BIOLOGICAL CONTROL OF BACTERIAL WILT OF POTATOES CAUSED BY *PSEUDOMONAS SOLANACEARUM*[1]

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Abstract

Microbial antagonism was investigated as a possible method for control of bacterial wilt of potatoes (*Solanum tuberosum* L.), caused by *Pseudomonas solanacearum*. Seed potato tubers were treated with a selected antagonistic bacterial isolate and coated with $CaCO_3$. An amendment was also prepared, containing the same bacterial isolate. The bacterium used as a biological control and designated as isolate BC8 caused strong inhibition of *P. solanacearum* in both "in vitro" assays and growth cabinet conditions. In order to test the antagonistic capacity of isolate BC8 under field conditions, a completely randomized design was established in a soil naturally infested with *P. solanacearum* that included 12 treatments repeated 10 times, each one with 5 replications. The field experiment was planted on November 26, 1986 and the rate of wilt symptoms for each treatment was recorded periodically. Three tubers from each plant in each treatment were assayed for either presence of *P. solanacearum* or the antagonistic isolate BC8. Treatments that included isolate BC8 gave the lowest amount of wilted plants and fewest tubers latently infected as well. Using this system, about 80% of the tubers assayed from plants growing in the naturally infested soil were colonized by the antagonistic isolate BC8 and free of *P. solanacearum*. It was established that the pathogen was still present in the soil after 2 years of non-potato cropping and that latent infections play an important role in the dispersal of *P. solanacearum*. The system of delivering bacteria in an amendment, as used in this work, proved to be much more efficient in the biological control of *P. solanacearum* than just coating the seed potato tubers with the antagonistic isolate BC8.

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[1]Joint Research Project between the Universidad Austral de Chile (Valdivia) and the Instituto de Investigaciones Agropecuarias (La Platina, Santiago). Funds for this study were provided by a Grant from the Asociacion Nacional de Productores de Semillas (ANPROS-Santiago).
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Accepted for publication February 8, 1989.
ADDITIONAL KEY WORDS: Chile, Race 3, strain BC8.

Compendio

Antagonismo microbiano fue investigado como posible método para controlar la marchitez bacteriana en papas (*Solanum tuberosum* L.), causada por *Pseudomonas solanacearum.* Tubérculos semillas de papas fueron tratados con una cepa antagonista bacteriana seleccionada y cubiertos con $CaCO_3$. También se preparó una enmienda la cual incluía la misma cepa. La bacteria utilizada como control biológico y designada como cepa BC8 indujo una fuerte inhibición de *P. solanacearum* tanto en ensayos "in vitro" como en pruebas en cámaras climáticas. Para comprobar la capacidad antagonista de la cepa BC8, se estableció un ensayo de campo completamente al azar el cual incluyó 12 tratamientos con 10 repeticiones y cada uno con 5 replicaciones. El ensayo se sembró el 26 de Noviembre de 1986 y periódicamente se tomaron notas sobre el desarrollo de marchitez en cada tratamiento. Tres tubérculos por planta en cada tratamiento fueron analizados tanto para presencia de *P. solanacearum* como cepa BC8. Los tratamientos que incluyeron la cepa BC8 demostraron la menor incidencia de marchitez y la menor cantidad de infecciones latentes en tubérculos. Utilizando este sitema alrededor del 80% de los tubérculos analizados a partir de plantas en el suelo infestado en forma natural, estaban colonizadas con la cepa BC8 y libres de *P. solanacearum.* Se estableció que el patógeno estaba aun presente en el suelo despues de 2 años de ausencia de siembra de papa y que las infecciones latentes juegan un rol importante en la diseminación de *P. solanacearum.* El sitema de esparcir bacterias en una enmienda tal como se uso en este trabajo, fue mucho mas eficiente para controlar biológicamente a *P. solanacearum* que simplemente peletizar los tubérculos con la cepa antagonista BC8.

Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* E.F. Smith, is one of the most important bacterial diseases of plants and affects mainly species of the *Solanaceae* family. Economic crops such as potato, tomato, tobacco, green peppers, and eggplant are severely affected in many countries. The disease is widespread in potatoes throughout Central and South America where Race 3 is the most commonly found variant, particularly in the moderately cool highlands. Under field conditions, isolates of *P. solanacearum* are present as races that show temperature adaptation and host specificity (3). Because of the many plant species and geographic regions affected, bacterial dissemination through latent infections, and the persistence of the pathogen in the soil, this disease is considered among the limiting factors in potato production in the world tropical belt (17).

P. solanacearum was recently detected for the first time in Chile (9, 12). The original presence of Race 3 strains in potatoes stored in the Metropolitan Region, and the subsequent detection of the pathogen affecting potato plants growing in the V, VI, and VII Regions (1), located in the Central

Valley, indicate the fast spread of the pathogen. This represents a potential and real menace to the Southern Regions of the country where the certified seed area is located and where most potatoes are produced.

The first breeding program for resistance in potatoes to *P. solanacearum* started at the University of Wisconsin in association with the International Potato Center (Peru). Resistance to bacterial wilt is influenced by a complex of pathogen-host-environment interactions (17). A major problem in temperate regions is the cool adapted Race 3, some of which strains latently infect potato tubers. Under these particular conditions, commercial cultivars of *Solanum tuberosum* are very susceptible, especially when pathogen populations burst inside plants growing under field temperatures close to 28 C (10).

Unsuccessful efforts have been made through the years to control bacterial wilt, *e.g.*, crop rotation, or use of certified seed and the release of resistant varieties. More recently, the manipulation of soil microorganisms as biological agents of control of plant pathogens has become a very active research field. Investigations suggest that under particular conditions some soil bacteria produce siderophores that chelate iron and make it unavailable for plant pathogens (20, 21). Some substrates as sources of potential bacterial antagonists to control plant pathogens have been used, including soil organic matter (25), and periderm (13) stem, daughter tubers (27), and roots of potatoes (6).

We reported early (7), the finding of several bacterial strains that "in vitro" caused strong inhibition of the Chilean Race 3 of *P. solanacearum.* A particular strain designated as BC8 was selected because it maintained an excellent inhibition to the wilt bacterium in controlled experiments conducted under growth chamber conditions (5).

The primary objective of this research was to explore the possibility of controlling bacterial wilt of potatoes in a naturally infested soil by means of bacterial antagonism. The plan was to determine whether delivering an antagonistic bacterial isolate in an amendment or on the surface of potato tubers could reduce wilt in potato plants and latent infection in daughter tubers.

Material and Methods

Source of Bacterial Strains. To study the inhibition of bacterial wilt on potato plants growing in a naturally infested soil, an antagonistic strain of *P. solanacearum* was used (isolate BC8), originally isolated from potato and tested under "in vitro" and growth chamber conditions in experiments that were previously reported (5, 7). Isolate BC8 was used for coating potato tubers as well as delivered in a especially prepared amendment. For the seed tuber treatments used as controls, and adult plant inoculations, a Chilean isolate of *P. solanacearum* (Race 3) was used. This isolate was originally obtained from diseased potato tubers stored in the Metropolitan Region (9).

Preparation of bacterial inoculants. The bacterial suspensions of isolate BC8 for both seed potato tuber coating and amendment were prepared as follows: 800ml of CNE broth (26) were inoculated with a suspension of the BC8 strain and incubated in a Lab-Line Environmental shaker at 120 oscillations per min. After 18 h of growth the broth was centrifuged in 250 ml bottles at 8,000 rpm for 15 min in a IEC refrigerated centrifuge Model B-20A using a model 872 rotor. The bacterial sediment was resuspended in a 1:5 dilution of CNE broth. The suspension of *P. solanacearum* was prepared from casamino-acid peptone glucose agar plates (CPG) (26) incubated at 28 C for 48 h. After this period, 10 ml of 1:5 diluted CPG broth is added to each plate, mixed and the bacterial suspension is placed in sterile test tubes with a Pasteur pipette. For inoculations of the treatments listed in Table 1, 1.5 l of bacterial suspension of *P. solanacearum* and isolate BC8 were used.

Preparation of soil amendment. From the area of Pelchuquin, located 40 km north of Valdivia, a high organic soil (pH 4.5) was collected and transported to our laboratory. This material was steamed in wooden flats at 100 C for 1 h, then placed and sealed inside black plastic bags. Each bag contained 2700g of soil and 300g of $CaCO_3$ (final pH 6.5). A 50 ml syringe was used to add 100 ml of bacterial suspension of isolate BC8 by puncturing

TABLE 1. — *Treatments used in the field experiment to test the ability of isolate BC8 to biologically control* Pseudomonas solanacearum, *conducted in a naturally infested soil of a farm located in Lonquen, Metropolitan Region (Santiago, Chile).*[1]

Treatment	Presence of:		Delivery system of bacteria
	Isolate BC8	*P. solanacearum*	
T 1	+	−	Pellet[2]
T 2*	−	−	Pellet
T 3	+	−	Amendment[3]
T 4*	−	−	Amendment
T 5	+	+	Pellet
T 6	−	+	Pellet
T 7	+	+	Amendment
T 8	−	+	Amendment
T 9	+	Stem[4]	Pellet
T10	−	Stem	Pellet
T11	+	Stem	Amendment
T12	−	Stem	Amendment

* = Non-inoculated controls (T2 and T4)
1 = Every treatment with 10 replications (5 repetitions each)
2 = Bacteria delivered around the seed tubers
3 = Amendment added to the furrow below the seed tuber
4 = Field stem inoculation of adult potato plants (T9 to T12)

the sealed bags that finally contained a concentration of 1×10^8 CFU of bacteria/g of soil. The addition of *P. solanacearum* suspension to positive control bags was performed in the same manner and in a similar concentration. Negative control bags were inoculated with sterile water only. This procedure was used for preparation of treatments 3, 4, 7, 8, 11 and 12 (Table 1). The final humidity in each bag, after the bacterial suspensions were added was 32%. The bags were prepared two days in advance of the potato field experiment.

Tuber pelletization. Potato tubers of cv. "Corahila" (*Solanum tuberosum* L.) were obtained from the Remehue-Inia Experimental Station located in Osorno. Six hundred tubers were hand washed with tap water and surface disinfected during 5 min in a 5% solution of commercial sodium hypoclorite, and then washed in distilled water. The tubers were air dried and wrapped with sterile paper towels that were kept inside paper bags for pelletization. Equal volumes (150 ml) of arabic gum free of inhibitors, and the bacterial suspension were mixed inside a cylinder and added to a sterile tray. The tubers of each treatment were put on the tray and hand mixed with the suspension, left to air dry and finally coated with $CaCO_3$. This procedure was used for preparation of treatments 1, 2, 5, 6, 9, and 10 (Table 1). The final concentration of both isolate BC8 and *P. solanacearum* was 2×10^9/CFU/ml and 1×10^9/CFU/ml respectively on the surface of each treated and coated tuber. Tuber pelletization was conducted two days in advance of the potato field experiment.

Field experiment. The field experiment designed to test the ability of isolate BC8 to biologically control *P. solanacearum* was a completely randomized design with 12 treatments, 10 repetitions, each one with 5 replications. A total of 300 pelletized tubers was used and 300 planted along with the amendment. Figure 1 illustrates both delivery systems and Table 1 indicates the 12 different treatments used. Treatments 9 to 12 were used for stem inoculation of adult plants with *P. solanacearum* about 30 days after the planting date, using materials and methods described by Ciampi (11).

The field experiment was established on November 26th, 1986, on a farm whose soil was naturally infested with *P. solanacearum.* This field was especially designated for this experiment by the Servicio Agricola y Ganadero (Santiago, Chile). The farm is located in Lonquén (Metropolitan Region), a farming area close to the capital, Santiago. Inspections to detect wilt field symptom development in growing potato plants, were conducted on December 29, 1986; January 6, 14, and 29, February 11, and 28, 1987. The harvest of potato tubers from the field experiment was conducted on March 17, 1987. For this purpose the foliage of each individual plant was cut, and tuber harvest was conducted plant by plant. The number of tubers per plant was recorded and 3 tubers per plant, randomly selected, were used for detection of latent infections caused by *P. solanacearum* and presence of antagonistic isolate BC8 as well. These determinations were conducted during the

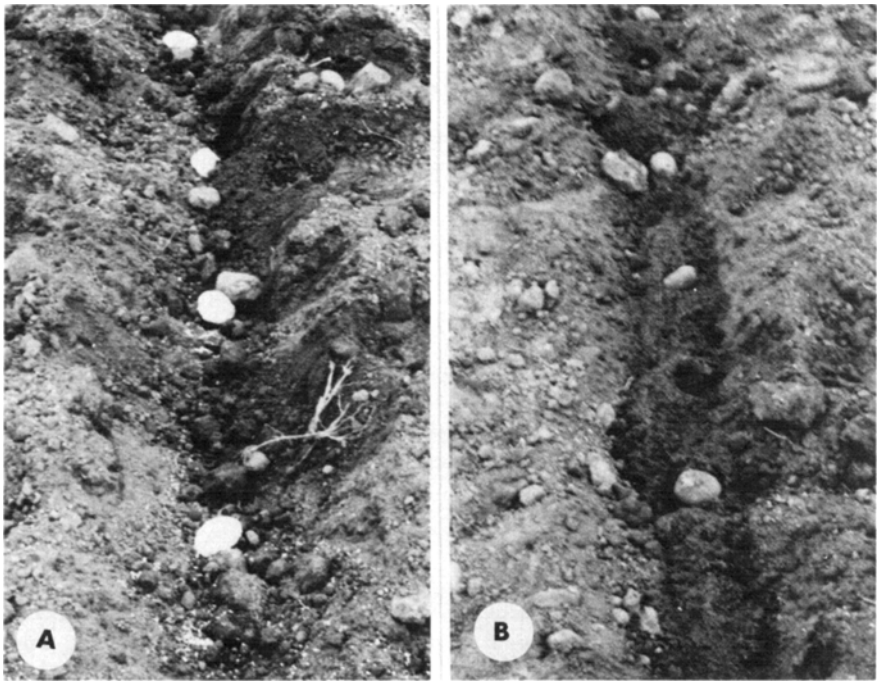

FIG. 1. Pictures showing the delivery systems of bacterial isolate BC8 antagonistic *P. solanacearum*. Left (A), seed potato tubers coated with a bacterial suspension and covered with $CaCO_3$. Right (B), amendment containing the suspension of isolate BC8 placed in the furrow and potato tubers on top.

months of March and April, 1987, according to material and methods described by Ciampi (8) and Bustamante (5). Some of the field and laboratory results were statistically analyzed using SPSS (Statistical Package for Social Sciences) through the main computer of the Austral University of Chile (Valdivia).

Results

1) Field observations of wilt development and symptoms in growing potato plants. Results of wilt development in potato plants growing in the naturally infested soil of a farm are presented in Table 2. According with the last observation conducted on February 2nd, 1987 (94 days after planting) 177 potato plants with wilt symptoms were detected among the 12 treatments.

Treatments 1 and 3 (Table 2), reflect the response of potato plants protected by isolate BC8 to infection by *P. solanacearum*. In these treatments only 3 and 2 wilted plants were found, respectively, in which isolate BC8 was

TABLE 2. — *Field development of wilt symptoms on potato plants growing in a* P. solanacearum *naturally infested soil in the locality of Lonquen, Metropolitan Region (Santiago, Chile).*[1]

		Date of observations and number of plants wilted[2]:						
Treatments	Population	12.29.86 (33)	01.06.87 (41)	01.14.87 (49)	01.29.87 (64)	11.02.87 (77)	02.28.87 (94)	TTTD[3]
T 1	50	0	0	0	0	0	3	10.6 b
T 2	48	0	0	0	0	0	10	27.0 b
T 3	43	0	0	0	0	1	2	6.5 b
T 4	43	0	0	0	1	1	5	12.6 b
T 5	49	21	25	28	38	39	41	71.2a
T 6	49	12	17	23	41	44	44	76.8a
T 7	42	2	2	5	12	12	12	26.6 b
T 8	36	6	9	15	22	23	27	64.8a
T 9	50	1	1	1	1	1	5	14.5 b
T10	49	0	0	0	0	0	9	17.1 b
T11	43	0	1	1	2	2	8	15.3 b
T12	46	0	0	0	2	5	11	23.5 b
Total	548	42	55	73	119	128	177	

1 = Treatments T1 to T12 accordingly to Table 1.
2 = In parentheses and below each date the number of days after planting.
3 = Tukey Test for Tranformed Data of 02.28.87 (D.H.S. 1% = 35.0).

delivered in pelletized tubers (T1) and in the amendment (T3). Treatments 2 and 4 reflect the response of potato plants to natural infection. In both cases there was no inoculation of isolate BC8, and tubers were coated with $CaCO_3$ alone (T2) and amendment alone (T4). The number of wilted plants in these treatments was 10 (T2) and 5 (T4), higher than the protected treatments T1 and T3.

Treatments 5 and 7 (Table 2), show the response of potato plants to joint artificial inoculation of both isolate BC8 and *P. solanacearum.* On the other hand, treatments 6 and 8 show the drastic effect of *P. solanacearum* when delivered alone, either in the surface of coated tubers (T6) or amendment (T8). In this group of four treatments, the lowest number of wilt symptoms was found in T7 with 12 plants and the highest in T6 with 44 plants.

Treatments 9 and 11 (Table 2) show the response of stem inoculation of adult plants with *P. solanacearum*; these plants were treated with isolate BC8 as a pellet in T9 and amendment in T11. In both cases there were few plants with wilt symptoms (5 and 8 respectively). On the other hand, treatments 10 and 12 show the effect of stem inoculation on plants growing from pelletized tubers with $CaCO_3$ alone (T10) and on amendment alone (T12). The numbers of wilted plants found in both treatments was low as well (9 and 11 respectively).

The analysis of variance of data taken from the last field observation conducted on 02.28.87 and included in Table 2, is presented in Table 3. Figures show that there is no variation inside the treatments but, there are statistical differences among the 12 treatments conducted in the field test.

2) Detection of P. solanacearum *and antagonistic isolate BC8 inside potato tubers.* About 1,500 potato tubers harvested from treatments 1 to 12 were assayed for either presence of *P. solanacearum* and isolate BC8. The results of these determinations were presented in Table 4. Treatments 1 and 3 representing isolate BC8 delivered in the surface of pelletized tubers and in the amendment yielded 37 and 15 tubers infected by *P. solanacearum,* and 68 and 103 tubers

TABLE 3. — *Analysis of variance of wilt symptoms in potato plants 94 days after planting growing in a* Pseudomonas solanacearum *naturally infested soil. Data from Table 2.*

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F value
Treatments[1]	11	70,084.9	6.371,4	15,32**
Experimental Error	108	44,914.7	415,9	
Total	119	114,999.6		

[1] = Treatments 1 to 12 accordingly to Table 1.
**Significant at 1% level.

TABLE 4. — *Number of tubers with isolate BC8 and* Pseudomonas solanacearum *isolated from the interior of healthy appearing potato tubers harvested from plants growing in a naturally infested soil.*[1]

Treatment	Tubers Analized	Presence of:[2]				TTTD[3]
		P. solanacearum		Isolate BC8		
	No.	No.	%	No.	%	
T 1[4]	143	37	25.9	68	47.6	30.0 fg
T 2	143	68	47.6	0	0.0	43.8 cde
T 3	128	15	11.7	103	80.5	19.2 g
T 4	127	59	46.5	2	1.6	43.0 cde
T 5	105	77	73.3	16	15.2	58.4 b
T 6	107	94	87.8	0	0.0	71.8a
T 7	112	27	24.1	76	67.9	29.7 fg
T 8	75	70	93.3	0	0.0	81.4a
T 9	146	47	32.2	63	43.1	34.3 ef
T10	143	86	60.1	8	5.6	51.4 bcd
T11	133	28	21.0	93	69.9	26.6 fg
T12	137	81	59.1	8	5.8	50.4 bcd
Total	1,499	689	45.96	437	29.16	

[1] = Three tubers per plant were assayed for each treatment.
[2] = Tubers that yielded no presence of either isolate BC8 nor *P. solanacearum* are not included in this table.
[3] = Tuckey Test for Tranformed Data for presence of *P. solanacearum* only (DHS 1% = 13.2).
[4] = T1 to T12 accordingly to Table 1.

colonized by isolate BC8, respectively. The lowest latent infection incidence by *P. solanacearum* was found in T3 (11.7%) and the highest colonization by isolate BC8 was also detected in T3 (80.5%).

Treatments 2 and 4 (Table 4) had high amounts of latent infections by the pathogen (47.6 and 46.5% respectively) and the lowest colonization of isolate BC8 (0 and 1.6% respectively). In these treatments no inoculations were made with antagonistic isolate BC8 and so reflect the natural presence and infection capacity of *P. solanacearum* present in the soil.

Treatments 5 and 7 (Table 4) show on daughter tubers the effect of joint inoculation of *P. solanacearum* and antagonistic isolate BC8 either in pellet (T5) or amendment (T7). In these treatments, 73.3 and 24.1 tubers latently infected, and 15.2 and 67.9 tubers colonized by isolate BC8 were detected. On the other hand, inoculated controls of *P. solanacearum* delivered alone in pellet (T6) or amendment (T8) yielded no presence of isolate BC8. Both treatments showed the highest amount of tubers latently infected (87.8 and 93.3% respectively).

Results obtained from treatments 9 to 12 in Table 4, reflect stem inoculation of adult potato plants with *P. solanacearum*, for positive control

purposes. In these treatments isolate BC8 was delivered as amendment or pellet (T9 and T11). Treatments 10 and 12 represent stem inoculation alone. In this group of four treatments *P. solanacearum* was high in treatment 10 and 12 (60.1 and 59.1% respectively), and colonization of isolate BC8 was high in treatments 9 and 11 (43.1 and 69.9 respectively).

Analysis of variance performed with data presented in Table 4 is shown in Table 5. Figures indicate that there are statistical differences among the 12 treatments.

TABLE 5. — *Analysis of variance performed between tubers latently infected with* Pseudomonas solanacearum *and tubers protected with isolate BC8. Data from Table 4.*

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F value
Treatments[1]	11	38,779.6	3,525.4	59.8**
Experimental Error	108	6,370.5	59.0	
Total	119	45,150.1		

[1] = Treatments 1 to 12 accordingly to Table 1.
**Significant at 1% level.

3) Relation between field wilt symptoms and latently infected tubers with P. solanacearum. The amount of plants wilted under field conditions and the amount of latently infected tubers with *P. solanacearum* found for each treatment are shown in Figure 2. Data were obtained from Table 2 (observation of 02.28.87) and Table 4 (presence of *P. solanacearum*). The number of wilted plants in all the treatments is lower than the amount of latently infected tubers. In the first four treatments a small amount of wilted plants was observed but the number of latently infected tubers was very high in T2 and T4, both non-inoculated treatments used as natural controls (Table 1). This is an indication that the pathogen is still present in the soil and can readily infect plants and may become established inside potato tubers.

Isolate BC8 when delivered through a pelletization process (T1, T5 and T9) yielded higher number of both wilted plants and infected tubers; on the contrary, when this isolate is delivered in an amendment (T3, T7 and T11), the number of wilted plants and infected tubers is low. This is a clear indication that coating tubers is not as efficient as delivering the biocontrol agent with an amendment.

The relationship between wilted plants in the field and latent infections in daughter tubers is also possible to observe in Figure 2. When the number of wilted plants is high (T5, T6, T8, T10 and T12) the number of latently

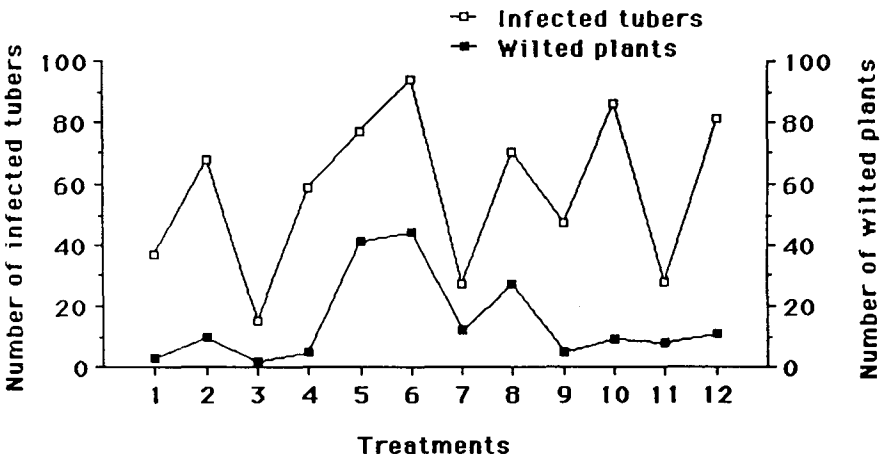

FIG. 2. Number of potato plants wilted under field conditions and number of infected tubers in each treatment. Plants wilted as detected 94 days after planting (Table 2). Number of tubers infected as detected for *Pseudomonas solanacearum* (Table 4).

infected tubers is also high. In these treatments, *P. solanacearum* was artificially inoculated. Also, it is possible to observe that the number of plants growing in treatments used as controls showed a relatively high number that wilted (T2, T4); and that the number of latently infected tubers is lower than the artificially inoculated treatments. These facts show that *P. solanacearum* is well established in these soils and that latent infections occur naturally.

When the number of wilted plants is lower such as in treatments 3, 7 and 11, the number of latent infections is also lower (Figure 2). Isolate BC8 was used as a protective amendment against infection in these treatments. The best results were obtained in T3, the treatment that delivered isolate BC8 along with the amendment. This is shown by fewer plants with wilt symptoms in the field (2), and by the lowest number of latently infected tubers with *P. solanacearum* (15).

4) Efficiency of antagonistic isolate BC8 to protect plants and tubers from infection by P. solanacearum. The number of tubers latently infected with *P. solanacearum* and the number of tubers protected with isolate BC8 for each treatment are shown in Figure 3. Data to compose this figure were obtained from Table 4. It is possible to observe that when isolate BC8 is present in many tubers such as in treatments 1, 3, 7, 9 and 11, the number of tubers with presence of *P. solanacearum* is low. On the other hand, when *P. solanacearum* is present in many tubers such as in treatments 1, 4, 5, 6, 8, 10, 11 and 12, the number of tubers with isolate BC8 is low. Regression analysis between the amount of tubers infected with *P. solanacearum* and isolate BC8 was made and is shown in Figure 4. The coefficient of regression ($r = 0.92$),

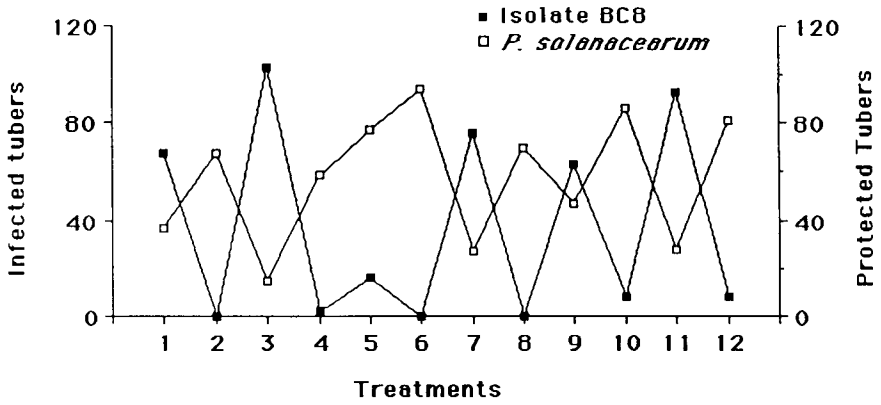

FIG. 3. Numbers of tubers assayed for each treatment either with isolate BC8 (protected tubers) and with *Pseudomonas solanacearum* (infected tubers). Data obtained from Table 4.

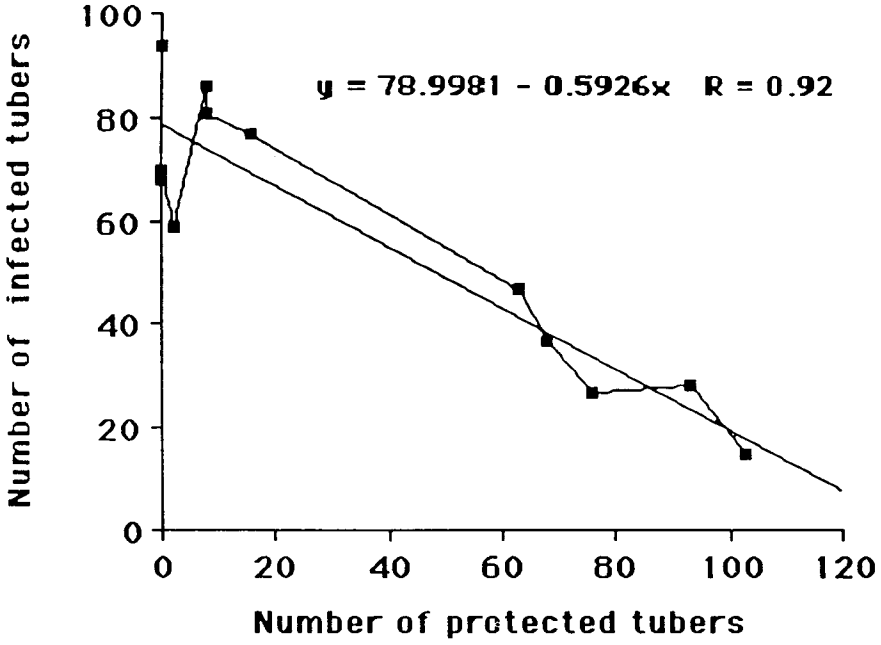

FIG. 4. Regression analysis between the number of tubers protected with isolate BC8, and the number of latently infected tubers with *Pseudomonas solanacearum*. Data obtained from Table 4.

indicates a high relation between the increasing number of tubers with presence of isolate BC8 and the decreasing amount of tubers with *P. solanacearum.* The larger the number of tubers colonized by isolate BC8 the lower the number of tubers infected with *P. solanacearum.*

Discussion

The most challenging step in any biotechnological application is field success, as well as to solve a practical agricultural problem especially when previous experiments are encouraging. In many cases "in vitro" and semi-controlled assays are most convincing, and will keep the research open but, as many end in failure under field conditions, this will mean the end of the project. Detection of antibiosis on agar media provides an initial screening for the search of antibiotic-like compounds producing bacteria, but does not mean that antibiosis will necessarily occur in the soil environment (2). Field experiments are the last instance in which the plant pathogen and the antagonistic microorganism must be tested for any biological control system.

Our research was originated from a solid background. First, during the study of antagonistic-pathogen interactions, "in vitro" experiments provided evidence of a strong inhibition of *P. solanacearum* using a local isolate designated as BC8 (7). Second, a set of experiments was conducted in growth cabinets and the inhibition of the pathogen was maintained. The work was conducted using sterilized soil and potato seedlings kept at 28 C, conditions favorable for both *P. solanacearum* and antagonistic isolate BC8 (5). Third, represented by the field test, is the practical approach of our system and is the purpose of this report whose results are discussed here.

Our results are very encouraging since incidence of bacterial wilt of potatoes was significantly reduced in a naturally infested soil (T3 in Table 4). This was achieved by the addition to the soil of an amendment containing high populations of the antagonistic isolate BC8. This is the first report of *P. solanacearum* (Race 3) suppression in potato plants growing in naturally infested soil, as well as inhibition of latent infections in daughter tubers.

Field trials, such the one used in this research in which root inoculation occurs naturally, have been recommended for testing antagonistic bacteria to protect potato plants from *P. solanacearum.* Kempe and Sequeira (19) were able to obtain significant reduction of disease severity and incidence by treating the tubers with antagonistic bacteria. Their results, as well as ours, indicate that even under conditions highly favorable for disease progress, protecting potato tubers with antagonistic bacteria may result in a significant reduction in severity of bacterial wilt.

The mechanism of inhibition of *P. solanacearum* by isolate BC8, may be explained through several reports and a recent review (20). Siderophores have been pointed out as responsible for detrimental effects on pathogen populations. Some plant-related bacteria produce the siderophore pseudobactin,

which can efficiently complex iron in soils making it unavailable to pathogens, thus inhibiting their growth. However, we suggested earlier that probably pyocin-like substances may be responsible for this action as well (7).

Potato plants growing in the presence of isolate BC8 and that later were stem inoculated with *P. solanacearum* (T11 in Table 4), showed a substantial reduction of latent infections. This fact indicates that isolate BC8 is responsible for the decline of the pathogen in the soil and it protects potato plants from root infection. However, an interesting view must be pointed out in relation to the interaction between *P. solanacearum* and our isolate BC8. How and why is a pathogen recently introduced to Chile (9) affected by a local bacterial isolate (7)? Most plant pathologists working in biological control of any disease, will search for potential antagonists where the disease has been present for an extended period of time. Also, it is recommended that the antagonistic bacteria be used in the same area where obtained.

The antagonistic isolate BC8, obtained from potato in Southern Chile (7), is able to survive in soils of the Metropolitan Region that are quite different from those commonly found in Southern regions of the country and is still able to protect potato plants from infection by *P. solanacearum.* This saprophytic adaptation, which is a distinct feature and commonly found in soil bacteria, makes possible the survival of isolate BC8, thanks to the organic compounds already present in the new soil environment and probably ones present in the amendment as well.

From evolutionary and ecological aspects, both *P. solanacearum* and isolate BC8 were not sharing the same habitat and environment, nevertheless latent infections are being repressed. It is possible that a good antagonist may be found in "novel" locations, distant from where the pathogen is present. In our case, this non sharing habitat is more important to the repressor or antagonistic microorganism since its decline or low numbers in the soil would favor the pathogen. This effect can be interpreted from T5 (Table 4), where *P. solanacearum* and isolate BC8 were placed together in coated tubers, a system that did not provide efficient protection to the whole rhizosphere. On the other hand, T2 which reflects the natural soil infection by *P. solanacearum,* yielded 47.6% of the tubers latently infected. This natural infection was reduced to 11.7% (T3), evidence that supports the testing of organic amendments as delivery systems for bacterial antagonists in future field experiments.

The presence of isolate BC8 inside potato tubers may be indicating that there is more than an affinity between isolate BC8 and *S. tuberosum.* Perhaps this isolate can penetrate the plant from the soil via root system to become established in potato tubers and therefore protect plants from infection of *P. solanacearum,* thus reducing the number of latently infected tubers. This induced resistance remains clear in T3 (Table 4) that showed the presence of isolate BC8 in 80.5% of the tubers assayed. On the other hand, in those treatments where isolate BC8 was not delivered, its presence in tubers was

very low or undetectable (T2, T4, T6, and T8 in Table 4). Our results also show that isolate BC8 does not occur naturally in soils of the Metropolitan Region, but when present, can penetrate roots and colonize potato plants.

The system of amendment (T3 in Table 4), containing isolate BC8 was far more effective than when this isolate is delivered in coated tubers (T1). These results indicate clearly that: a) movement of isolate BC8 from the coated mother tuber to the rhizosphere is slow or scant under the conditions prevalent during this field test; and b) that the antagonistic agent delivered in the amendment to the soil, protected the rhizosphere from infection by naturally occurring isolates of *P. solanacearum* already present in the soil.

Periodic wilt disease outbreaks have occurred where the initially low disease levels in a region build up to epiphytotic proportions and expand to adjacent areas (4). Latently infected potato tubers play an important role in the dissemination of *P. solanacearum* (10, 11). Probably this means of disease transmission was responsible for the introduction of the pathogen to Chile. The epidemiological effects and extreme importance of latent infections can be deduced in T2 (Table 4). This treatment reflects the natural soil conditions and it remains absolutely clear that *P. solanacearum* survives well in the soils of the Metropolitan Region, a new micro-environment for the pathogen. Healthy potato tubers grown in infested soils, such as the one used for this experiment, yielded a high number of latently infected daughter tubers, which were responsible for disease transmission.

Tubers planted in a naturally infected soil with *P. solanacearum* will show few plants with wilt symptoms but close to 50% of the tubers obtained from these plants will be latently infected. Therefore, for the situation occurring in these soils, detection of latent infections is more important than observation of bacterial wilt in growing potato plants. Studies show that Race 1 of *P. solanacearum* can become established in soils of Costa Rica and that the inoculum level is maintained for several years, even in the absence of susceptible crops (18).

The farm where this field experiment was conducted had records of bacterial wilt on potatoes 2 years before and, in the interim, no potato or tomato plants were allowed to grow. However, isolates of *P. solanacearum* were still present, and readily infected potato plants and tubers. This is not surprising since most pseudomonads, especially plant pathogens, have the capability to survive as saprophytes in the absence of the host and can utilize a vast variety of naturally occurring organic compounds as energy and carbon sources. Races 1 and 3 of *P. solanacearum* survive in many soils without a host and have evolved sufficient survival mechanisms to persist in compatible soils (22).

The mechanism of soil survival of *P. solanacearum* has been studied in several occasions and it is difficult to understand and interpret (15, 18, 24). Probably soil populations of *P. solanacearum*, in absence of the target hosts,

will decrease to low numbers and still be able to survive for years and readily penetrate through the roots when the proper host is again present (22). In the interior of the potato plant, *P. solanacearum* will increase in numbers and induce latent infections in potato tubers (10). Epidemiological facts were determined by our results.

The inhibitory characteristics of soils suppressive to *P. solanacearum* have been studied and reported in previous years (7, 16, 22). These kinds of soils represent a valuable source of research in order to detect soil microorganisms usable as biological control agents of bacterial wilt. In compatible soils, *P. solanacearum* Race 1 multiplies and levels off at about 10^3 CFU/gr, although decline is difficult, and probably the pathogen enters a resting phase (22). Our data indicate that the pathogen was present in the soil in sufficient amounts to induce bacterial wilt in plants and latent infections in tubers; and that these compatible soils become suppressive or incompatible, with the addition of the amendment containing isolate BC8.

From our results it is evident that isolate BC8 did not provide 100% bacterial wilt inhibition to plants nor latent infection protection to all daughter tubers. More testing must be encouraged and supported. Future field work must include a system in which isolate BC8 is delivered in larger concentrations in the amendment. Also, it is important to distribute this product more evenly in the soil in order to cover and include all the rhizosphere of the growing potato plant. The antagonistic bacteria could also be delivered in high concentrations directly to the infested soil either as a freeze-dry product or wet suspension. Whichever approach is chosen, experimentation must continue because there are increasing hopes of biological control *P. solanacearum* in recently infested soils in Chile and eventually to stop the spread of the pathogen to other potato producing areas of the country.

Acknowledgment

We are very grateful for the technical assistance of Mr. Gaston Vergara, Statistician.

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