيوية ضد بعض مسببات أمراض النبات الفطرية

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أجريت تلك الدراسة بهدف الحصول عل سلالة بكتيرية ذات نشاط مضاد لنمو مسببات أمراض النبات ودراسة طبيعة المقاومة الحيوية لها. تم إنتخاب سلالة بكتيرية سميت TB281 من بين عدة عزلات، تم الحصول عليها من أرض منزرعة حديثا بمزرعة كلية الزراعة- جامعة قناة السويس، لقدرتها العالية على تثبيط أو ايقاف مسببات أمراض النبات الفطرية المستخدمة فى هذا البحث. أوضحت الدراسة أن تلك العزلة أحد أنواع جنس *Bacillus* حيث أنها موجبة لصبغة جرام، عصوية الشكل، متحركة، وذات قدرة على تكوين جراثيم داخلية، غير هوائية الخ من صفات هذا الجنس. أظهرت تلك العزلة تأثيرا مضادا لنمو العديد من الفطريات الممرضة للنبات وكان من أكثر الفطريات تأثرا بتلك السلالة فطر *Sclerotinia sclerotiorum* . أوضحت الدراسة الحالية أيضا أن تلك السلالة ذات قدرة على تكوين مركبات ذات تأثير تثبيطى وتكوين سيانيد الهيدروجين فى بيئة نموها. بالإضافة الى ذلك فإن تلك السلالة ذات قدرة على تكوين بعض الإنزيمات المحللة لمكونات الجدر الخلوية، إنزيمات الجلوكانيز والسليوليز، تحت الظروف المعملية *in vitro* . أوضحت النتائج أيضا أن تلك السلالة ذات قدرة معنوية على حماية نباتات الطماطم من الإصابة بفطر *Fusarium oxysporun* f. sp. *lycopersici* إذا تمت المعاملة بتلك السلالة قى أوقات معينة من نمو بادرات الطماطم. بناء عل ما سبق، يستنتج من تلك الدراسة أن العزلة البكتيرية عامل مقاومة حيوية جيد ضد مسببات أمراض النبات حيث أنها تملك ميكانيكيات مختلفة لمقاومة غيرها من الكائنات الحية الدقيقة الممرضة للنبات تحت الظروف المعملية والحقلية.