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Induced Resistance Against Tomato Late Blight Disease Using Bioagents

A.Z. Aly*; H. Buchenauer; M.I. Abou-Zaid*; M.S. Shalaby*** and M.M.M. Atia***

* *Agric. Bot. Dept., Fac. Agric., Zagazig Univ., Zagazig, Egypt.*

** *Phytomedicine Instit., Hohenheim Univ., Stuttgart, Germany.*

*** *Efficient Productivity Instit., Zagazig Univ., Zagazig, Egypt.*

The antagonistic effect of different microorganisms (bacteria, fungi and actinomycetes) against *Phytophthora infestans* (Mont.) de Bary under laboratory and greenhouse conditions were investigated. The results obtained revealed that, *Pseudomonas fluorescens* CW1 was the most effective bacterial isolate in reducing *P. infestans* mycelial growth followed by CW2 isolate. On the other hand, *Bacillus* spp. (B2) had a good bioagent activity. Culture filtrate (CF) and bacterial suspension significantly inhibited the release of zoospores and cysts germination compared with the check. *Pseudomonas fluorescens* CW2 isolate had the highest effect, while *B. subtilis* (1) was the lowest one. *B. subtilis* TH isolate produced the highest amount of bio-surfactant followed by *B. subtilis* (209), while *Bacillus* spp. (B2) produced the lowest amount. Different isolates of *P. fluorescens* produced salicylic acid (SA) with different concentrations in their culture media. SA production was responsible for inducing resistance against different plant pathogens. Some of the bacterial isolates tested controlled the tomato late blight under greenhouse conditions as a direct effect on detached and/or intact leaves at 1, 2 and 7 days after application. *Trichoderma harzianum* isolates were the most effective bioagents in reducing the growth of the late blight pathogen followed by *T. viride* and *T. hamatum* isolates. The differences between all fungal isolates and the check treatment were significant. Under greenhouse conditions tomato late blight was controlled when tomato plants treated with the mixture of the pathogen and certain of *T. harzianum* isolates in the same time of inoculation (as a direct effect) and/or 1, and 7 days from application. Among the actinomycetes tested, No. 0 isolate was the most effective one in reducing the growth of the late blight pathogen followed by A1, while A3 was the least effective one.

Key words: *Bacillus* spp., bioagent, induced resistance, late blight, *Phytophthora infestans*, *Pseudomonas fluorescens*, salicylic acid and *Trichoderma* spp.

Late blight disease caused by *Phytophthora infestans* Mont. de Bary is one of the most destructive diseases of tomato which often killing foliage and fruits (Hartman and Haung, 1995). Several investigators used the phylloplane or rhizoplane flora as a bioagent against late blight pathogen and other several plant pathogenic fungi or bacteria (Jindal *et al.*, 1988).

Penicillium aurantiogriseum, Fusarium equiseti, Mucor hiemalis, Trichoderma hamatum, T. koningii, Epicoccum purpurascens and *Stachybotrys atra* were used to control *P. infestans* under laboratory and greenhouse or field conditions (Jindal *et al.* 1988).Moreover, many workers, *i.e.* Eliseeva *et al.* (1995), Filippov & Kuznetsova (1995), Sadlers (1996) and Gulati *et al.* (1998) used *Pseudomonas* spp. and *Bacillus subtilis* to control the same pathogen.

Recently some plant growth promoting rhizobacteria (PGPR) are able to induce systemic resistance (ISR) in plants against root and foliar diseases (Wie *et al.*, 1991; Hoffland *et al.*, 1996; Buchenauer, 1998; Chen *et al.*, 1999 and Enebak & Carey, 2000).

The mode of action of induced resistance by the bacteria include: chitinolytic activity by *Serratia marcescens* (Akatsumi *et al.*, 1993) or lipopolysaccharide (LPS) produced by *P. fluorescens* (Leeman *et al.*, 1995) as well as enhanced lignification and total peroxidase activity in cucumber by *Bacillus pumilus* (Jetiyanon *et al.*, 1997), but not chitinase activity. Induction of pathogeneses related protein (PR-1), chitinase and ß,1-3,gluconase in the intracellular fluid of leaves in addition to increase salicylic acid (SA) level in the plants by *P. fluorescens* were reported by Maurhofer *et al.* (1994) and Velazhahan *et al.* (1999).

Bacillus subtilis metabolites induced systemic resistance against biotrophic fungi by stimulating plants ability to compensate the damaging effects of the pathogens on plant metabolism leading to prolonged maintenance of assimilation rates (Bochow & Doley, 1998 and Podile & Laxmi, 1998). De Meyer and Höfte (1997) found that, SA produced by *P. aueruginosa* 7 NSKZ was necessary to induce resistance in bean plants against *Botrytis cinerea.*

Thus, this investigation was planned to isolate tomato phyllospheric microorganisms and investigate their antagonistic effect against *P. infestans* under laboratory and greenhouse conditions. Induced local and systemic resistance by the selected microorganisms against tomato late blight disease under greenhouse conditions was also undertaken.

Materials and Methods

1. Isolation of different microorganisms from tomato phyllosphere:

Tomato leaflets were collected from untreated and Previcur-N treated tomato plants (3ml/l as a recommended dose). One gram of leaflets from each treatment was transferred to Erlenmeyer-flask (250 ml) containing 99 ml of sterile distilled water. Flasks were thoroughly shaken on a mechanical shaker for 30 minutes at 125 rpm. The flask gave an approximately 10^{-2} dilution, and serial dilutions from 10^{-2} to 10^{-6} were prepared using sterile distilled water.

One ml from 10^{-6} dilution was mixed with 9 ml nutrient agar medium in Petri dish and incubated at 30 ± 2°C for 3 days to count the total bacterial flora. Three plates were prepared for each dilution. The total fungal count was detected using 10^{-5} dilution on Martin's medium (Leader *et al.*, 1960) and/or PDA medium. Actinomycetes were counted on Jensen's agar medium (Jensen, 1930). Identification

of fungal genera was carried out according to Barnett and Hunter (1981) and Wbster (1980) while the actinomycetes and bacteria were identified according to their shape, pigmentation and culture characteristics according to Buchanon *et al.* (1974).

Other bacterial isolates used in this work were obtained from laboratory of Prof. Dr. Buchenauer, Phytomedicine Inst., Hohenheim Univ., Stuttgart, Germany (German isolates).

2. Biological control:

2.1. Effect of microorganisms on mycelial growth of P. infestans under laboratory conditions:

The interaction between the previously isolated microorganisms (bacteria; fungi and actinomycetes) and the mycelial growth of *P. infestans* isolate D previously isolated by Aly *et al.*(2000) was studied under lab. conditions. Petri dishes(9 cm in diameter) containing rye agar medium amended with 3g yeast extract (Cohen, 1994) were inoculated in the center with disk (9 mm in diameter) taken from the edges of 7 days old agar cultures of *P. infestans* isolate D.

Three days after incubation at 18 ± 2°C plates were inoculated with the previously isolated bacteria and actinomycetes by streaking on the surface of the media beside the fungal growth with the aid of dual culture method. In case of fungi, plates were inoculated with agar disks (5 mm∅) of the isolated fungi at the distance of 1.5 cm from the edge of the plates. Plates inoculated with *P. infestans* alone were used as a check. The plates were incubated at 18 ± 2°C. Three plates were used for each treatment. When the plates of check were filled with the mycelial growth of *P. infestans* (about 7-10 days after incubation) the mean diameter of the mycelial radial growth in different treatments was measured. The percentage of growth was calculated from the following formula: A/B X 100.

A = The mean diameter of the growth in the treatment.

B = The mean diameter of the growth in the check.

2.2. Effect of bacterial culture filtrates on P. infestans zoospores release:

This experiment was designed to study the effect of different bacterial, *i.e.* 6 isolates of *P. fluorescens* and 7 isolates of *B. subtilis*, culture filtrates on the mycelial growth of *P. infestans* on rye agar medium. Bacterial isolates were grown on King's B (King *et al.*,1954) liquid medium for 7 days and incubated at 28 ± 2°C. The resulted filtrates were obtained through Roth separation filter (25 mm).

Sporangial suspension of *P. infestans* ($8x10^4$) was mixed with an equal volume of each bacterial culture filtrate. In check treatment the same volume of sterilized distilled water was added to the sporangial suspension. Suspensions were incubated at 4°C for 16 hr. Number of empty and normal sporangia were microscopically determined using a haemocytometer slide.

2.3. Effect of bacterial culture filtrates on cysts germination:

Zoospores were added to equal volumes of the bacterial culture filtrates or sterilized distilled water for the check, and incubated at 18±2°C for 16 hrs. Number of germinated and non-germinated cysts were microscopically determined using a haemocytometer slide.

2.4. Effect of bacterial suspension on P. infestans zoospores release and cysts germination:

2.4.1. Preparation of bacterial suspension:

The aforementioned bacterial isolates were grown on King's B liquid medium and incubated at 28 ± 2°C for 24 hours in shaker incubator at 120 rpm. Growth obtained was blended for 1 min and the resulted suspensions containing bacterial cells and their filtrates in the liquid media were adjusted to be 10^8 CFU/ml. Un-inoculated liquid King's B medium was used as a check treatment.

2.4.2. Effect of bacterial suspension on release of zoospores:

The sporangial suspension of *P. infestans* ($2x10^4$) was separately mixed with an equal volume of each bacterial suspension, and or to the same volume of King's B liquid medium. The mixture was then incubated at 4 °C for 16 hr. The number of empty and normal sporangia were microscopically determined using a haemocytometer slide.

2.4.3. Effect of bacterial suspension on cysts germination:

Sporangial suspension of *P. infestans* was incubated at 4 °C to encourage the release of zoospores. Zoospores were mixed with an equal volume of bacterial suspensions and with King's B liquid medium to serve as a check. The zoospore suspensions were then incubated at 18 ± 2°C for 16 hr. The number of germinated and non-germinated cysts were microscopically determined using a haemocytometer slide.

2.5. Salicylic acid (SA) production in bacterial culture medium:

King's B liquid medium was inoculated by five *P. fluorescens* isolates and one *B. subtilis* isolate. After 24 hr and 48 hr of incubation, SA content in the culture filtrate was determined according to the methods descried by De Meyer and Höfte (1997), with some modifications. Culture filtrate was centrifuged at 14000 rpm for 10 min at 4°C. One ml supernatant was mixed with 0.5 ml of 100% methanol and 50µl trichloroacetic acid (TCA) 5%. The volume was then adjusted to be 5 ml with de-ionized water and re-centrifuged at 14000 rpm for 10 min at 4°C. SA-contents were analysed by HPLC and calculated using the Kontron Data System 450-MT2/DAD.

2.6. Detection of the biosurfactant produced by the bacteria:

The aim of this study was, to test the ability of four *B. subtilis* isolates for production of biosurfactants (rhamnolipids) in the culture medium.

A specific mineral salt medium was used for this study as described by Siegmund and Wagner (1991). Different bacterial isolates tested were inoculated at the surface of the medium, then incubated at 28± 2°C and checked daily. The clear zone around the bacterial growth was measured (mm) and photographed.

2.7. The effect of bacterial isolates tested on the tomato late blight disease severity under greenhouse conditions:

The most effective bacterial isolates on mycelial growth, release of zoospores and cysts germination of *P. infestans* were selected to investigate their effect, under controlled greenhouse conditions, against tomato late blight disease. The bacterial

isolates were grown on 100 ml of King's B liquid medium in 500 ml Erlenmeyer flasks on a rotary shaker (100 rpm) for 24 hr at 28±2°C. The bacterial suspension was adjusted to contain 10^8 CFU/ml and used to study its effect on disease incidence. Tomato plants (5-6 leaf stage) were sprayed with 30 ml/plant of each bacterial suspension. The sprayed plants were covered with plastic box under controlled greenhouse conditions (18 ± 2°C and 100 % RH).

Detached and/or intact leaves sprayed with the bacterial cell suspensions, were inoculated after 1 and 7 days from spraying with 6 droplets (10µl)/leaflet of zoospores suspension as mentioned by Cohen (1994). All plants (intact leaves) were sprayed with 30 ml/plant of $8x10^4$ zoospores/ml of *P. infestans*. Also the detached leaves were inoculated with 6 droplets (10µl) of the mixture of the bacterial cell and fungal zoospores suspension (1:1 v/v) to test the direct effect on the disease incidence.

Inoculated leaflets and/or plants were incubated at 18 ± 2°C and 100% RH under plastic boxes in the greenhouse with a light and dark 16 and 8 hrs daily.

The disease incidence was evaluated after 7-10 days. Number and diameter of necrotic lesions (mm) as well as the blighted area/leaflet was determined. The percentage of protection was calculated as follows: Protection (%)= 100 x A/B While, A= disease (%) in treated plants and B= disease (%) in untreated plants. (100 x blighted area in treated/blighted area in the untreated [check])

2.8. Induced resistance against tomato late bight using different bacterial isolates:

The local (L) and systemic (S) effect of different bacterial suspensions were studied as leaf treatment on tomato plants. The number and diameter of necrotic lesions, as well as the blighted area / leaflet were evaluated. The percentage of tomato late blight disease protection on detached leaves was also studied.

Different bacterial suspensions, except of *B. subtilis* No. 209 and fusaric acid sensitive, were tested against tomato late blight on detached leaves and/ or intact leaves under greenhouse conditions. Bacterial suspensions and /or the same volume of distilled water were sprayed to the upper and lower surface of leaves No. 2 and 3.

The sprayed tomato leaves (leaf No. 2 and 3) and/or the upper unsprayed leaf (leaf No. 4) were detached immediately after spraying with bacterial suspension and before inoculation with the pathogen (after 7days). Inoculation, incubation and disease incidence were done as mentioned before.

3. The effect of Trichoderma spp. isolates on the tomato late blight disease under greenhouse conditions:

The aim of this study was to investigate the ability of the previously isolated two *Trichoderma* spp. isolates (T2 and T6) to control tomato late blight when applied at different times before inoculation (0, 1 and 7 days).

The fungal isolates were grown on 200 ml of biomalt liquid medium (Hirte *et al.*, 1989) in 500 ml Erlenmeyer flasks on a rotary shaker (100 rpm) for 7 days at 28±2°C.

The liquid culture obtained was mixed in a blender and adjusted to be 10^6 CFU/ml. Tomato plants (5-6 leaf stage) were sprayed with 30 ml/plant of each fungal suspension. The sprayed plants were covered with a plastic box and kept in the greenhouse at 18 ± 2°C and 100 % RH.

After 1 and 7 days from fungal spraying, detached leaves were inoculated with 6 drops (10µl)/leaflet of the zoospores suspension as mentioned before.

Also healthy detached leaves were inoculated with 6 droplets (10µl) of the mixture containing the same volume of the test fungal culture and *P. infestans* zoospores suspension to evaluate its direct effect on the disease severity. Inoculated leaflets were incubated and the disease incidence was assessed after 7-10 days as mentioned before.

Results and Discussion

Antagonistic reaction between different bacterial isolates and *P. infestans* was studied under lab. and greenhouse conditions.

Pseudomonas fluorescens, isolate CW1, was the most effective bacterial isolate in reducing growth of the late blight pathogen followed by *P. fluorescens,* isolate CW2, while *P. fluorescens,* isolate WB24 was the least effective one. Moderate effect was obtained with *P. fluorescence* isolate WB15, compared with the check treatment. On the other hand, *Bacillus* spp. (B2) had a good bioagent activity (Table 1).

Culture filtrates of the bacterial isolates tested varied in their bioagent activity against the release of zoospores from *P. infestans* sporangia and cysts germination. Data in Table (2) indicate that culture filtrate (CF) significantly inhibited the release of zoospores and cysts germination compared with the check. It was also clear that, cyst germination was sensitive to all the bacterial isolates tested than zoospores release with significant difference.

Suspension of different bacterial isolates varied in their bioagent activity against zoospores release of *P. infestans* sporangia (Table 3). Data indicate that, bacterial suspension significantly inhibited the release of zoospores. Many of bacterial suspension isolates (B2; CW2; *B.s.*45; WB34; WB52; CW1; KMPCH; *B.s.*209 and WB15) completely inhibited the release of zoospores from *P. infestans* sporangia, while *B. subtilis* TH was the lowest one. Bacterial suspension was effective than CF on zoospores release but the opposite was true in case of cysts germination .

Many bacterial genera had a good bioagent activity against wide range of Oomycetes fungi and others (Eliseeva *et al.,* 1995; Filippov & Kuzentsova, 1995; Sadlers, 1996; Wie *et al.,* 1996;Bochow and Doley, 1998; Buchenauer, 1998 and Gulati *et al.,* 1998).

The mode of action of the bacteria in their suspension and /or their culture filtrates against mycelial growth, zoospores release and cysts germination might be due to induce anti-fungal compounds in its culture (Niderman *et al.,* 1995)

Table 1. Effect of different bacterial isolates on the percentage of *Phytophthora infestans* mycelial linear growth reduction

Bacterial isolate		Mycelial growth (%)
Pseudomonas fluorescens	CW 1*	30.38
	CW2*	47.8
	WB 34*	64
	WB15*	58.14
	WB 24*	75.22
	WB52*	55.89
	KMPCH**	65
	B 17	61.33
Bacillus subtilis	Isolate No 1	63.45
	Isolate No 45	58.44
	TH isolate	44.44
	No 208*	63.45
	Fusaric acid sensitive	72.55
	No 209*	62.22
	B2	53.33
	B3	93.33
	B4	80
	B5	78.33
	B6	66.7
Bacillus spp.	B7	73.33
	B8	93.33
	B9	78.33
	B10	80
	B11	69.33
	B12	100
	B13	52.22
	B18	68.11
	B30	64.04
	B54	78.22
	B90	75.96
	B97	68.89
	B309	65.74
	B311	73.74
Serratia sp.	B316	68.85
Check		100

LSD at 0.05 = 7.899

* German isolates.

** KMPCH (*P. aeruginosa*).

and/or laytic enzymes production (celluolytic, gliconolytic, chitinolytic, ß-1,3-gluconase) as mentioned by Ng & Webster (1997) or production of bio-surfactant (Stanghellini and Miller, 1997).

Some of the bacterial isolates tested produced high SA concentration in their culture medium which, was found to be affected by incubation period. Bacterial isolate WB52 produced the highest SA content followed by WB34 isolate, while *B. subtilis* 1 isolate was the lowest one (Table 4). Other isolates tested hadn't the ability to produce SA in the medium tested. *P. aeruginosa* 7 NSKZ produced SA and induced resistance in bean plants against *Botrytis cinerea* (De Mayer and Höfte, 1997).

Table 2. Effect of different bacterial culture filtrates on zoospores discharge and cysts germination of *Phytophthora infestans*

bacterial culture filtrate tested	Empty sporangia (%)	Germinated cysts (%)
Check	100	100
CW1	0	20
CW2	0	0
WB 15	0	0
WB 34	0	0
WB 52	0	0
KMPCH	0	0
B. subtilis fusaric acid sensitive	100	0
B. subtilis TH	19.52	0
B. subtilis 1	0	0
B. .subtilis 45	0	0
B. subtilis 208	0	0
B. subtilis 209	29.73	0
B2	0	0
Mean	2.91	1.9
LSD at 0.05	0.353	0.158

Table 3. Effect of different bacterial suspensions on zoospores discharge and cysts germination of *Phytophthora infestans*

Bacterial suspension tested	Empty sporangia (% of the check)	Germinated cysts (%)
Check	100	100
CW1	0	0
CW2	0	0
WB15	0	0
WB 34	0	0
WB 52	0	0
KMPCH	0	0
B. subtilis fusaric acid sensitive	8.93	32.95
B. subtilis TH	19.52	43.6
B. subtilis 1	18	100
B. subtilis 45	0	14.33
B. subtilis 208	15.24	0
B. subtilis 209	0	100
B2	0	53.32
Mean	2.4	31.73
LSD at 0.05	0.453	12.55

Table 4. Salicylic acid (SA) content produced by different bacterial isolates in king's B medium

Bacterial isolate	SA content (µg/l) after	
	24 h	48 h
Check	0.00	0.00
CW1	119.30	4313.50
WB15	1277.38	8919.00
WB34	2934.50	9110.50
WB52	7446.25	13101.00
KMPCH	105.08	36.45
*B.subtilis*1	74.50	0.00

Bacillus. subtilis isolate TH produced the highest amount of bio-surfactant followed by *B. subtilis* 209, while, B2 isolate produced the lowest amount (Table 5 and Fig. 1). Other bacterial isolates tested had no ability to produce bio-surfactant under this experiment. Similar results were detected by Stanghellini and Miller (1997).

Table 5. Ability of some bacterial isolates to produce bio-surfactant grown for 7 days in specific medium

Bacterial isolate	Diam. (mm) of clear zone around the colony
B. subtilis TH	13
B. subtilis 209	12
B. subtilis fusaric acid sensitive	10
B2	5

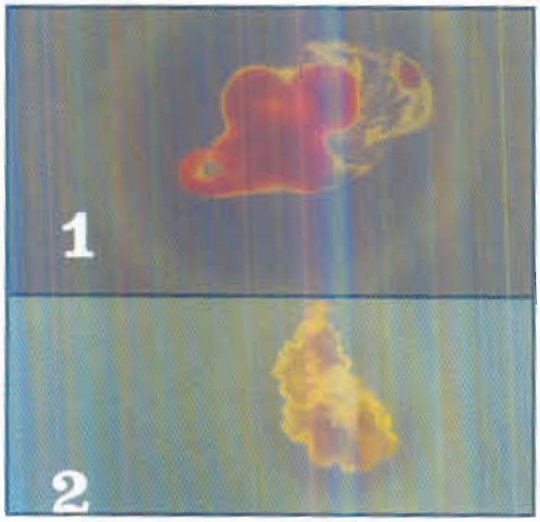

Fig. 1. Production of bio-surfactant in specific bacterial culture medium
1- (*B. subtilis* fusaric acid sensitive)
2- *Bacillus* sp. (isolate B2).

Some of the bacterial isolates tested controlled the tomato late blight under greenhouse conditions as a direct effect or 1, 2 and 7 days after application (Tables 6, 7, 8 and 9). Data in Table(6) show that, isolates CW1 and CW2 completely controlled tomato late blight disease under greenhouse condition followed by KMPCH ; B2 and *B. subtilis* fusaric acid sensitive without significant differences between them, while *B. subtilis* 209 and *B. subtilis* 1 had no effect on controlling the disease. Spraying tomato leaflet with bacterial isolates WB15; WB52; B2; *B. subtilis* 209 and *B. subtilis* TH; after 1 day and then 7 days later inoculated

Table 6. Effect of different bacterial isolates tested on tomato late blight disease severity (as a direct effect)

Bacterial isolate	No. of lesion	Ø of lesion (mm)	Blighted area (mm^2)	Protection (%)
Check	5.22	4.33	76.83	0
CW1	0	0	0	100
CW2	0	0	0	100
WB15	2.5	1.33	3.47	95.48
WB 34	0.5	3.5	4.81	93.74
WB 52	0.5	3.5	4.81	93.74
KMPCH	0.44	1.33	0.61	99.2
***B .subtilis* fusaric acid sensitive**	0.25	0.68	0.09	99.88
***B. subtilis* TH**	2	2	6.28	91.83
***B. subtilis* 1**	5.22	4.33	76.83	0
***B. subtilis* 45**	1	3.842	11.58	84.93
***B. subtilis* 208**	0.25	2.75	1.48	98.01
***B. subtilis* 209**	5.22	4.33	76.83	0
B2	0.08	0.68	0.03	99.96
LSD at 0.05	0.797	1.18	9.43	1.868

with the pathogen were the most effective in controlling tomato late blight disease, without significant differences between them (Table 7). Data presented in Table (8) reveal the effect of bacterial isolates on tomato late blight disease on detached leaves, one day after spraying bacteria. Data indicate that, *B. subtilis* 208; B2; CW2 andWB52 isolates were the most effective, in this respect. B2; CW1 and *B. subtilis.* 208 were the most effective bacterial isolates on tomato late blight disease 7 days after spraying bacteria and 7 days after spraying *P. infestans* on the intact leaves(Table 9). It was also clear that, the number and diameter of lesions as well as blighted area/leaflet were lower in treated leaves than the check (Tables 6, 7, 8 and 9).

The effect of the bacterial isolates tested on the disease reduction might be due to induce anti-fungal compounds and/or laytic enzymes in their culture (Akatsumi *et al.* 1993). It might be also due to chitinase and ß-1,3-gluconase enzymes (Velazhahan *et al.*, 1999). The bacterial metabolites (*B. subtilis* metabolites) also induced systemic resistance against biotrophic fungi (Steiner and Schönbeek, 1995) through production of promoting plant growth (Ng & Webster, 1997).

CW2 and CW1 sprayed 2 days before inoculation were the most effective to control tomato late blight disease as a local and systemic effect respectively, while, WB 34 was the lowest one (Table, 10). Sprayed tomato plants with bacterial isolates B2 and CW1 and inoculated with *P. infestans* after 7 days, were the most effective in controlling tomato late blight disease (Table, 11), whereas, KMPCH was the lowest effective one under greenhouse conditions. On the other hand, the same bacterial isolates were the most effective on treated leaves, while, WB 34 , CW1 and CW2 were the most effective on the upper untreated leaves when the bacterial

Table 7. Effect of spraying different bacterial isolates tested one day before spray inoculation tomato intact leaves with *P. infestans* zoospore suspension on tomato late blight disease

Bacterial isolate	No. of lesion	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)
Check	26.33	17.5	6329.89	0
CW1	20.33	11.28	2030.6	67.92
CW2	20	11.42	2047.54	67.65
WB 15	11	9.42	766.2	87.89
WB 34	19	10.25	1567.01	75.24
WB 52	18	9.75	1343.23	78.78
KMPCH	18	12.83	2325.92	63.25
B. subtilis fusaric acid sensitive	18	14.67	3040.9	51.96
B. subtilis TH	19	10.25	1567.00	75.24
B. subtilis 1	21	12.56	2600.56	58.92
B. subtilis 45	15.67	11.28	1565.15	75.27
B. subtilis 208	18	12.83	2325.92	63.25
B. subtilis 209	16	10.9	1492.25	76.43
B2	17	9.69	1235	80.2
LSD at 0.05	1.212	2.683	1105.54	13.738

Table 8. Effect of different bacterial isolates tested on tomato late blight disease severity on detached leaves one day after spraying bacteria

Bacterial isolate	No. of lesion	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)
Check	3.67	8	184.38	0
CW 1	2.33	1	1.83	99.00
CW 2	2.33	1	1.83	99.01
WB 15	4	4	50.24	72.75
WB 34	2	3.17	15.78	91.44
CW 52	2.33	6	65.85	64.29
KMPCH	4.7	3.67	49.69	73.05
B. subtilis TH	2.33	6	65.83	64.24
B. subtilis 1	3.67	0.67	3.86	97.91
B. subtilis 45	3.33	1.33	6.95	96.23
B. subtilis 208	2.67	1.5	4.72	97.44
B 2	1	0.33	0.09	99.95
LSD at = 0.05	1.527	1.596	76.985	13.7

Table 9. Effect of different bacterial isolates tested on tomato late blight disease severity7 days after spraying bacteria and 7 days after spraying *P. infestans* on the intact leaves

Bacterial isolate	No. of lesion	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)
Check	51	7.6	2312.42	0
CW1	1	2.5	4.91	99.78
CW 2	5.5	4.33	80.90	96.50
WB 15	12.5	5.55	302.24	86.93
WB 34	7	5.24	150.87	93.48
KMPCH	40	5.5	949.85	52.92
***B. subtilis* TH**	6	7	230.79	90.02
***B. subtilis* 45**	3	6.67	104.77	45.47
***B. subtilis* 208**	3	2	9.42	99.59
B2	1	2.5	4.41	99.79
LSD at 0.05	3.219	1.38	444.64	5.951

Table 10. Effect of different bacterial isolates tested on tomato late blight disease severity on detached leaves two days after spraying bacteria

Bacterial isolate	Treated leaves as a local effect (L)				The upper untreated leaves as a systemic effect (S)			
	No. of lesion	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)	No. of lesion	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)
Check	2.67	11.25	265.26	0	1	5	19.63	0
CW1	2.67	3.33	23.24	91.24	0	0	0	100
CW2	0	0	0	100	0	0	0	100
WB 15	3	4	37.68	85.80	0	0	0	100
WB 34	2.67	10	209.60	20.99	1	5	19.63	0
KMPCH	1.46	3.95	17.88	93.26	0.41	1.2	0.02	99.89
B. subtilis TH	0.67	5	13.15	95.04	0	0	0	100
B. subtilis 45	1.5	2	4.71	98.22	0.33	1	0.26	98.68
B. subtilis 208	1.5	2	4.71	98.22	0.33	1	0.26	98.68
B2	0	0	0	100	0.33	1	0.26	98.68
LSD at 0.05 for:								
Bacteria (B) =	1.01	2.42	42.77	15.78				
B X L & S =	1.49	3.42	60.49	22.31				
L X S =	0.47	1.1	19.27	7.1				

isolates were sprayed after 7 days and the pathogenic fungus was inoculated with droplets. Bioagent could induce systemic resistance (ISR) against fungi, bacteria and virus, by lipopolysacchariedl (LPS), sidrophores, jasmonic acid (JA) and ethylene perception (Maurhofer *et al.*, 1994; Duijff *et al.*, 1997; Van Loon *et al.*, 1997 and Chen *et al.*, 1999). Induced systemic resistance (ISR) could be also induced by PR proteins such as PR-1, chitinase and ß,1-3,gluconase in the intracellular fluid of leaves (Chen *et al.*,1999). Plant growth promoting rhizobacteria (PGPR) strain of *B. subtilis* and Pseudomonas treatment increased in phenylalanine ammonia-lyase (PAL) and peroxidase activity (Podile & Laxmi, 1998 and Chen *et al.*, 2000).

Table 11. Effect of different bacterial isolates tested on tomato late blight disease severity in detached leaves 7 days after bacteria spraying

Bacterial isolate	Treated leaves as a local effect (L)				The upper untreated leaves as a systemic effect (S)			
	No. of lesion	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)	No. of lesion	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)
Check	3.67	8	184.38	0	5	7.56	224.33	0
CW1	0.33	0.67	0.20	99.94	0	0	0	100
CW2	0.33	1	0.26	99.86	0	0	0	100
WB 15	3.33	5.67	84.04	54.42	2	2	6.28	97.2
WB 34	1	4.67	17.12	90.71	0	0	0	100
KMPCH	3.6	2	11.3	93.87	4	5	78.5	65.01
B. subtilis TH	1.33	6.33	41.83	71.81	0.67	1.33	0.93	99.59
B. subtilis 45	1.83	2.55	9.34	94.93	2.33	2.55	11.89	94.70
B. subtilis 208	3.33	5.67	84.04	54.42	4	8	200.96	10.42
B2	0.33	0.67	0.35	99.81	0.67	1	0.53	99.77
LSD at 0.05 for:								
Bacteria (B) =	1.37	2.2	41.28	17.63				
B X L & S =	1.94	3.12	58.38	24.93				
L X S =	0.58	0.93	17.38	7.42				

Trichoderma harzianum isolates were the most effective bioagents in reducing the growth of the late blight pathogen followed by *T. viride* and *T. hamatum* isolates (Table 12). The differences between all fungal isolates and the check treatment were significant. Such results were detected by using *Trichoderma* spp. with different plant pathogenic organisms which found by De Meyer *et al.* (1998); Han *et al.* (2000) and Howell *et al.* (2000).

Under greenhouse conditions *T. harzianum* controlled tomato late blight as a direct effect or 1, and 7 days after application (Table 13). This effect might be due to induce anti-fungal or lytic compounds by *Trichoderma* in its culture or mycoparasitism (Handlesman and Park, 1989) or competition (Sivan and Chet, 1989). Also might be due to induction of plant defense to control the pathogen. This treatment also increased peroxidase activity in the plants (Howell *et al.*, 2000). The fungal bioagent *T. hamatum* st., 382 induced systemic resistance in radish (Han *et al.*, 2000).

Actinomycetes isolate No. A0 was the most effective isolate in reducing the growth of the late blight pathogen followed by actinomycetes isolate A1 (Table 14). The differences between all actinomycetes isolates and the check treatment were significant. Actinomycetes are known to produce one or more antibacterial, anti-fungal, antiviral, antiprotozoal, and antitumour or anti-microbial materials (Waksman and Henrici, 1943). *Streptomyces violaceus-niger* strain YCED-9 is known as an anti-fungal bioagent, had antagonistic effect to many different classes of plant pathogenic fungi. It produced antimicrobial compounds (antifungal activity) such as guanidyl, nigericin and geldanamycin. Also produced hydrolytic enzymes (chitinase and ß-1,3-gluconase) as described by Trejo *et al.* (1998).

Table 12. Antagonistic effect between different fungal isolates and tomato late blight pathogen *Phytophthora infestans*

Fungal isolate	Mycelial growth (%)
Trichoderma sp.	50
T. viride	50
T. hamatum	50
T. harzianum T2	46.67
T. harzianum T6	46.67
Fusarium sp.	66.67
Aspergillus sp. 1	66.67
Aspergillus sp. 2	66.67
Penicillium sp.	66.67
Isolate No. 16	46.67
Isolate No. 3	48

LSD at 0.05 = 1.176

Table 13. Effect of different *Trichoderma* sp. isolates on control tomato late blight disease in detached leaves after different time of application

Time of application	*Trichoderma* isolates tested	No. of lesion (mm)	Ø of lesion (mm)	Blighted area (mm²)	Protection (%)
At the same time (0)	Check	6	5.83	160.09	0
	Trichoderma T2	0	0	0	100
	Trichoderma T6	1.62	1.68	3.59	97.76
One day later	Check	6	5.83	160.09	0
	Trichoderma T2	0	0	0	100
	Trichoderma T6	0	0	0	100
seven days later	Check	6	5.83	160.09	0
	Trichoderma T2	0.5	0.38	0.06	99.96
	Trichoderma T6	1.54	0.88	0.94	99.41
	Treatments	0.685	0.737	16.26	4.13

Table 14. Antagonistic effect between different actinomycetes isolates and tomato late blight pathogen *Phytophthora infestans*

Actinomycetes isolate	Mycelial growth (%)
Isolate No. A0	63.33
Isolate No. A1	64
Isolate No. A2	66.67
Isolate No. A3	76.67
Isolate No. A4	68.4

LSD at 0.05 = 1.07

References

Akatsumi, K.; Hirata, A.; Yamamoto, M.; Hirayae, K.; Okuyama, S. and Hibi, T. 1993. Growth inhibition of *Botrytis* spp. by *Serratia marcescens* B2 isolated from tomato phylloplane. *Ann. Phytopathol. Soc. Japan*, **59**: 18-25.

Aly, A. Z.; Buchenauer, H.; Abou-Zaid, M. I. and Atia, M. M. 2000. Studies on tomato late blight disease. *J. Environ. Res.*, **2**: 229-245.

Barnett, H.L. and Hunter, B.B. 1981. Illustrated Genera of imperfect fungi. Mc Millan Publishing, London, 218 pp.

Bochow, H. and Doley, S. 1998. Mechanisms of tolerance induction in plants by root colonizing *Bacillus subtilis* isolates. *Modern Fungicides and Antifungal Compounds.* 12th Internat. Reinhardsbrunn Symp. May, 1998, Germany.

Buchanon, R.E; Gibbons, N.E.; Cowan, S.T.; Hoh, J.C.; Liston, I.; Murray, E.G.D.; Niven, C.F.; Ravin, A.W. and Stanier, R.Y. 1974. Bergy's Manual of Determination Bacteriology, 8th Ed. Williams and Wilkns Company, B. Himore, U.S.A.

Buchenauer, H. 1998. Biological control of soil-borne diseases by rhizobacteria. *J. Plant Dis. and Protec.*, **105**: 329-348.

Chen, C; Belanger, R.; Benhamou, N. and Paulituz, T. 1999. Role of SA in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber. *Eur. J. Plant Pathol.*, **105**: 477-486.

Chen, C.; Belanger, R.; Benhamou, N. and Paulituz, T. 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacterium (PGPR) and *Pythium aphanidermatum*. *Physiological and Molecular Plant Pathol.*, **56**: 13-23.

Cohen, Y. 1994. Local and systemic control of *Phytophthora infestans* in tomato plants by Dl-3-amino-n-butanoic acids. *Phytopathology*, **84**: 55-59.

De Meyer, G. and Höfte, M. 1997. Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathology*, **87**: 588-593.

De Meyer, G.; Bigirimana, J.; Elad, Y. and Höfte, M. 1998. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *Eur. J. Plant Pathol.*, **104**: 279-286.

Duijff, B. J.; Gianninazzi-Pearson, V. and Lemanceau, P. 1997. Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS17r. *New Physiol.*, **135**: 325-334.

Eliseeva, L.G.; Latushkin, V.V. and Lichko, N.M. 1995. New biological control methods against potato diseases. *Zashchita Rastenil (Moskva)*, **1**: 12-17. (C.f. *Rev. Pl. Pathol.*, **74**: 5).

Enebak, S.A. and Carey, W.A. 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. *Pant Dis.*, **84**: 306-308.

Filippov, A.V. and Kuznetsova, M.A. 1995. Different influence of some biofungicides on dynamics of potato plant susceptibility to *Phytophthora infestans* (Mont.) de Bary. *Mikolo. Fitopatol.*, **28**: 64-69. (C.f. *Rev. Pl. Pathol.*, **74**: 6399).

Gulati, M.K.; Koch, E. and Zeller, W. 1998. Isolation of antifungal metabolites produced by flourescent psedomonas, antagonist of red core disease of strawberry. *Modern Fungicides and Antifungal Compounds.* 12th Internat. Reinhardsbrunn Symp. May, 1998, Germany.

Han, D.Y.; Coplin, D.L.; Bauer, W.D. and Hoitink, H.A. 2000. A rapid bioassay for screening rhizosphere microorganisms for their ability to induce systemic resistance. *Phytopathology*, **90**: 327-332.

Handlesman, J. and Park, J.I. 1989. Mechanism in biocontrol of soilborne plant pathogens. Pp. 27-61 in Vogue, T. Nester E.W. (eds.). *"Plant Microbial Interaction"*. Vol.3. McGrwohill, New York.

Hartman, G.L. and Haung Y.H. 1995. Characteristics of *Phytophthora infestans* isolated and development of late blight on tomato in Taiwan. *Plant Dis.*, **79**: 849-852.

Hirte, W.F.; Walter, C.; Grunberg, M.; Sermann, H. and Adam, H. 1989. Selection of pathotypes of *Verticillium lecanii* for various harmful insects in glasshouses and aspects of the biotechnological spore production. *Zentralblatt für Mikrobiologie*, **144**: 405-420.

Hoffland, E.; Hakulinen, J. and Van Pelt, J.A. 1996. Compares ion of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* spp. *Phytopathology*, **86**: 757-762.

Howell, C.R.; Hanson, L.E.; Stipanovic, L. and Puckhaber, L S. 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathology*, **90**: 248-252.

Jensen, H.L. 1930. Actinomycetes in Danish soils. *Soil Sci.*, **30**: 59-77.

Jetiyanon, K.; Tuzun, S. and Kloepper, J.W. 1997. Lignification, peroxidase reaction and superoxidase dismutase as early plant defense associated with PGPR-mediated induced systemic resistance. Pp. 265-268 in: Ogoshi, A., Kobayashi, K.; Homma, Y.; Kodama, F.; Kodo, N. and Akino, S. (eds.). *"Plant Growth Promoting Rhizobacteria. Present Status and Future Prospects"*. Proc. 4th Internat. Sapporo, Japan.

Jindal, K.K.; Singh, H.; Madhu, Meeta, and Meeta, M. 1988. Biological control of *Phytophthora infestans* on potato. *Indian J. Plant Pathol.*, **6**:59-62. (C.f. CAB Abstracts, 1990-1991).

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

Leader, F.J.; Curl, J.H.; Bond, J.H. and Fribourg, H.A. 1960. *Methods for Studying Soil Microflora-Plant Disease Relationship.* Burgess Publishing Comp. St. Minneapolis, Minn.

Leeman , M.; Van Pelt, J.A.; Den Ouden F.M.; Heinsbroek, M., Bakker, H.M. and Schippers, B. 1995. Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens. Phytopathology,* **85**: 1021-1027.

Maurhofer, M.; Keel, C.; Hass, D. and Defago, G. 1994. Pyoluterorin production by *Pseudomonas fluorescens* CHAO is involved in the suppression of Pythium damping-off of cress but not of cucumber. *Eur. J. Plant Pathol.,* **100**: 221-232.

Ng, K.K. and Webster, J.M. 1997. Antimycotic activity *of Xenorhabdus bovienii* (Enterobacteriaceae) metabolites against *Phytophthora infestans* on potato plants. *Canad. J. Plant Pathol.,* **19**: 125-132.

Niderman, T.; Genetet, I.; Bruyere, T.; Gees, R.; Stintzi, A.; Legrand, M.; Fritig, B. and Mosinger, E. 1995. Pathogenesis-related PR-1 proteins are anti-fungal. Isolation and characterization of three 14-kilodalton proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against *Phytophthora infestans. Plant Physiol.,* **108**: 17-27.

Podile, A.R. and Laxmi, V.D.V. 1998. Seed bacterization with *Bacillus subtilis* AF1 increase phenylalanine ammonia-lyase and reduces the incidence of Fusarium wilt in pigeonpea. *J. Phytopathol.*, **146**: 255-259.

Sadlers, H.M. 1996. Use of bacteria in controlling fungal diseases. *Gemuse Munchen,* **32**: 180-181. (C.f. CAB Abstracts 1998-1999).

Siegmund, Inka and Wagner, F. 1991. New method for detecting rhamnolipids excreted by *Pseudomonas* spp. during growth on mineral agar. *Biotechnology Techniques* , **5**: 265-268.

Sivan, A. and Chet, I. 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporium* on rhizosphere colonization. *Phytopathology,* **79**: 489-501.

Stanghellini, M.E. and Miller, R.M. 1997. Biosurfactants their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Dis.*, **81**: 4-12.

Steiner, U. and Schönbeek, F. 1995. Induced resistance against biotrophic fungi. *Modern fungicides and antifungal compounds,* 11th Internat. Symp., May 14–20 , 1995 Germany.

Trejo, Estrad, S.R.; Paszczynski, A. and Crawford, D.L. 1998. Antibiotics and enzymes produced by the biocontrol agent *Streptomyces violaceusniger* YCED-9. *J. of Industrial Microbiol. and Biotechnol.*, **21**: 81-90. (C.f. CAB Abstracts 1998/8-1999/01).

Van Loon , L.C.; Bakker, P.A. and Pieterse, C.M.J. 1997. Mechanisms of PGPR-induced resistance against pathogens. In: Ogoshi, A., Kobayashi, K.; Homma, Y.; Kodama, F.; Kodo, N. and Akino, S. (eds.). "*Plant Growth Promoting Rhizobacteria. Present Status and Future Prospects*". Proc. 4th Internat. Sapporo, Japan.

Velazhahan, R.; Samiyappan, R. and Vidhyasekaran, P. 1999. Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of laytic enzymes. *J. Pant Dis. and Protect.*, **106**: 244-250.

Waksman. S.A. and Henrici, I. 1943. Family Strepotomycetaceae, Genus Streotomyces. Pp. 275-305 in *Shorter Bergey's "Manual of Derterminative Bacteriology"* Holt, J.G. (Ed.), 1984, (8th ed.). The Williams and Wilkins Company, Baltimore.

Webster, J. 1980. *Introduction to Fungi*. 2nd Edition. Cambridge University Press. Cambridge, New York , Port Chester. 665 pp.

Wie, G.; Kloepper, J.W. and Tuzun, S. 1991. Induced systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. *Phytopathology*, **81**: 1508-1512.

Wie, G.; Kloepper, J.W. and Tuzun, S. 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. *Phytopathology*, **86**: 221-224.

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