A Hypersensitive Reaction Induced in Tobacco Leaves by a Compatible (Race 1) Isolate of Pseudomonas solanacearum

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Supported in part by National Science Foundation Grant GB31584 to the College of Agricultural and Life Sciences, University of Wisconsin (Project 1474), and the Instituto Colombiano Agropecuario (ICETEX).

ABSTRACT

A hypersensitive response (HR) is obtained when tobacco leaves are infiltrated with suspensions of incompatible (race 2) or avirulent strains of *Pseudomonas solanacearum*. In contrast, most compatible (race 1) strains induce a necrotic lesion that develops slowly, allowing spread of bacteria to adjoining tissues. We now report that certain race 1 isolates can cause a typical HR when infiltrated at approximately 10^8 cells/ml into leaves of the tobacco cultivar Cuba 12. Although a low level of pathogenicity on tobacco was correlated with induction of the HR by most of these race 1 isolates, one (S-123) was highly pathogenic. This isolate multiplied rapidly in tobacco leaves until the population reached 2×10^7 cells/1 cm^2 leaf disk; at this point, the HR was induced and populations declined precipitously thereafter. A comparison of the physiological characteristics (such as growth and acid production with different carbohydrate substrates, production of polygalacturonase, etc.) of isolates capable or incapable of inducing the HR did not reveal any specific features that could be associated with this property.

Phytopathology 65:731-733

Additional key words: bacterial wilt, incompatibility.

The leaf-infiltration technique developed by Klement, et al. (8) provided a useful means to differentiate the three main races of *Pseudomonas solanacearum* E. F. Sm. (9). A hypersensitive reaction (HR) was obtained in tobacco (*Nicotiana tabacum* L. 'Bottom Special') leaves infiltrated with incompatible strains of *P. solanacearum* (race 2). On the other hand, with all compatible (race 1) isolates that had been tested, infiltrated tissues remained symptomless for 48 hours, then necrosis developed slowly in the center of the infiltrated area, and the bacteria spread to the adjoining tissues.

During a comprehensive study of the physiological characteristics of nineteen race 1 isolates of *P. solanacearum* from various geographical locations and different hosts (3) it was determined that, contrary to expectations, a few isolates caused a typical HR when infiltrated into leaves of the tobacco cultivar Cuba 12. This included a number of isolates that had been reported to cause a compatible, necrotic response when infiltrated in leaves of the susceptible cultivar Bottom Special (9). These isolates had been stored in distilled water (7) for several years, but produced fluidal, slimy colonies on TZC medium (6), a characteristic of highly virulent cultures.

The objective of this work, therefore, was to determine the pathogenicity to tobacco, growth pattern in leaf tissues, and physiological properties of these HR-inducing isolates of *P. solanacearum*.

Tobacco cultivar Cuba 12 plants were grown in sand culture in a growth chamber at 28 C, 50% relative humidity, and 19,375 lx light intensity on a 12-hour

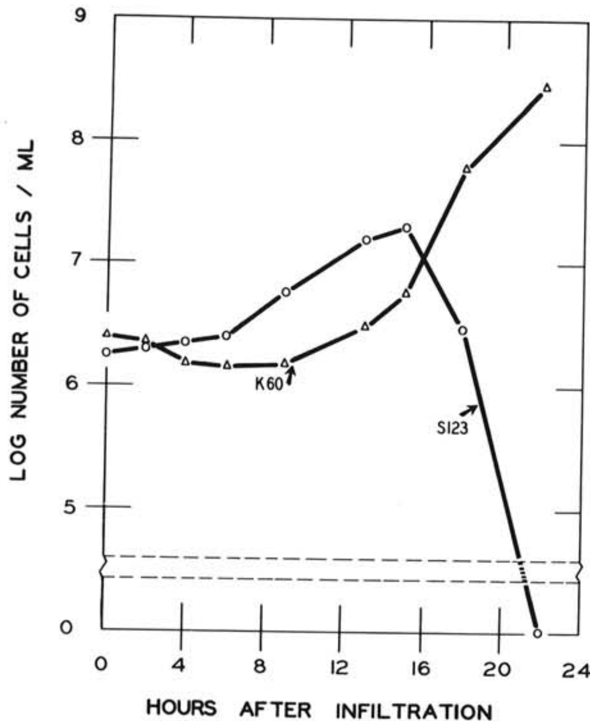

Fig. 1. Bacterial populations after infiltration of tobacco leaves with compatible strains of *Pseudomonas solanacearum* that induce the hypersensitive (isolate S-123) and slow-necrotic (isolate K-60) reactions. Cells/ml = cells/cm^2 leaf area.

photoperiod. Single plants were grown for 1 month in clay pots and were watered daily with Hoagland's nutrient solution. Bacteria were grown on TZC medium (6) for 48 hours at 30 C. Suspensions containing approximately 10^8 bacteria per ml were injected into the intercellular spaces of tobacco leaves with a hypodermic syringe fitted with a fine (30-gauge) needle (8). An area 3-5 cm^2 in each of 25 intercostal areas from several leaves was infiltrated with each of seven isolates (Table 1). The HR was indexed 24 hours after infiltration according to the following scale: 1 = no symptoms; 2 = one-fourth; 3 = one-half; 4 = three-fourths; and 5 = all of the infiltrated area presenting collapsed tissue.

For pathogenicity tests, 1-month-old tobacco plants were inoculated by placing a drop of a bacterial suspension containing 10^8 cells/ml on the axil of the third fully expanded leaf from the top and thrusting a needle downward through the drop and into the stem. Disease indices, which were recorded at 3-day intervals for 21 days, were based upon the following scale: 1 = no symptoms; 2 = wilting of the inoculated leaf only; 3 = one-third of the leaves wilted; 4 = two-thirds of the leaves wilted; and 5 = all leaves wilted. All experiments were repeated two or three times.

Isolates K-74, S-225, S-236, S-123, and S-240 induced a typical HR on tobacco leaves; partial or total collapse of the tissues was evident by 12 hours after infiltration; bleaching and desiccation began at approximately 24 hours (Table 1). On the other hand, isolates G-11 and K-60 caused a normal compatible response in which necrosis became visible at the center of the lesion within 48 hours after inoculation, and bacteria moved to adjoining tissues thereafter. Pathogenicity tests indicated that most isolates that induced the HR either were not pathogenic or had a very low level of pathogenicity to tobacco, whereas those that induced the compatible response were highly virulent and caused death of the plants by 12 days after inoculation (Table 1). An exception was isolate S-123; it induced the HR, but was highly pathogenic on tobacco as well.

Because of the unexpected results obtained with isolate S-123, populations of that bacterium and those of K-60 were determined in two separate experiments at 2- to 6-hour intervals after infiltration of tobacco leaves. One 1 cm^2 diameter disk was removed with a cork borer from each of five infiltrated areas at each sampling time and each disk was comminuted with a Ten Broeck tissue grinder in 1 ml distilled water. Bacterial populations in this suspension were determined by standard dilution plating on TZC.

Both isolates showed a lag period of 6-8 hours and multiplied exponentially thereafter (Fig. 1). However, with S-123, the HR was induced when populations reached 2×10^7 cells per leaf disk; populations declined rapidly thereafter. With K-60, on the other hand, populations continued to multiply unhindered and reached 4×10^8 cells per leaf disk 22 hours after infiltration without causing any visible symptoms. Necrosis appeared in the center of the infiltrated area by

TABLE 1. Origin, pathogenicity to tobacco (cultivar Cuba 12), and hypersensitive reaction (HR)-inducing properties of various race 1 isolates of *Pseudomonas solanacearum*

Isolate	Location	Original host	Disease index[a] at 12 days	HR index[b] at 24 hours
G-11	Santander, Colombia	Tobacco	5.0	1.0
K-60	Wake County, North Carolina, U.S.A.	Tomato	5.0	1.0
K-74	Worth County, Georgia, U.S.A.	Tomato	2.0	4.5
S-123	Coto, Costa Rica	*Eupatorium odoratum*	5.0	5.0
S-225	Lupuna, Peru	Tomato	1.5	5.0
S-236	Nambour, Australia	Tomato	1.3	4.8
S-240	Ingham, Australia	Tobacco	2.2	4.8

[a]Figures represent average indices of five plants 12 days after stem inoculation. Index ranged from 1 = no symptoms, to 5 = complete wilting.

[b]Figures represent average indices of 25 inoculum-infiltrated leaf areas. Index ranged from 1 = no symptoms, to 5 = full HR.

48 hours when populations reached approximately 10^{10} cells per leaf disk; by this time, the bacteria had spread to adjoining parenchyma tissues and into the xylem elements. With S-123, no bacteria could be isolated at the edge of the lesions (4-7 mm away from the necrotic tissue) by 72 hours after infiltration.

A comparison of the capacity of the seven isolates of *P. solanacearum* to utilize a large number of C sources, following the methods described by Hayward (4), did not reveal any specific characteristics that could be associated with the HR-inducing properties of these isolates. On the basis of their ability or failure to utilize three disaccharides (maltose, lactose, and cellobiose) and three hexose alcohols (mannitol, dulcitol, and sorbitol) added at 0.1% (w/v) to Hayward's basal medium, isolates K-60 and K-74 belong to biotype 1, and isolates S-123, S-225, S-236, and S-240 belong to biotype 3. It was first thought that all isolates classified in biotype 3 might induce the HR, but isolate S-222 [from eggplant (Harris Culture No. 200 in Kenya], which belongs in biotype 3, failed to induce the HR.

All isolates were very similar in their ability to utilize a wide variety of amino acids and other organic acids as sole sources of C. No differences were noted in their ability to produce polygalacturonase in Husain and Kelman's (5) basal medium.

That certain isolates of race 1 should induce the HR when infiltrated in tobacco leaves was not unexpected. Isolates sometimes lose virulence after prolonged storage, although retaining some of their fluidal colony characteristics in culture. Also, there are several reports of race 1 isolates which cannot attack tobacco (2, 7). Following the hypothesis first presented by Klement, et al. (8), isolates which are not pathogenic to tobacco should induce the HR. Isolate S-123 appears to be an exception to the rule that compatible (pathogenic) strains of bacteria do not induce the HR on tobacco. This isolate multiplied rapidly before the HR was induced, unlike incompatible strains which generally do not multiply before the HR is induced (9). However, Cook (1) reported that incompatible tomato strains of *Xanthomonas vesicatoria* induced the HR in two cultivars of *Capsicum annuum* after progressive increases in bacterial numbers took place 6-24 hours after inoculation.

From our results, it is clear that the leaf-infiltration technique used to differentiate between compatible and incompatible strains of *P. solanacearum* (9) cannot be relied upon entirely. Pathogenicity tests must be carried out to verify the presumed incompatibility of certain isolates. This, of course, limits the usefulness of the infiltration technique. However, in all tests carried out thus far, isolates that give a compatible response by this method are also highly pathogenic when stem-inoculated on susceptible tobacco cultivars (3).

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