Disease control / Moyens de lutte

Comparative screening of bacteria for biological control of potato late blight (strain US-8), using in-vitro, detached-leaves, and whole-plant testing systems

F. Daayf, L. Adam, and W.G.D. Fernando

Abstract: Forty-three bacteria were isolated from the phylloplane and (or) rhizosphere of potato and canola plants and tested for their ability to control the pathogen *Phytophthora infestans* (strain US-8) causing late blight on potato. This study revealed the benefit of using more than one system when searching for biocontrol activity. In this regard, the complementarity of the three systems chosen (in-vitro culture media, detached leaves, and whole plants) in selecting and identifying potential modes of action, provided a useful insight into the different types of biocontrol activity present. Bacteria with biocontrol activity were from the genera *Bacillus*, *Pseudomonas*, *Rahnella*, and *Serratia*. Mechanisms of inhibition characterized included those occurring directly, through antibiosis, and (or) indirectly, through the induction of plant defense systems.

Key words: Phytophthora infestans, late blight, biological control, potato, canola.

Résumé : Quarante-trois bactéries, isolées de la phyllosphère et (ou) de la rhizosphère de plants de pomme de terre et de canola, ont été testées pour vérifier leur efficacité contre le *Phytophthora infestans* (souche US-8), l'organisme pathogène responsable du mildiou de la pomme de terre. Cette étude a révélé l'avantage d'utiliser plus d'un système lors de la recherche d'agents de lutte biologique. Dans cette perspective, la complémentarité des trois systèmes choisis (le milieu de culture in vitro, le système de feuilles détachées et le système utilisant la plante entière) pour la sélection et l'identification de modes d'action potentiels a été utile pour se faire une idée sur les différents modes d'action en présence. Les bactéries identifiées, possédant une activité de contrôle biologique, appartiennent aux genres *Bacillus*, *Pseudomonas*, *Rahnella* et *Serratia*. Les mécanismes d'inhibition caractérisés comprennent ceux agissant soit directement, par l'intermédiaire de la production d'antibiotiques, et (ou) indirectement, par l'induction de systèmes de défense chez la plante.

Mots clés : Phytophthora infestans, mildiou, lutte biologique, pomme de terre, canola.

Introduction

Potato is the fourth most important food in the world and the most important vegetable in North America (Desjardins et al. 1995). Difficulty in growing this crop commercially can be attributed to its susceptibility to diseases, of which late blight caused by *Phytophthora infestans* de Bary is considered the most important (Chycoski and Punja 1996; Fry and Goodwin 1997; Goodwin et al. 1994a; Shattock et al. 1990). For more than a decade, controlling late blight has become increasingly demanding because of the emergence of new strains of the pathogen, especially the US-8 genotype (Fry et al. 1991, 1992, 1993; Goodwin et al. 1994a, 1994b), which is known to be more aggressive and resistant to the fungicide metalaxyl (Daayf et al. 2001; Kato et al. 1997; Lambert and Currier 1997). Repeated use of chemicals has resulted in fungicide resistance in the pathogen, as documented for metalaxyl (Daayf and Platt 1999, 2000; Daayf et al. 2000), and increased the costs of crop production. Moreover, the public has expressed concerns about the heavy reliance on chemicals in plant protection strategies. Despite these issues, safe and environmentally friendly products for plant protection represent an insignificant portion of the pesticide market, which is dominated by synthetic chemicals. If new alternative methods, such as bi-

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ological control, could be developed to minimize the need for synthetic chemicals in food crop production, a more sustainable agricultural system could be created.

Biological-control treatments consisting of living microorganisms or abiotic products can provide disease protection essentially through one or more of the following: (i) production of antibiotics or other molecules that are deleterious to the pathogen's development, (ii) competition with the pathogen for nutrients and space, or (iii) induced plant resistance. As a result of such a diversity in the mechanisms of action, it is relatively difficult to choose adequate methods for screening of biocontrol agents and, by consequence, to identify reliable and sustainable biocontrol solutions. Previous studies that searched for biocontrol agents generally tested potential candidates for their ability to: (i) inhibit the pathogen in vitro on culture media (Foldes et al. 2000) and (or) (ii) protect pretreated seeds (Handelsman et al. 1990; Williams and Asher 1996), seedlings or plants (Gees and Coffey 1989; Larkin and Fravel 1998), or fruit (Korsten et al. 1995) from further infections. Biocontrol agents' characteristics other than protection against infection, such as the ability to promote plant growth (Berg et al. 2001) or to colonize plant tissue (Nautiyal 1997), were also evaluated in parallel. However, systems tested were generally limited to one or two screening methods (Foldes et al. 2000; Gees and Coffey 1989; Han et al. 2000; Handelsman et al. 1990). In addition, most screening studies concentrated solely on the biocontrol efficacy of the treatments, whereas only few did investigate both their efficacy and mechanisms of action.

In the potato – late-blight system, biocontrol studies are very limited, especially those involving the US-8 genotype, which is currently the predominant strain in most of Canada and many parts of the U.S.A. Choosing the most adequate method for screening of the best biocontrol agents may be challenging because it would be difficult to predict which test would allow the selection of the best treatment without knowing in advance mechanisms of action of the tested treatments. For this reason, the simultaneous use of multiple screening methods appears as an alternative of choice to circumvent such a difficulty. In addition, it would likely identify biocontrol agents with more than one biocontrol mechanism, which would represent a considerable advantage because additional control mechanisms represent further barriers against infection.

The objectives of this study were to:

(i) compare the efficacy of selected bacteria in controlling potato late blight, using three different systems: (1) in-vitro culture media, (2) detached potato leaves, and (3) whole potato plants; and
(ii) identify bacteria with different mechanisms of action (i.e., antibiosis versus induced plant resistance).

Materials and methods

Isolation of bacteria

Bacterial isolates for this study originated from single CFU (colony-forming units) obtained after dilution plating of purified bacterial colonies, isolated from the phylloplane and the rhizosphere of potato and canola grown in Manitoba, Canada. Plant material and soil, randomly collected from potato ('Russet Burbank') and canola ('Westar' and 'Cresor') fields and canola stubble previously placed at four different depths (0, 2, 5, and 10 cm) in canola rhizosphere, were used as bacterial sources.

Plant (1 g) or soil (10 g) material was placed into a 250-mL Erlenmeyer flask containing 100 mL of sterile, distilled water. The flask was shaken briefly and left to stand for 10–15 min. After 20 s of sonication (Branson® ultrasonic cleaner, Branson Cleaning Equipment Company, Shelton, Conn., U.S.A.), isolations were carried out with standard dilution plating techniques (Tortora et al. 1992) on half-strength nutrient agar medium (Difco Laboratories, Detroit, Mich., U.S.A.) amended with 100 ppm of cyclohexamide (Sigma, St. Louis, Mo., U.S.A.).

Effect of bacteria on growth of *P. infestans* in vitro

Bacteria recovered from potato and canola sources were cultured on nutrient agar and incubated for 2 to 3 days at 20°C. Bacterial suspensions were prepared by adding 100 µL of sterile, distilled water to the colony and mixing. Four replicates of 5-µL aliquots of the bacterial suspension were placed 90° apart on the perimeter of each of two Petri dishes each (9 cm in diameter) of clarified rye agar (CRA) and V8 – potato dextrose agar (V8–PDA). Petri dishes were incubated overnight at room temperature. A 5-mm plug of *P. infestans* US-8 strain isolated from potato in Manitoba in 2001 was transferred to the center of each CRA or V8–PDA Petri dish. This isolate was chosen for its fast in-vitro growth and high aggressiveness on potato (results not shown). The Petri dishes were incubated at 20°C and duplicate diameters of fungal colonies were measured at 90° with a ruler when the control colony reached full growth (85 mm). This occurred 7 days after incubation on V8–PDA media and 21 days on CRA media. The inhibitory effect of the bacteria against *P. infestans* was estimated based on the percent relative growth:

$$[1] \quad \% \text{ relative growth} = (B/P) \times 100$$

where B is the growth of *P. infestans* when challenged with the bacteria and P is the growth of the control *P. infestans*. The experiment was repeated once.

Effect of bacteria on *P. infestans*, using detached potato leaves

Living bacteria and bacterial-broth supernatant were employed separately to evaluate their effects on late-blight development. Bacteria suspension tests were conducted on leaflets of 'Russet Burbank', with three plants as replicates. Bacterial inoculum was prepared by transferring two loops from a culture growing on a nutrient agar into 15 mL of sterile, distilled water and adjusting to a final concentration of 10^8 CFU/mL; the inoculum was applied to a leaflet by dipping. After 5 h of incubation at room temperature, *P. infestans* was applied by placing 20-µL droplets (10^4 zoospores/mL) to the underside of each leaflet. Treated leaflets were placed on moist, sterile filter paper in a covered Petri dish and transferred to a moist chamber (>95% RH). For bacterial-broth supernatant tests, nutrient broth was prepared in 250-mL flasks, autoclaved, and then inoculated

with two loops of bacteria. After 3 days of continuous incubation on a shaker (150 r/min; 1 r = 2π rad) at room temperature, the medium was spun down at 4500 r/min for 10 min to pellet the bacteria. The supernatant was decanted and frozen. Four treatments were compared in this experiment: (i) water pretreatment and water application (WW), (ii) water pretreatment and pathogen application (WP), (iii) bacterial pretreatment and water application (BW), and (iv) bacterial pretreatment and pathogen application (BP). The pretreatments were applied by dipping the leaflets in water or bacterial supernatant solutions. Inoculation with *P. infestans* was performed as described above. In control treatments, water application consisted of 20-μL droplets deposited on the lower leaf surface. Inoculated leaves, which were treated either with bacteria or water, were scanned using the image analysis software Assess® (Lamari 2002) to estimate the area infected by *P. infestans*. Percent inhibition provided by each bacterium was calculated as follows:

$$[2]\ \%\ \text{inhibition} = 100 - [(BP/WP) \times 100]$$

where BP and WP represent the infected area on leaves that were pretreated with a bacterium or with sterile, distilled water, respectively, and then inoculated with *P. infestans*. Treatments were rated based on the percent inhibition they provided: 1, <10%; 2, 10–20%; 3, 21–40%; 4, 41–75%; 5, >75%.

Biolog identification procedure

Bacteria demonstrating biocontrol potential (15 isolates) were selected for further identification and whole-plant studies. Single colonies were obtained by applying the bacterial streaking method, and the following tests were done: (i) Gram stain (Biolog Inc., Hayward, Calif.), rated as Gram positive or Gram negative; (ii) bacteria were streaked onto Biolog universal growth (BUG) agar medium (Biolog Inc.). Approximate bacterial numbers were quantified with a turbidimeter, and 150 μL of this solution was poured into each of 96 wells in Biolog microplates. The plates were incubated at 32°C for 16–24 h and then read with an automated plate reader or assessed visually, to provide identification to genus or species level.

Local and systemic effects of bacteria on disease expression on whole plants

Six-week-old 'Russet Burbank' plants grown in clay pots (10 cm in diameter) in a soil–sand–peat–perlite mix (4:4:4:1, v/v) were used. One terminal and one primary leaflet, at leaf position 4 from first fully expanded leaf, were treated on each of three plant stems, either with water or bacterial suspension. This was repeated on five plants. Bacterial suspensions consisted of two loops of bacteria suspended in 15 mL of sterile, distilled water, which were adjusted to an absorbance of 0.14 at 640 nm (10^8 CFU/mL) before treatment. Treatments consisted of 1 mL of bacterial suspension spread on the leaf surface with a sterile hockey stick. Control leaves received sterile, distilled water. Treated and adjoining (untreated) leaves were both tagged. Plants were incubated 24 h at 22°C (day) and 17°C (night), under a 16-h photoperiod, before inoculation with *P. infestans* of the US-8 genotype. Inoculation was performed by placing four 15-μL drops of zoospore suspension (10^4 zoospores/mL) on tagged (both treated and untreated) leaves of both control and treated plants. After inoculation, all plants were left for 48 h in a misting chamber (100% RH) and then transferred to the growth room (22°C (day) and 17°C (night), 16-h photoperiod). Six days later, tagged leaves were removed, scanned and analysed with Assess (Lamari 2002) to estimate the infected area. Percent protection by bacteria was calculated separately on treated (local protection) and untreated (systemic protection) leaves as follows:

$$[3]\ \%\ \text{local protection} = 100 - [(BPt/Pt) \times 100]$$

where BPt is the infected area on leaves pretreated with a bacterium and then inoculated with *P. infestans*, and Pt is the infected area on their counterparts, from control plants that received sterile, distilled water and were then inoculated with *P. infestans*;

$$[4]\ \%\ \text{systemic protection} = 100 - [(BPu/Pu) \times 100]$$

where BPu is the infected area on untreated leaves inoculated with *P. infestans* (from plants treated with a bacterium), and Pu is the infected area on corresponding untreated leaves inoculated with *P. infestans* (from control plants).

Data analysis

Analysis of variance was performed on all data and, when appropriate, means were compared based on the least significant difference (LSD, $P = 0.05$).

Results

Isolation of bacteria

More than 200 bacterial isolates were obtained. For purity assessment, they were plated onto Luria Bertani agar: 15 g of agar (Difco Laboratories), 10 g of tryptone peptone (Becton Dickinson Microbiology Systems, Sparks, Md., U.S.A.), 5 g of yeast extract (Sigma), and 5.0 g of NaCl per liter of solution. Pure cultures were stored in Luria Bertani broth amended with 20% glycerol (v:v) at –80°C. Based on preliminary inhibition tests on *Sclerotinia sclerotiorum* (Lib.) de Bary, *P. infestans*, and *Rhizoctonia solani* Kühn (results not shown), 43 bacteria (32 from canola, 9 from potato, and 2 provided by Dr. P. Thonart, Belgium) were selected for this study.

Effects of bacteria on growth of *P. infestans* in vitro

In general, the inhibitory activity of most bacteria towards *P. infestans* was higher on CRA (Fig. 1A) than on V8–PDA media (Fig. 1B), respectively. For example, 18 bacteria (DF34 to P2, Fig. 1B) did not inhibit *P. infestans* on V8–PDA, while they provided 2 to 52% inhibition on CRA (Fig. 1A). However, most bacteria that provided ≥50% inhibition (11 out of 15) did so on both media (Figs. 1A and 1B). Eleven bacteria (P1, B2, Y1, B3, W1, M1, B1, C1, DF35, DF40, and W2) provided 50 to 89% and 53 to 86% inhibition on CRA and V8–PDA, respectively. The remaining four bacteria, DF33, DF39, DF28, and DF37, which

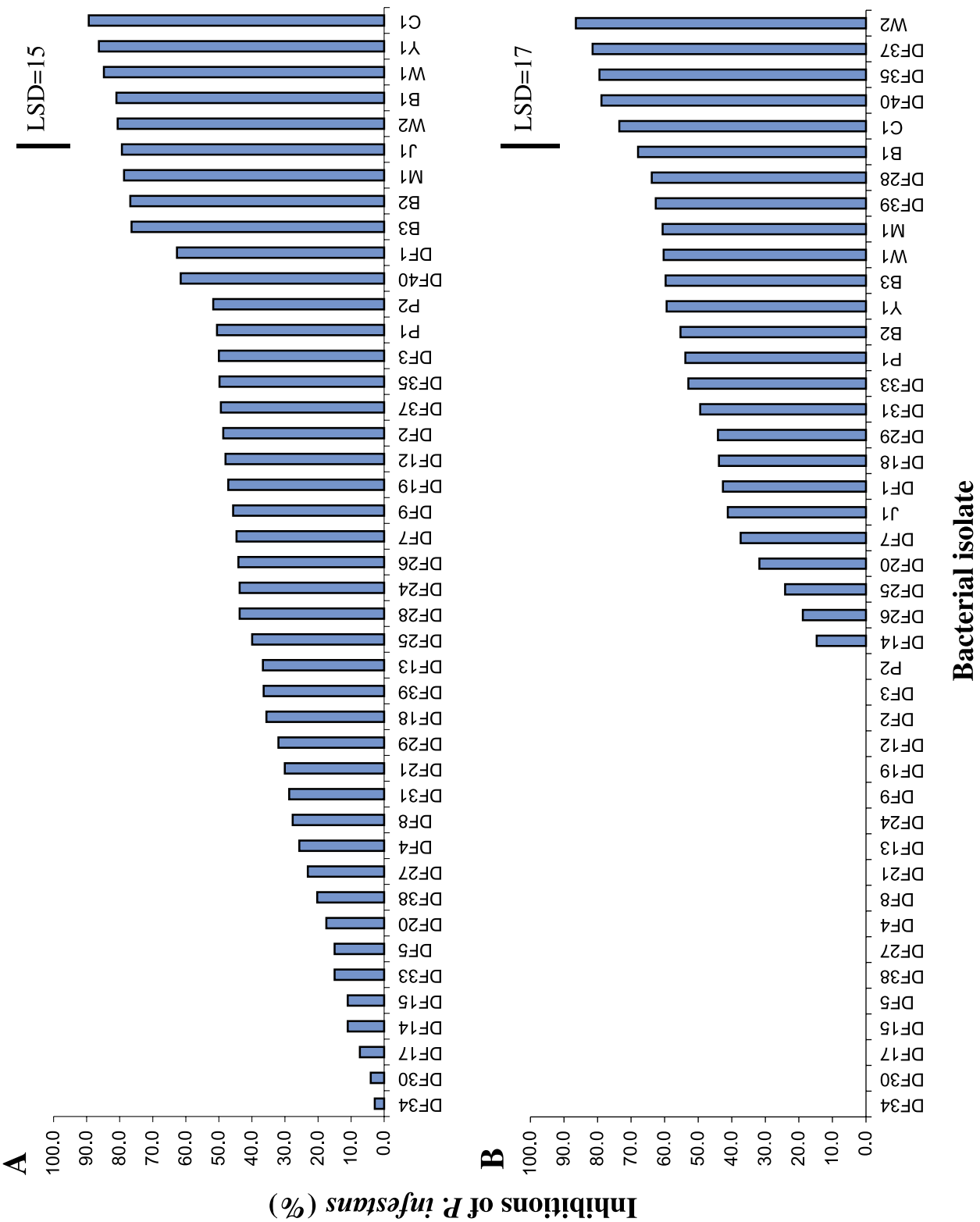


Fig. 1. Effects of bacteria on in-vitro growth of *Phytophthora infestans*, expressed as percent inhibition. (A) Growth on clarified rye agar. (B) Growth on V8 – potato dextrose agar. 100% represents complete growth inhibition while 0% represents no inhibition.

provided 15, 36, 43, and 49% inhibition on CRA, respectively, were more inhibitory on V8–PDA (52, 62, 63, and 82%, respectively). On the other hand, DF3, P2, J1, and DF1, which provided 0, 0, 41, and 42% inhibition of *P. infestans* on V8–PDA, were more active on CRA (50, 51, 79, and 63%, respectively).

In general, four groups of bacteria were identified, based on their inhibitory effects against *P. infestans* on the two test media: (1) bacteria with none or little inhibitory effects on both media; (2) bacteria that were highly inhibitory to *P. infestans* on both media; (3) bacteria that showed no inhibition on V8–PDA, but had 20–60% inhibition on CRA; and (4) bacteria that were more inhibitory on V8–PDA than on CRA.

Effects of selected bacteria on *P. infestans*, using detached potato leaves

When living bacteria were applied directly to detached leaves, only a limited number of isolates provided a level of protection high enough (>40%) to be further considered as potential biocontrol agents. DF1 and DF14 showed the highest level of inhibition to *P. infestans*, with protection rates of 5 and 4, respectively (Table 1). Few other bacteria provided a moderate level of protection (DF18, DF30, DF34, DF35, P2, and W2), while others (DF2, DF3, DF9, DF13, DF14, DF31, B1, B2, and P1) had the lowest level of inhibition. All the remaining bacteria provided no to negligible protection against *P. infestans* (Table 1).

For ease of handling, 29 bacteria out of the original 43, including representatives from all groups providing different levels of protection described above, were selected for supernatant studies. Supernatants from DF1 and DF14 provided similar levels of protection as their corresponding bacterial suspensions and were among the best treatments, especially DF1 (Table 2). On the other hand, DF4 and DF5, which did not provide any protection when applied as a bacterial suspension, were very effective as culture supernatants. Supernatants from DF7, DF8, and DF12 were highly inhibitory to *P. infestans*, whereas the corresponding living bacteria were ineffective on detached leaves. All the other culture supernatants were as effective as their corresponding bacteria in terms of inhibiting *P. infestans* development on detached potato leaves (Table 2).

Local and systemic effects of selected bacteria on disease expression, using whole plants

Based on their previous performance on culture media and on detached leaves, 15 bacteria were selected for testing on whole plants (B1, B2, B3, C1, DF1, DF3, DF35, DF37, DF40, J1, M1, P1, W1, W2, and Y1).

All the bacteria, in this sudy, provided local protection of potato leaves against *P. infestans*, with a range continuum from 18 to 60% reduction in infection rate (Fig. 2A). A group of nine bacteria provided less than 40% inhibition (W1, DF3, DF37, B2, DF40, Y1, C1, M1, and DF35), while another group of six bacteria provided between 40 and 60% inhibition (DF1, B3, J1, P1, W2, and B1).

The selected bacteria were also tested for their ability to provide protection in untreated adjacent leaves. From the first group of bacteria, only W1 provided a significant protection in leaves adjacent to the treated leaves, whereas infection levels on untreated leaves from plants treated with DF37, B2, and DF35 were not significantly different from the corresponding control leaves, and DF3 was only slightly different (Fig. 2B). By contrast, most of the second group of bacteria (P1, W2, and B1) provided significant systemic protection, with 50 to 70% reduction in infection rate as compared with control leaves.

Table 1. Protection level against *Phytophthora infestans*, provided by living bacteria on detached leaves of potato plants.

Protection rating*	Bacterial isolates
1	DF4, DF5, DF7, DF8, DF12, DF15, DF17, DF19, DF20, DF21, DF24, DF25, DF26, DF27, DF28, DF29, DF33, DF37, DF38, DF39, DF40, B3, C1, J1, M1, W1, Y1
2	DF2, DF3, DF9, DF13, DF31, B1, B2, P1
3	DF18, DF30, DF34, DF35, P2, W2
4	DF14
5	DF1

*Based on the percent inhibition of *P. infestans* provided by each bacterial treatment: 1, <10%; 2, 10–20%; 3, 21–40%; 4, 41–75%; 5, >75%.

Table 2. Protection level against *Phytophthora infestans*, provided by bacterial-culture supernatant on detached leaves of potato plants.

Protection rating*	Bacterial isolates
1	DF15, DF21, DF33
2	DF2, DF3, DF13, DF19, DF20, DF35, DF38, B1, B2
3	DF9, DF17, DF18, DF31, DF34, DF37, DF39, P1, P2, W1
4	DF7, DF8, DF12, DF14
5	DF1, DF4, DF5

*Based on the percent inhibition of *P. infestans* provided by each bacterial treatment: 1, <10%; 2, 10–20%; 3, 21–40%; 4, 41–75%; 5, >75%.

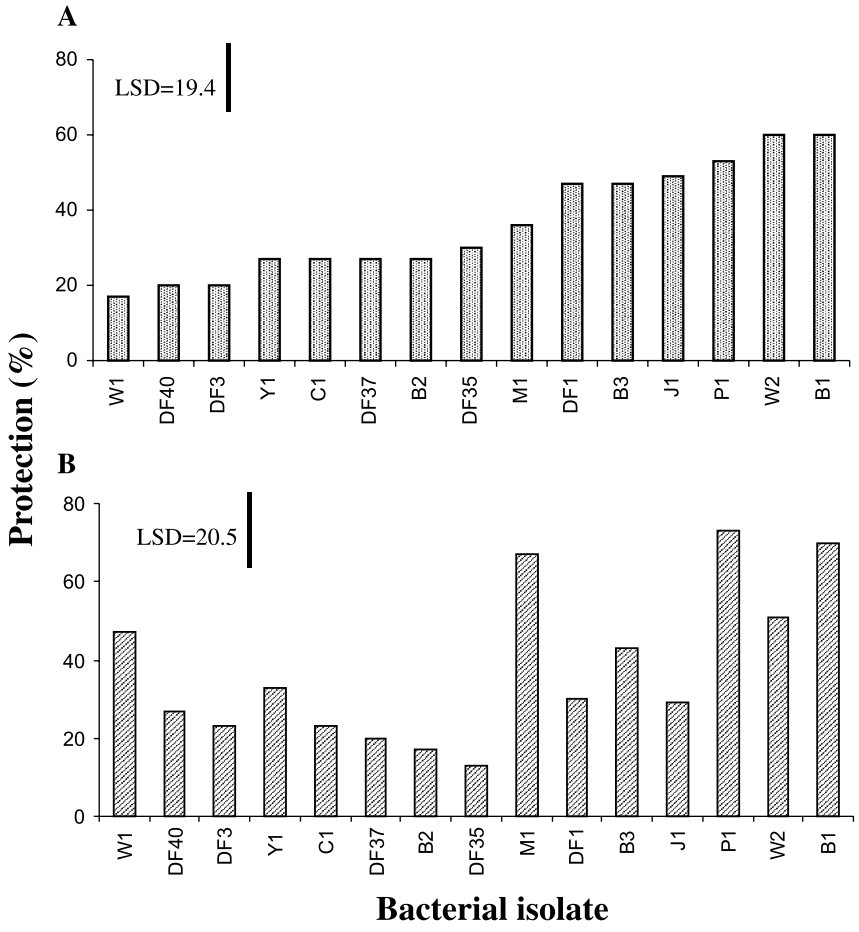
Bacteria identification with the Biolog system

The bacteria of interest were identified as *Bacillus subtilis* (Ehrenberg) Cohn (B1, B3, and J1), *Serratia plymuthica* (Lehmann & Neumann 1896) Breed et al. (DF1), *Bacillus pumilus* Meyer & Gottheil (B2, M1, W1, and Y1), *Pseudomonas fluorescens* Migula (DF35, DF37, and DF40), *Pseudomonas viridilivida* (Brown) Stapp (DF3), *Rahnella aquatilis* Izard et al. (W2), and *Bacillus amyloliquefaciens* (ex Fukumoto) Priest et al. (C1) (Table 3). P1 and P2, both *Pseudomonas putida* (Trevisan) Migula, were kindly provided by Dr. Philippe Thonart, Belgium.

Discussion

This study demonstrated that the higher the number of experimental systems for screening and characterizing bacteria as biocontrol agents, the greater the likelihood of identifying bioactivity. Of all the systems employed, the whole-plant test was the most convincing, but the other tests provided an indication of alternative mechanisms of action that

Fig. 2. Percent local (A) and systemic (B) protection of potato plants provided against *Phytophthora infestans* by bacterial treatments. See [3] and [4] in Materials and methods for definitions of percent local and percent systemic protection and their parameters, respectively.



could operate in a successful treatment. In previous studies based on only one or two methods for the screening of biocontrol agents (Foldes et al. 2000; Gees and Coffey 1989; Han et al. 2000; Handelsman et al. 1990; Korsten et al. 1995; Williams and Asher 1996), it was rather difficult to reach a conclusion on the mechanism of action. In addition, in the majority of tests where more than one screening method was tested, a step-by-step process was implemented, where only successful treatments from the first step were tested in the following step (Kempf and Wolf 1989; Korsten et al. 1995; Nautiyal 1997; Swadling and Jeffries 1996; Walker et al. 1998). The latter approach automatically eliminates other potential biocontrol agents whose mechanism(s) of action is (are) not useful in the first step. For example, when an in-vitro plate test is the first line of screening, it is generally designed to screen biocontrol agents with antibiotic activity (Burkhead et al. 1995) and eliminates those with resistance-inducing capacity that have no in-vitro antagonism against the pathogen. Another problem of in-vitro tests with culture media is the variability in production of antibiotics by microorganisms in different culture media. In our study, many differences were observed between the two types of culture media in the in-vitro tests. Clearly, a single culture medium may not be adequate in identifying certain kinds of antibiosis ability. Complementary in-vitro tests that would bring an insight into antibiotic production by bacteria include the use of cell-free and heat-killed extracts of their liquid cultures (Walker et al. 1998) or their culture-filtrate supernatant as in the present study. In our study, many of the bacteria that showed direct antagonism toward *P. infestans* on Petri dishes were also effective as bacterial-culture supernatant, which suggests possible antibiotic production by these bacteria. Many reports are available about antibiotic-producing microorganisms tested for biocontrol capacity (Behal 2000; Fravel 1988; Muninbazi and Bullerman 1998; Rothrock and Gottlieb 1984). However, only few antibiotics have been developed as commercial compounds in agriculture. Some of the reasons include their inconsistent efficacy in the field, their instability, and the costs related to their production (Jespers and De Waard 1993).

Although some bacteria (*Pseudomonas fluorescens* DF37, *B. pumilus* W1) were effective inhibitors of *P. infestans* in vitro, they provided no protection on detached leaves when applied as a bacterial suspension, nor did they have an effect on treated leaves when challenged with *P. infestans*. On the other hand, on detached leaves, their culture filtrates provided protection against *P. infestans*. *Bacillus pumilus* W1 induced 47% systemic protection, even though it did not provide any local protection against *P. infestans* on ei-

Table 3. Comparative protection levels provided by the most promising bacteria against *Phytophthora infestans* on culture media and detached leaves or whole plants of potato.

		Culture media (% inhibition*)		Detached leaves (protection rating†)		Whole plants (% protection‡)	
Isolate	Species	V8–PDA	CRA	Bacteria	Filtrate	Local	Systemic
B1	*Bacillus subtilis*	68	81	2	2	60	70
W2	*Rahnella aquatilis*	87	81	3	3	60	51
P1	*Pseudomonas putida*	54	51	2	3	53	73
J1	*Bacillus subtilis*	41	79	1	—	49	29
DF1	*Serratia plymuthica*	43	63	5	5	47	30
B3	*Bacillus subtilis*	60	76	1	—	47	43
M1	*Bacillus pumilus*	61	79	1	—	36	67
DF35	*Pseudomonas fluorescens* G	79	50	3	2	30	13
B2	*Bacillus pumilus*	55	77	2	2	27	17
DF37	*Pseudomonas fluorescens* F	82	49	1	3	27	20
C1	*Bacillus amyloliquefaciens*	74	89	1	—	27	23
Y1	*Bacillus pumilus*	59	86	1	—	27	33
DF3	*Pseudomonas viridilivida*	0	50	2	2	20	23
DF40	*Pseudomonas fluorescens* G	79	62	1	—	20	27
W1	*Bacillus pumilus*	60	85	1	3	17	47

Note: —, data not available.

*See [2] in Materials and methods for definition.

†Based on the percent inhibition of *P. infestans* provided by each bacterial treatment: 1, <10%; 2, 10–20%; 3, 21–40%; 4, 41–75%; 5, >75%.

‡See [3] and [4] in Materials and methods for definitions of local and systemic protection, respectively.

ther detached leaves or whole plants. Although no explanation can be suggested at this time, except that systemic protection was observed, it appears that culture filtrates of these bacteria were effective locally while bacterial suspensions were not. This lack of efficacy of bacteria in presence of the pathogen may suggest a possible antagonism of *P. infestans* against these bacteria. For example, bacterial suspensions of DF4, DF5, and DF8 were not effective against *P. infestans* on either plates or potato leaves, whereas their culture filtrates were very effective. This suggests the activity of possible secondary metabolites or staling products present in their culture filtrates. It also suggests that these bacteria are perhaps weak competitors when challenged with *P. infestans*, or that the latter has a mechanism to counteract their effects, i.e., antibiotic detoxification as previously described in potato (Desjardins and Gardner 1989) and other systems (Blount et al. 1992; Miao and Van Etten 1992; Mundodi et al. 2001).

Our results demonstrated differences among bacteria within tests with culture media, detached leaves, and whole plant, as well as between tests for each bacterium. There was no relation between inhibition levels on V8–PDA and those on CRA media. Similarly, no correlation was found between inhibition of *P. infestans* in vitro and that on detached potato leaves or on whole plants. In fact, most bacteria inhibited *P. infestans* to some degree, but almost every bacterial isolate showed a different combination of activity in the three tests. This might be explained by the fact that different types of biocontrol activity can be provided by the same bacterial strain.

For example, *B. subtilis* B1 and *Rahnella aquatilis* W2 both strongly inhibited *P. infestans* on CRA and V8–PDA and provided the best rates of local protection on whole plants and among the best rates of systemic protection. Antibiosis ability in vitro suggested production of antibiotic moieties. Nevertheless, the level of systemic protection also indicated that these bacteria could induce some level of plant defense response. This is supported by the relatively moderate protection (levels 2 and 3) obtained on detached leaves when using these bacteria (Table 3). In this case, we concluded that the level of protection obtained on a living plant is higher than the corresponding level obtained on detached leaves, which are only maintained in a "survival state" on Petri dishes. This is also supported by the results obtained with *Pseudomonas putida* P1, which provided relatively little inhibition of *P. infestans* on both CRA and V8–PDA and a moderate level of protection on detached leaves, but induced a high level of systemic protection on leaves away from treated leaves. This bacterium was also previously shown to induce systemic resistance in cucumber (Ongena et al. 1999, 2000). The moderate level of protection obtained on detached leaves might be due to the relatively poor antibiotic activity of this strain, as determined by in-vitro media studies, and the incomplete response to resistance induction that is found in detached-leaves tests in general. *Serratia plymuthica* DF1, on the other hand, is an example of a bacterium that can produce high levels of antibiotics, as evidenced by the in-vitro and the detached-leaves studies (Table 3), but that did not induce systemic protection.

Detached leaves appear to be useful to quickly confirm antibiotic activity previously shown in vitro. However, this method shows some limitations when the main mechanism of action in place is plant's induced resistance. This is probably due to the limited resources available to detached leaves maintained in Petri dishes. An alternative test would

be with tissue or cell cultures on media with appropriate nutrient sources. The tests based on whole plants, in contrast, are likely the most appropriate for screening of biocontrol agents, because they are the closest to natural conditions, and are more realistic when the biocontrol mechanisms of action need to be studied.

Many of the bacteria tested in vitro had strong inhibitory effects against *P. infestans*, especially on CRA. Those bacteria may be potential agents to control late blight, and our results suggest antibiosis to be a probable mode of action, especially as their culture supernatants were very effective in controlling late blight. These bacteria, along with those that showed a capacity to induce plant resistance, will be used in plant–pathogen biocontrol model systems for more in-depth studies of the biochemical and molecular mechanisms of phytoprotection.

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