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A PRELIMINARY INVESTIGATION INTO THE ADAPTIVE
SIGNIFICANCE OF AVIRULENCE GENES IN THE BACTERIUM
Xanthomonas campestris pv. vesicatoria.

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ABSTRACT

In plant-pathogen interaction, the hypersensitive response is the host's resistance response which prevents pathogen spread and multiplication. This response results from the interaction between a pathogen with a dominant avirulence gene and a host with a specific complementary resistance gene. The adaptive significance of the pathogen avirulence gene to the pathogen in this host-pathogen relationship is not evident. This research presents preliminary data on the adaptive significance of avirulence genes in Xanthomonas campestris pv. vesicatoria.

INTRODUCTION

Models of the specificity of the host-pathogen interactions state that only a pathogen with a specific avirulence gene can trigger the resistance response in a host which must carry a specific complementary resistance gene (Ellingboe 1979; 1981). In this context, the avirulence gene allows the recognition of the pathogen by the host. It is unlikely that the function of the pathogen avirulence gene is merely to cause recognition of the pathogen by the host, since the resistance response which ensues is deleterious to the pathogen. It is therefore of interest to investigate any possible adaptive significance of pathogen avirulence genes.

The bacterial spot disease of pepper and tomato caused by Xanthomonas campestris pv. vesicatoria provides a good model system for this investigation. This bacterium has been divided into three groups or strains on the basis of the nature of the interaction with pepper and/or tomato, and these are as follows: the tomato strain, the pepper strain, and the pepper-tomato strain (Reifschneider et al., 1985; Minasavage et al., 1990). The tomato strain is virulent on tomato only, while the pepper strain is virulent only on pepper. Both tomato and pepper are susceptible to the pepper-tomato strain. Within the pepper and pepper-tomato strains, races of the pathogen can be distinguished on the basis of their ability to induce disease on particular pepper lines (Hibberd, et al., 1987; Hibberd, et al., 1987).

There is evidence that resistance in pepper to Xanthomonas campestris pv. vesicatoria is controlled in a gene-for-gene fashion. The avirulence gene, avrBS1, of race-2 isolates of the pepper-tomato group, corresponds to the BS1 gene for resistance in pepper (Stall et al., 1986). The avirulence gene locus is responsible for inducing the hypersensitive or resistance response in pepper carrying the BS1 resistance gene. This locus is located on a self-transmissible plasmid that also encodes copper resistance (8).

In this study, the fitness of an isolate of X. campestris pv. vesicatoria with a mutation within the coding sequence of the avirulence (avrBS1) gene is assessed.

MATERIALS AND METHODS

Host and pathogen cultures: The pepper lines Early Calwonder IOR, PI 163189, PI 164471, PI 123-1-7, PI 244670 and PI 163184 were used as hosts in this study. Early Calwonder IOR contains a specific resistance gene (BS1), effective against race-2 isolates of the pepper-tomato strains of X. campestris pv. vesicatoria used in this study. Isolate 81-23M13 had completely overcome the resistance encoded by BS1, inducing the development of water-soaked lesions. Detailed analysis of 81-23M13 has revealed that a 1.2 Kb transposon or insertion element is inserted in the coding region of the avirulence gene, avrBS1 (Kearney, 1988). Isolates were grown on NYGA or in NYGB. NYGA consists of oxoid bacteriological peptone (5 g/l), yeast extract (3 g/l), glycerol (20 g/l) and agar (10 g/l). NYGB contained the constituents of NYGA except agar.

Plant inoculation: Plants were inoculated with a suspension (107 cells/ml) of 81-23 or 81-23M13 or a mixed suspension of both by vacuum infiltration. Plants infected with 81-23 or 81-23M13 were used to assess the pathogenicity of the respective isolates. Pathogenicity was assessed 7-10 days following inoculation. Each leaf was scored on a 0-4 scale where 0, 1, 2, 3 and 4 represent 0%, 25%, 50%, 75% and 100% reduction in photosynthetic area, respectively.

Seedlings of the cultivar Early Calwonder IOR were also stab-inoculated in the stem with a sterile pin previously inserted in a colony of the pathogen grown NYGA. All inoculated seedlings were incubated in a humid chamber under the plant growth conditions described.

Assessment of colonization: Plants inoculated with the mixed bacterial suspension were used to investigate the ability of 81-23 and 81-23M13 to colonize the host material. Bacteria were isolated from pepper leaves after 10 days by comminuting leaf sections in sterile distilled water using an ethanol-sterilized pestle and mortar. 100 ul of an appropriate dilution were planted into NYGA and incubated at 25-28C for 3-5 days. One hundred

colonies from each treatment were screened to determine the proportion of 81-23 or 81-23M13 by stab-inoculating Early Calwonder IOR seedlings.

RESULTS

The pathogenicity of isolates 81-23M13 on five susceptible pepper lines is shown in Table 1. With the exception of PI 163189, all cultivars were more susceptible to 81-23 than to 81-23M13.

Table 1. Pathogenicity* of isolates 81-23 and 81-23 M13 of Xanthomonas campestris pv. vesicatoria on 5 pepper varieties.

Isolate	PATHOGENECITY				
	PI 23-1-7	PI 244670	PI 163184	PI 164471	PI 163189
81-23	3.5	3.1	2.9	2.1	1.6
81-23 M13	1.8	1.6	2.0	1.4	1.5
Control	0	0	0	0	0
LSD (5%)	0.4	0.5	0.3	0.6	0.7

* Pathogenicity is expressed as the mean area (0-4) of leaves covered by disease lesions of 20 replicate plants per isolate.

Control plants were treated with sterile distilled water.

The isolates gave characteristic hypersensitive or susceptible reactions with the pepper cultivar Early Calwonder IOR. Isolate 81-23 induced a hypersensitive response characterized by a localized necrotic lesion at the point of inoculation. The susceptible response induced by 81-23M13 was identified by a spreading water-soaked lesion. On this basis it was possible to distinguish isolates colonizing the plants as either 81-23 or 81-23M13. Table 2 gives an estimate of the proportion of 81-23 and 81-23M13 on the susceptible pepper varieties. Isolate 81-23 represented a significantly greater proportion of bacteria colonizing all host cultivars except PI 163189.

DISCUSSION

There is a current interest in the molecular genetics of host-pathogen relationships with particular emphasis on the modification of crops to suit our needs. This effort requires a detailed understanding of the role of the relevant genetic functions in both host and pathogen with respect to their

survival and continuity. There is some information available on the role of host resistance genes in a few host-pathogen systems. The active induction of phytoalexins, hydrolytic enzymes, gels, callose or wall modification are all features of the host's resistance response to infection.

Table 2. Comparative* colonization of five pepper varieties by isolates 81-23 and 81-23 M13 10 days after inoculation with mixed inocula.

Isolate	PERCENTAGE COLONIZATION				
	<u>PI 23-1-7</u>	<u>PI 244670</u>	<u>PI 163184</u>	<u>PI 164471</u>	<u>PI 163189</u>
81-23	61	87	79	70	54
81-23 M13	39	13	21	30	46

- * A mixed suspension (10^7 cells/ml) of equal amounts of isolates 81-23 and 81-23 M13 was used to inoculate five pepper varieties by vacuum infiltration. At ten days following inoculation bacteria were reisolated and 100 colonies selected randomly and screened on Early Calwonder 10R to determine the proportion of the isolates in each sample.

The function of the pathogen avirulence gene is understood only in the context of the gene-for-gene relationship. In this relationship, the avirulence gene induces the hypersensitive response in a host with a complementary resistance gene. Avirulence genes can therefore restrict the host range of the pathogen within the susceptible plant species.

This study represents the start of an investigation into other possible functions of avirulence genes. Isolates 81-23 and 81-23M13 are identical except for the insertion of a transposable element within the coding sequence of the *avrBS1* gene of the latter. Differences in fitness of 81-23M13 relative to 81-23 are therefore assumed to be due to a lack of a functional *avrBS1* in the former. The extent of colonization and disease lesion development in the susceptible pepper varieties were used as indices of the fitness of 81-23 and 81-23M13. The data (Tables 1 and 2) generally show that the *avrBS1* gene may confer advantages on its bearer when in a compatible relationship with a host. More detailed analysis of the role of the *avrBS1* and other avirulence genes of the *X. campestris* pv. *vesicatoria* is forthcoming.

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