Mutation in the *avrBs1* Avirulence Gene of *Xanthomonas campestris* pv. *vesicatoria* Influences Survival of the Bacterium in Soil and Detached Leaf Tissue

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ABSTRACT

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The role of the *avrBs1* avirulence gene of *Xanthomonas campestris* pv. *vesicatoria* in survival of the bacterium was investigated by testing two strains that differ in the structure and function of the gene in 37 different soil types of Barbados and detached leaf tissue of four pepper genotypes. One strain carried a mutation in the *avrBs1* gene and lacked avirulence activity, while the other expressed wild-type *avrBs1* activity. In 30 to 32

soil types and all leaf tissue tested, the mutant strain persisted longer and more abundantly than the wild-type strain over a 2- to 6-week period. During this time, the mutant strain generally replaced the wild-type strain completely in soil initially infested with a mixture of equal amounts of each strain and by a factor of 6.7 in similarly infested pepper leaves. Nine selected soil factors, namely pH, clay and cation content, and percent nitrogen, carbonates, carbon, K⁺, Na⁺, and Ca²⁺ did not affect bacterial survival significantly.

Additional keywords: bacterial spot of pepper.

The function of pathogen avirulence was originally defined in the context of the gene-for-gene hypothesis (10,11), which is commonly expressed as the requirement of an avirulence gene for inducing the incompatible response in a host plant carrying a corresponding disease-resistance gene (7,8). However, recent reports indicate that pathogen avirulence genes may confer selective value. The first definitive study of this nature reported the requirement of an avirulence gene from *Xanthomonas campestris* pv. *vesicatoria*, the bacterial spot pathogen of pepper and tomato, for full expression of virulence on a compatible pepper genotype (20). Avirulence genes from a number of other phytopathogenic bacteria have been reported to enhance virulence on compatible hosts (6).

A recent study has indicated that avirulence genes may also influence pathogen survival in the saprophytic stage of disease cycles. Ward (37) reported that pepper race 1 and tomato race 1 strains of *X. campestris* pv. *vesicatoria* predominated in pepper and tomato field soils in Barbados, despite many instances of higher levels of bacterial spot infection by pepper-tomato race 2 and pepper-tomato race 3 pathotypes. Since inheritance of avirulence genes is the basis of race classification of *X. campestris* pv. *vesicatoria* (17,24), it is possible that avirulence gene(s) of race 1 strains are associated with survival of the bacterium in the soil phase of its life cycle.

X. campestris pv. *vesicatoria* is suitable for investigating the selective value of avirulence genes in survival of the bacterium in soil. Interactions between single dominant genes have been used to classify the bacterium into races or pathotypes of which pepper race 1 and pepper-tomato races 2 and 3 are the most common (24, 25,38). Disease-resistance genes *Bs1*, *Bs2*, and *Bs3* restrict races of the pathogen carrying the *avrBs1*, *avrBs2*, and *avrBs3* avirulence genes, respectively. Genes *Bs3* and *Bs1* confer resistance to pepper race 1 and pepper-tomato race 2, respectively, while gene

Bs2 restricts these races, as well as pepper-tomato race 3. Loss of avirulence gene activity can occur by insertional inactivation by a transposable element (19), loss of plasmid, or a base-pair mutation (24), enabling the bacterium to overcome host resistance.

In addition to a well-defined physiological race structure, *X. campestris* pv. *vesicatoria* has a significant existence in soil, particularly when associated with crop debris (5,13). Bacterial spot of pepper is typically initiated in the field under hot and humid conditions after infested soil and plant debris are splashed by wind or rain onto plants. Following a phase of bacterial growth and multiplication on leaves, fruits, blossoms, or stems, the bacterium may be returned to soil by defoliation, blossom and fruit drop, or plowing fields of infested crop plants. Bashan and Okon (5) reported that *X. campestris* pv. *vesicatoria* is capable of surviving in artificially infested field soil for at least 18 months in the absence of host plants. Several soilborne xanthomonads, including *X. campestris* pv. *vesicatoria*, have been implicated in the occurrence of plant diseases (5,13,14,18,29).

Numerous biological, physical, and chemical factors affect bacterial survival in soil, and these have been reviewed (4,9,15,21,28). Of these factors, the clays have some of the most profound effects on bacterial survival in soil (22,32). Generally, the clays, namely bentonite, illite, montmorillonite, and vermiculite, are believed to coat bacterial cells to varying extents and, in this manner, protect them from desiccation, high temperature, and pH changes (22). They also stimulate bacterial respiration, presumably by providing mineral nutrients (22,32). There is no known information on the role of soil factors in survival of *X. campestris* pv. *vesicatoria*. In the current study, the effect of wild-type and mutant *avrBs1* avirulence genes on survival of *X. campestris* pv. *vesicatoria* in soil, as well as in detached pepper leaf tissue, was investigated.

MATERIALS AND METHODS

Pathogen and host culture. Near-isogenic pepper (*Capsicum annuum* L.) cultivars Early Calwonder (ECW), ECW10R, ECW20R,

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and ECW30R provided leaf tissue used in this study. Cultivar ECW lacks resistance to bacterial spot of pepper, whereas 'ECW10R', 'ECW20R', and 'ECW30R' contain the *Bs1*, *Bs2*, and *Bs3* disease-resistance genes, respectively (17). Plants were cultured as previously described (25,38) and used when 4 weeks old.

X. campestris pv. *vesicatoria* strains 81-23, 81-23 M13, and 81-23 M13a were used in this study. Strain 81-23 is a pepper-tomato race 2 pathotype (19,31), and strain 81-23 M13 is a pepper race 1 variant derived from it by the spontaneous insertion of a 1.2-kb insertion element, *IS476*, in the coding sequence of the avirulence gene *avrBs1* (19). Strain 81-23 M13 was used to derive strain 81-23 M13a, which is resistant to rifampicin (50 µg ml⁻¹), ampicillin (50 µg ml⁻¹), and tetracycline (10 µg ml⁻¹). Strain 81-23 M13a was previously produced by initially plating strain 81-23 M13 at high titers onto nutrient yeast extract glycerol agar (NYGA) (34) amended with a single antibiotic and then recovering survivors. The process was repeated with each remaining antibiotic using surviving bacteria from each preceding treatment to eventually recover mutants, including strain 81-23 M13a, resistant to the antibiotics, either singly or when combined. Strain 81-23 M13a was previously shown to be similar to the parental strain 81-23 M13 with respect to pathogenicity towards pepper (L. W. O'Garro, H. Gibbs, and A. Newton, unpublished data). Media used routinely for pathogen culture were NYGA, nutrient yeast extract glycerol broth (NYGB) (34), and Tween medium B (23).

Preparation of inocula. Suspensions for inoculation were prepared by growing bacteria overnight in NYGB at 25 to 28°C in a rotary shaker at 220 rpm. Cells were collected by centrifugation at 15,000 × g, washed twice in sterile distilled water (SDW), and resuspended in SDW. Suspensions containing 5 × 10⁸ cells ml⁻¹ were then prepared following dilution and plate assays.

Assessment of pathogen survival in soil. Samples of the 37 soil types of Barbados were used in the current study. The soil types were originally described by Vernon and Carroll (35), and samples used in this investigation were provided by the Chemistry Department, Cave Hill Campus of the University of the West Indies, Barbados. Each soil type was tested previously for a number of chemical and physical parameters using standard methods (2,3,12,36), and results of these tests have been reported (1). Soil was sieved to obtain the 2.0-mm fraction and then dehydrated at 80°C for 24 h. Two dehydrated samples (3.0 g) of soil were each transferred to separate blackened cylindrical bottles (5 × 6 cm) and mixed thoroughly with a suspension (3 ml) of either a 1:1 mixture of strains 81-23 and 81-23 M13a or the individual strains. Soil serving as controls was treated with SDW only. Bottles containing treated soil were capped loosely and kept in an incubator maintained at 28 to 30°C. Moisture loss from soil was replaced daily by adding an equal weight of SDW.

Bacteria were reisolated shortly after infestation and at 4- to 6-day intervals by suspending soil (0.3 g) in SDW (10 ml), shaking the suspension at 3,000 oscillations per minute for 15 min, and then plating aliquots (100 µl) of the diluted suspensions in quadruplicate onto Tween medium B (23). Plates were incubated at 25 to 28°C for 7 days to obtain *X. campestris* pv. *vesicatoria* appearing as circular, raised, yellow colonies surrounded by zones of white crystals. The number of colonies obtained was used to determine pathogen survival, which was assessed as the percent initial inoculum recovered per gram of two replicates of each soil type at each sampling time. The duration of survival of each strain in each soil type and the corresponding detectable amounts of initial inoculum remaining were also determined. The experiment was conducted three times.

To assess survival of individual strains in soil tested with the mixed inoculum, colonies of *X. campestris* pv. *vesicatoria* isolated on Tween agar as described above were replica-plated using velvet pads onto NYGA amended with rifampicin (50 µg ml⁻¹), ampicillin (50 µg ml⁻¹), and tetracycline (10 µg ml⁻¹) for the selection of strain 81-23 M13a.

Linear regressions were used to examine the relationship between the log of pathogen survival and time, and the slopes of corresponding linear expressions were used to compare the rates at which populations of strains 81-23 and 81-23 M13a change in each soil type tested with individual strains or the mixed inoculum. The significance of differences between slopes was assessed by least significant difference (5%) values and Student's *t* test following analysis of variance. All statistical analyses were performed using the Minitab microcomputer program (Making Data Analysis Easier, State College, PA).

Confirmation of the identity of *X. campestris* pv. *vesicatoria* reisolated from infested soil was sought by testing a random sample of 10 to 15 colonies of the presumptive bacterium obtained from each soil type at each sampling time on 'ECW' by leaf infiltration (33) with a bacterial suspension (5 × 10⁵ cells ml⁻¹). Positive identification of *X. campestris* pv. *vesicatoria* was made by the presence of typical bacterial spot symptoms arising 2 to 4 weeks later under the host culture conditions described (38).

The possibility that soil factors including pH, clay and cation content, and percent nitrogen, carbonates, K⁺, Na⁺, Ca²⁺, and carbon (1) may influence survival of *X. campestris* pv. *vesicatoria* in soil was investigated also. In this investigation, a relationship was sought between the numerical values of each soil factor (1) and slopes of linearized survival curves of strains 81-23 and 81-23 M13a of the bacterium (Tables 1 and 2), examined by performing polynomial regression analysis and multiple regression analysis using the Minitab microcomputer program (Making Data Analysis Easier). Numerical values of soil factors and slopes were used as dependent and independent variables, respectively, in the former analysis.

Assessment of pathogen survival in detached leaf tissue. Intercellular spaces of the two youngest, fully expanded leaves of each plant of the ECW-type cultivars were fully infiltrated (33) with inoculum (5 × 10⁸ cells ml⁻¹) of strain 81-23, or strain 81-23 M13a, or both, prepared exactly as described above for infesting soil. Treated leaves were harvested immediately after inoculation, air-dried for 6 h, and then stored fully extended between sheets of sterile filter paper at 28 to 30°C. Bacteria were reisolated from leaves shortly after harvest and then daily for 7 days, followed by weekly intervals for 6 weeks. Reisolation was accomplished by grinding two randomly selected 20-mm² leaf discs from each treated leaf in SDW with an ethanol-disinfested mortar and pestle. The resulting suspension was diluted, and aliquots of 10-fold dilutions were plated in triplicate on NYGA and incubated at 25 to 28°C for 3 to 5 days to obtain colonies of *X. campestris* pv. *vesicatoria*. Colonies recovered from discs of 20 leaves of each pepper cultivar at each sampling time were used to assess survival of *X. campestris* pv. *vesicatoria*. The experiment was conducted three times.

To estimate survival of individual strains in leaves tested with inoculum composed of equal amounts of strains 81-23 and 81-23 M13a, colonies of *X. campestris* pv. *vesicatoria* isolated on NYGA were replica-plated onto NYGA amended with antibiotics as described above for the selection of strain 81-23 M13a.

The extent to which the functional integrity of the mutant and wild-type *avrBs1* genes of strains 81-23 M13a and 81-23, respectively, was maintained in infested soil and detached pepper leaves was also investigated by testing for changes in pathotype conferred by these genes. In this investigation, stems of 4-week-old 'ECW' and 'ECW10R' seedlings were stabbed with sterile tungsten pins (30) previously inserted in 5-day-old colonies of each presumptive pathotype of the bacterium cultured on NYGA (34). Using this procedure, approximately 2.6 to 4.5 × 10⁶ bacterial cells were introduced into each injured stem. Plants serving as controls were similarly tested with sterile pins dipped in SDW. All treated plants were kept for 24 h in humid conditions and then maintained under the host culture conditions previously described (38). Strain 81-23, which expresses the wild-type *avrBs1* gene, was identified by the

hypersensitive response induced by 'ECW10R' and characterized as an area of restricted necrosis coincident with the point of inoculation after 3 days, and the compatible response appeared as swelling, browning, and splitting, either alone or in combination, of stems of 'ECW' 6 to 7 days after infection. Strain 81-23 M13a, carrying the mutant *avrBs1* gene, induced the compatible response on 'ECW' and 'ECW10R' (19). A random sample of 338 colonies of each presumptive pathotype obtained at each sampling time from each of five randomly selected soils, namely soil types 16, 17, 19, 23, and 30, and the four ECW-type cultivars infested with individual strains of the bacterium as described before were tested. The presumptive pathotypes were recovered from infested soil and detached leaves on Tween medium and nutrient agar, respectively, following procedures previously described. Tests to determine the pathotype of each colony were replicated twice, and the experiment, which was conducted independently of those described earlier, was conducted twice.

The possibility that the antibiotic-resistance traits of *X. campestris* pv. *vesicatoria* strain 81-23 M13a may affect survival of the bacterium was also investigated. In this investigation, the 37 soil types and leaves of 'ECW' were tested with a suspension (5×10^8 cells ml⁻¹) of strain 81-23 M13a or strain 81-23 M13, which served

as control. The target bacteria were recovered using procedures described above for the isolation of *X. campestris* pv. *vesicatoria* from soil and detached leaves on Tween medium B and nutrient agar, respectively. Colonies of *X. campestris* pv. *vesicatoria* recovered from discs of three leaves and two replicates of each soil type, as previously described, were used to assess survival of the bacterium in detached leaf tissue and soil, respectively. The relationship between log of pathogen survival and time was examined by linear regression, and slopes of corresponding linear equations were used to compare rates of decline of strains 81-23 M13 and 81-23 M13a in each soil type as previously mentioned. This experiment preceded all others conducted to assess pathogen survival and was repeated once. In the repeat experiment, cultivars ECW10R, ECW20R, and ECW30R were also tested similarly to cultivar ECW, as described above.

RESULTS

Comparison of survival of strains 81-23 and 81-23 M13a in soil. The percent initial inoculum of *X. campestris* pv. *vesicatoria* strains 81-23 and 81-23 M13a recovered per gram of infested soil at the first sampling time was similar and varied from 93.8 to 104.5.

TABLE 1. Extent of survival and rate of decline of *Xanthomonas campestris* pv. *vesicatoria* strains 81-23 and 81-23 M13a in soils infested with individual strains of the bacterium

Soil type	Extent of survival				Rate of decline ^a (slopes)			Sig (5%)
	Strain 81-23 (wild type)		Strain 81-23 M13a (mutant)		Strain 18-23	Strain 18-23 M13a		
	Duration of survival (days)	Corresponding inoculum (% initial inoculum)	Duration of survival (days)	Corresponding inoculum (% initial inoculum)				
1	20	0.06	35	0.56	-0.20	-0.11	*b	
2	20	0.12	35	0.50	-0.21	-0.13	*	
3	42	0.11	35	0.62	-0.21	-0.12	*	
4	20	0.06	35	0.29	-0.21	-0.12	*	
5	20	0.63	35	0.22	-0.21	-0.10	*	
6	20	0.42	35	2.50	-0.22	-0.13	*	
7	20	2.28	42	0.01	-0.19	-0.10	*	
8	20	0.85	42	0.01	-0.21	-0.10	*	
9	20	0.64	42	0.01	-0.22	-0.11	*	
10	20	0.51	35	0.22	-0.22	-0.12	*	
11	20	0.83	42	0.02	-0.13	-0.21		
12	20	0.91	35	0.54	-0.22	-0.10	*	
13	20	1.78	42	0.03	-0.22	-0.15	*	
14	28	0.61	42	0.02	-0.18	-0.23		
15	20	0.51	35	2.00	-0.21	-0.10	*	
16	20	1.15	35	0.24	-0.21	-0.11	*	
17	20	0.16	42	0.03	-0.21	-0.10	*	
18	20	0.10	35	0.18	-0.21	-0.12	*	
19	20	0.66	42	0.67	-0.21	-0.12	*	
20	20	0.86	35	0.29	-0.13	-0.21		
21	16	0.64	35	0.43	-0.21	-0.11	*	
22	28	0.10	42	0.19	-0.21	-0.12	*	
23	28	0.01	35	0.36	-0.21	-0.11	*	
24	42	0.05	35	0.48	-0.21	-0.10	*	
25	42	0.01	42	0.08	-0.21	-0.11	*	
26	42	0.01	35	0.86	-0.16	-0.21		
27	16	0.61	35	0.56	-0.21	-0.12		
28	42	0.02	42	0.91	-0.21	-0.10	*	
29	16	0.65	42	0.64	-0.21	-0.13	*	
30	28	0.64	35	0.48	-0.19	-0.17		
31	20	0.01	42	0.93	-0.21	-0.12	*	
32	20	0.91	42	0.86	-0.21	-0.13	*	
33	16	0.01	42	0.92	-0.19	-0.10	*	
34	42	0.01	42	0.62	-0.21	-0.12	*	
35	20	0.61	35	0.76	-0.21	-0.10	*	
36	16	0.81	42	0.02	-0.21	-0.12	*	
37	20	1.76	35	1.05	-0.21	-0.13	*	
LSD (5%)					0.06	0.09		

^a Rates of decline were assessed as the numerical values of slopes associated with corresponding linear expressions derived from linear regression between the log of survival of each strain of the bacterium and time.

^b * indicates that amounts of strain 81-23 declined significantly faster than strain 81-23 M13a over a 42-day period according to Student's *t* test. For regressions, *n* = 37.

In contrast, no xanthomonads were detected in soil serving as controls. All presumptive xanthomonads isolated from soil and tested on pepper induced typical bacterial spot symptoms within 2 to 4 weeks.

The duration of survival of *X. campestris* pv. *vesicatoria* in soil infested with a 1:1 mixture of strain 81-23 and strain 81-23 M13a or the individual strains and the corresponding detectable amounts of initial inoculum remaining are shown in Tables 1 and 2. Strain 81-23 M13a was detectable for up to 35 to 42 days in amounts ranging from 0.01 to 2.0% of initial inoculum in soils infested with individual strains. In contrast, strain 81-23 was only recovered within this time from nine similarly infested soil types and in quantities up to 200 times less than strain 81-23 M13a (Table 1). In the other soil types, strain 81-23 was detectable for up to 20 days (Table 1). Average maximum survival times determined for strains 81-23 M13a and 81-23 in soil infested with inoculum constituted from equal amounts of each strain were 35.6 and 23.9 days, respectively. At these times, the mean percent initial inoculum remaining in soil was 0.31 for strain 81-23 M13a and 0.04 for strain 81-23 (Table 2).

Linear correlation coefficients derived for the relationship between the log of survival of strains 81-23 and 81-23 M13a in soil infested with the individual strains or the 1:1 mixture of strains and

time ranged from 0.82 to 0.99, and all were significant. Rates of decline, assessed as slopes associated with this linear relationship, are presented in Tables 1 and 2. In 32 of 37 soil types, strain 81-23 declined significantly faster than strain 81-23 M13a in soils tested with individual strains. In the remaining soil types, strain 81-23 M13a was reduced at similar or significantly greater rates relative to strain 81-23 (Table 1). Although both strains in soil infested with the mixed inoculum declined in abundance over a 42-day period, strain 81-23 was reduced proportionately faster than strain 81-23 M13a in 30 of 37 soil types (Table 2). The rate of decline of each strain was similar in four soil types, whereas strain 81-23 M13a declined proportionately faster than strain 81-23 in the remaining soil types (Table 2). Generally, the rate of decline of either strain in the 37 soil types was similar (Tables 1 and 2). Similar results were obtained each time the study was repeated and, consequently, only data from one experiment are shown (Tables 1 and 2).

There were no significant linear, quadratic, cubic, quartic, or quintic correlations between rates of decline of strain 81-23 or 81-23 M13a and the numerical values of each soil factor tested, on the basis of polynomial regression analysis (data not shown). The results of multiple regression analysis also indicated no significant linear relationship between the rates of bacterial decline and all pos-

TABLE 2. Extent of survival and rate of decline of *Xanthomonas campestris* pv. *vesicatoria* strains 81-23 and 81-23 M13a in soil infested with a mixture of equal amounts of the strains

Soil type	Extent of survival					Rate of decline ^a (slopes)		
	Strain 81-23 (wild type)		Strain 81-23 M13a (mutant)					Sig (5%)
	Duration of survival (days)	Corresponding inoculum (% initial inoculum)	Duration of survival (days)	Corresponding inoculum (% initial inoculum)		Strain 18-23	Strain 18-23 M13a	
1	35	0.01	42	0.28		-0.13	-0.06	*b
2	28	0.03	35	0.35		-0.17	-0.10	*
3	20	0.01	35	0.09		-0.16	-0.06	*
4	20	0.07	35	0.15		-0.19	-0.05	*
5	20	0.01	35	0.12		-0.09	-0.09	
6	20	0.02	35	0.26		-0.12	-0.05	*
7	20	0.01	35	0.04		-0.12	-0.05	*
8	20	0.05	35	0.06		-0.13	-0.06	*
9	20	0.05	35	0.10		-0.09	-0.08	
10	20	0.02	35	0.57		-0.19	-0.05	*
11	20	0.01	35	0.03		-0.08	-0.08	
12	20	0.01	35	0.02		-0.13	-0.05	*
13	35	0.15	35	1.34		-0.12	-0.06	*
14	20	0.02	35	0.02		-0.12	-0.08	*
15	20	0.05	35	0.28		-0.13	-0.06	*
16	28	0.10	35	0.26		-0.13	-0.05	*
17	20	0.11	35	0.55		-0.13	-0.05	*
18	20	0.02	35	0.41		-0.16	-0.05	*
19	20	0.05	35	0.13		-0.09	-0.04	*
20	20	0.01	35	0.61		-0.09	-0.11	
21	28	0.24	42	0.91		-0.10	-0.04	*
22	28	0.01	35	0.01		-0.12	-0.04	*
23	16	0.01	35	0.46		-0.15	-0.07	*
24	28	0.04	35	0.65		-0.15	-0.14	
25	28	0.02	35	0.01		-0.15	-0.06	*
26	35	0.01	35	0.25		-0.13	-0.07	*
27	28	0.01	35	0.22		-0.10	-0.15	*
28	28	0.01	35	0.91		-0.15	-0.06	*
29	28	0.01	35	0.54		-0.15	-0.06	*
30	35	0.01	35	0.12		-0.11	-0.09	
31	20	0.01	35	0.13		-0.10	-0.04	*
32	35	0.02	42	0.14		-0.13	-0.06	*
33	20	0.01	35	0.23		-0.13	-0.04	*
34	20	0.05	35	0.36		-0.13	-0.05	*
35	20	0.07	35	0.36		-0.15	-0.04	*
36	20	0.05	35	0.22		-0.16	-0.06	*
37	20	0.02	35	0.15		-0.07	-0.16	
LSD (5%)						0.09	0.09	

^a Rates of decline were assessed as the numerical values of slopes associated with corresponding linear expressions derived from linear regression between the log of survival of each strain of the bacterium and time.

^b * indicates that amounts of strain 81-23 declined significantly faster than strain 81-23 M13a over a 42-day period according to Student's *t* test. For regressions, *n* = 37.

sible combinations of soil factors tested (data not shown). Similar deductions were made from data of each experiment.

Comparison of survival of strains 81-23 and 81-23 M13a in detached pepper leaves. Survival of *X. campestris* pv. *vesicatoria* strains 81-23 M13a and 81-23 in detached leaf tissue of pepper cultivars ECW, ECW10R, ECW20R, and ECW30R from one experiment is shown in Figure 1. In all cultivar-strain interactions, bacteria declined over time, but the extent of reduction varied with the interaction. Strain 81-23 was detected for up to 5 weeks in 'ECW20R' and for up to 2 weeks in the other cultivars. In contrast, strain 81-23 M13a persisted for up to 5 to 6 weeks in all cultivars and in significantly greater abundance than strain 81-23. Strain 81-23 M13a also exceeded strain 81-23 by factors of 3.3, 4.4, 2.2, and 20.7 in leaf tissue of 'ECW', 'ECW10R', 'ECW20R', and 'ECW30R', respectively, by 6 days after treatment with the

1:1 mixture of strains. Similar results were obtained each time the experiment was repeated (data not shown).

In two experiments conducted, no change in pathotype of strain 81-23 M13a or strain 81-23 was detected in each collection of 338 strains of *X. campestris* pv. *vesicatoria* obtained from each ECW-type cultivar at each sampling time. The same result was obtained when similar collections of the bacterium from five soil types were tested.

In one experiment of the investigation into the effect of the antibiotic-resistance markers on survival of strain 81-23 M13a, linear correlations associated with the relationship between the log of survival of strain 81-23 M13a and its parental strain, 81-23 M13, in each soil type and time varied from 0.83 to 0.94 and from 0.78 to 0.98, respectively, and all were significant ($P \leq 0.05$). Rates of decline of these strains, assessed as the numerical values

Fig. 1. Survival of *Xanthomonas campestris* pv. *vesicatoria* strains 81-23 M13a (—▲—) and 81-23 (—△—) in detached leaves of pepper cultivars **A**, Early Calwonder (ECW); **B**, ECW10R; **C**, ECW20R; and **D**, ECW30R tested with individual strains of the bacterium. Data are the means $\pm$ standard error (bars) of 20 replicates.

of slopes of linear expressions as previously described, were similar in each soil type (Table 3). The two strains, when tested on detached leaves of 'ECW', were detectable for up to 42 days in amounts of 49.6 and 56.2 CFU per 20-mm² leaf disc. No significant differences between the survival of the two strains in soil or detached leaves of each ECW-type cultivars were detected in the other experiment (data not shown).

DISCUSSION

The current study reports, for the first time, on the adaptive significance of a pathogen avirulence gene during survival of a phytopathogenic bacterium apart from living host plants. The study was prompted by an observation of drastic differences between the relative abundance of races of *X. campestris* pv. *vesicatoria* recovered from bacterial spot-infected pepper or tomato and field soils in which the crops were grown (37). The current investigation utilized well-characterized wild-type and mutant *avrBs1* avirulence genes of *X. campestris* pv. *vesicatoria* that were tested on detached leaves of four pepper cultivars and 37 defined soil types of Barbados (35).

In the current study, spontaneous antibiotic-resistance traits were routinely used to distinguish strain 81-23 M13a from strain 81-23. These traits, which were acquired by strain 81-23 M13a, did not appear to affect survival or pathogenicity of the bacterium. This conclusion was based on comparisons of survival of strains 81-23 M13 and 81-23 M13a in 37 soil types and detached leaves of pepper cultivars ECW, ECW10R, ECW20R, and ECW30R, as well as pathogenicity on the two former cultivars. Despite these observations, it is, nevertheless, assumed that the antibiotic-resistance traits did not affect other, albeit undetected, gene function(s) capable of influencing survival of strain 81-23 M13a.

Similarly, no detectable changes in pathotypes conferred by the mutant and wild-type *avrBs1* genes were observed in populations of *X. campestris* pv. *vesicatoria* infesting soil and detached pepper leaves. On this basis, it is assumed that the functional integrity of each gene was not disrupted at frequencies or to extents that were detectable in the current study. It was further assumed that a copy of transposon *IS476* mostly remained inserted in the *avrBs1* gene of strain 81-23 M13a and was unlikely to affect other gene loci in ways that can influence survival of this strain. It has been suggested that *IS476* is not solely confined to the *avrBs1* gene of *X. campestris* pv. *vesicatoria* (19).

With the exception of the antibiotic-resistance traits, differences between strains 81-23 and 81-23 M13a are, therefore, assumed to be due only to a single change in structure or function of the *avrBs1* avirulence gene (19). It was, therefore, possible to investigate the effect of this change on survival of *X. campestris* pv. *vesicatoria* apart from living host plants. A conclusion of the current study is that strain 81-23 M13a, which carries the mutant *avrBs1* gene, has significantly greater capacity for survival in soil and dead leaf tissue than strain 81-23, expressing wild-type *avrBs1* avirulence gene activity.

There is also indication of the possible adaptive significance of the mutant *avrBs1* gene. The adaptive significance is best illustrated by results from tests on soil and leaf tissue with inoculum comprised of equal amounts of strains 81-23 and 81-23 M13a. In the ensuing interaction between the strains, probably for survival sites or nutrients, strain 81-23 M13a persisted longer and may, as a consequence, acquire a greater chance than strain 81-23 of establishing contact with a host plant to initiate pathogenesis.

On the basis of the greater survival capacity of strain 81-23 M13a in soil and dead leaves, it is tempting to predict that pepper race 1 strains of *X. campestris* pv. *vesicatoria* possess greater capacity to survive in the absence of living host plants than pepper-tomato race 2 pathotypes represented by strain 81-23. This contention may explain why race 1 strains of *X. campestris* pv. *vesicatoria* almost invariably predominate in most geographical areas (16,24,25,31,

38). Until recently, pepper-tomato race 2 strains of the bacterium predominated in Florida (31), but a sudden shift to a prevalence of pepper race 1 strains has been reported (28). The shift may have been initiated by introduction of race 1 strains (26) that subsequently competed successfully against the race 2 strains in soil between crops. The race 1 strains may also express greater virulence than pepper-tomato race 2 strains, as recently shown in Barbados by O'Garro and Tudor (25).

In the current study, the influence of pH and percent clay, carbonates, K⁺, Mg²⁺, Na⁺, Ca²⁺, carbon, and nitrogen of soil (1) on survival of *X. campestris* pv. *vesicatoria* was investigated and none appeared to have significant effect based on results of polynomial regression and multiple regression analyses. It is possible that survival of the bacterium in soil is influenced by a more complex interaction of soil factors.

The effect of soil on survival of *X. campestris* pv. *vesicatoria* reported in the current study may not be an exact representation of events affecting the bacterium in the soil phase of its natural life cycle. The soil samples tested would have lost an undetermined proportion of microflora due to heating, which could affect microbial survival in soil. Differences in the ability of soil to bind water, thus influencing available moisture, will also be critical to bacterial survival. In the current study, the soils tested were mostly heavy clays (1,35), with available water capacity typically varying from 11 to 18% (J. C. Hudson, unpublished data). Differences in soil

TABLE 3. Effect of antibiotic resistance on the survival of *Xanthomonas campestris* pv. *vesicatoria* strain 81-23 M13a in 37 soil types

Soil type	Rate of decline ^a (slopes)	
	Strain 81-23 M13a	Control ^b (strain 81-23 M13)
1	-0.13	-0.13
2	-0.11	-0.14
3	-0.12	-0.10
4	-0.10	-0.11
5	-0.12	-0.14
6	-0.12	-0.11
7	-0.13	-0.11
8	-0.12	-0.11
9	-0.13	-0.11
10	-0.10	-0.09
11	-0.18	-0.15
12	-0.13	-0.11
13	-0.16	-0.13
14	-0.19	-0.15
15	-0.09	-0.12
16	-0.12	-0.09
17	-0.13	-0.11
18	-0.10	-0.13
19	-0.11	-0.09
20	-0.18	-0.16
21	-0.12	-0.11
22	-0.09	-0.11
23	-0.12	-0.11
24	-0.12	-0.09
25	-0.11	-0.13
26	-0.18	-0.15
27	-0.11	-0.09
28	-0.11	-0.12
29	-0.12	-0.13
30	-0.19	-0.15
31	-0.14	-0.11
32	-0.15	-0.13
33	-0.09	-0.11
34	-0.09	-0.12
35	-0.11	-0.15
36	-0.11	-0.07
37	-0.16	-0.14

^a Rates of decline were assessed as the numerical values of slopes associated with corresponding linear expressions derived from linear regression between the log of survival of each strain of the bacterium and time.

^b Strain 81-23 M13a was derived from strain 81-32 M13. For regressions, *n* = 37.

moisture and microflora are not expected to affect the result, indicating a differential capacity of strains 81-23 M13a and 81-23 to survive in soil, since both strains were likely to be tested under identical conditions in each soil type.

The *avrBs1* gene of *X. campestris* pv. *vesicatoria* encodes a 50-kDa protein (27), and no information is available on its mode of action. Knowledge of this may provide an understanding of the role of *avrBs1* in survival of *X. campestris* pv. *vesicatoria* in the absence of suitable host plants. Many other avirulence genes of the bacterium, including *avrBsT*, *avrBs3*, and *avrBsP*, have also been identified and characterized (24). Research is also required to determine whether they encode functions other than pathogen avirulence.

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