Bacterial Blight of Beans Caused by Two Xanthomonas Pathogenic Types from Puerto Rico

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ABSTRACT

Field inoculations suggested that *Xanthomonas* bacteria pathogenic to southern pea (*Vigna unguiculata*) caused a bacterial blight of bean (*Phaseolus vulgaris*). Isolation and cross-inoculation studies of bacteria from the two diseased host species suggest that two pathogenic types of *Xanthomonas* affect beans in Puerto Rico. One type is pathogenic to bean only, while the other is pathogenic to both bean and southern pea. In pathogenicity tests on bean and cowpea with 67 Puerto Rican *Xanthomonas* isolates from *P. vulgaris* and *V. unguiculata*, 56 were similar to *X. vignicola* (ATCC 11648), nine to *X. phaseoli* (ATCC 13464). No differences in cultural, morphological, or physiological characters were apparent between isolates of the two pathogenic types. It is suggested that *Xanthomonas* cultures that are pathogenic to both bean and southern pea should be regarded as *X. vignicola*, as originally suggested by Burkholder.

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Dry beans (*Phaseolus vulgaris* L.) originated in middle America and evolved into one of the main staple foods of the inhabitants of the area (5). Common bacterial blight (*Xanthomonas phaseoli* E. F. Sm. Dows.) (abbreviation here = *Xp*) is an endemic disease of this crop in tropical regions (2, 3, 8, 9). During the rainy season, May to November in Puerto Rico, bacterial blight may cause complete loss of a bean crop. The AID/USDA Grain Legume Improvement Project was initiated at the Federal Experiment Station, Mayaguez, Puerto Rico, to develop sources of resistance to endemic diseases of grain legumes in the tropics.

In field-screening trials, two distinct types of bacterial blight symptoms were often observed on diseased bean plants. In a field trial where southern peas (cowpeas) [*Vigna unguiculata* (L.) Walp.] were being screened for resistance to bacterial stem canker (caused by *Xanthomonas vignicola* Burkh.) (abbreviation here = *Xv*), the plants of the bean cultivar Black Valentine, which bordered the southern pea trial, were severely diseased with bacterial blight. The source of inoculum was the crude sap obtained from southern pea cultivars which had bacterial blight and stem canker. Beans planted a few rows away from the trial were free from bacterial infection. Furthermore, the symptoms on Black Valentine leaflets were similar to those of one of the two types of symptoms associated with bacterial blight of beans, which occurs mainly in the tropical lowlands (10). This sequence of events suggested that more than one *Xanthomonas* strain or species was causing bacterial blight of beans in the tropics. This work presents data on the isolation and virulence of two types of *Xanthomonas* cultures that were pathogenic on beans.

MATERIALS AND METHODS.—Highly susceptible cultivars of *P. vulgaris* and *V. unguiculata* were used to test the virulence of bacterial cultures. These cultivars included: La Vega, a dry bean with dull black seeds; Bountiful, a bush bean; southern pea selection PR-V-70-10-R65 from USDA Plant Introduction (P.I.) accession 293474; and Early Ramshorn, a blackeye pea.

Bacterial isolates were grown on either Bacto Nutrient Agar (Difco Laboratories) or on YDC medium (6). Cultures were kept at 22-24 C. for two-to-four days before a turbid aqueous inoculum suspension was prepared. Isolates were stored as bacterial suspensions in distilled water at 12 C. Bacterial species used in the pathogenicity test were obtained from the American Type Culture Collection, and included *Xp* 9563, *X. phaseoli* var. *fuscans* (Burkh.) Starr & Burkh. (abbreviation here = *Xpf*) 13464, and *Xv* 11648.

Pathogen-free seeds were dusted with benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] before they were sown in steam-sterilized greenhouse soil mixture. Black plastic bags, 25 cm wide and 30 cm high, were used as pots. Six seedlings were permitted to remain in each pot and were placed on a greenhouse bench until 3 weeks old. Four seedlings per pot were inoculated with a given bacterial isolate, while two were kept as noninoculated test plants. In each inoculation trial, one set of seedlings was inoculated with sterile distilled water as a check for contamination. Inoculated seedlings were placed in an incubator made up of a greenhouse bench covered with a transparent polyethylene cage. Automatic misters were placed every 1.22 m (4 feet) in the center of the bench in the incubator. Inoculated seedlings were kept in the incubator for 7-10 days before disease reactions were recorded.

Seedlings were inoculated in two ways: (i) leaflet inoculation, which consisted of dusting leaflets with 50-μm Carborundum, and then either immersing a wooden

TABLE 1. Viruelnce of *Xanthomonas* isolated from Black Valentine bean (isolate 86) and southern pea P.I. accession 293474 (isolate 85)

Host	Tissue inoculated	Disease rating of isolate[a,b] 85	86
Vigna unguiculata			
P.I. 293474	Leaf	10	6
	Stem	10	5
Phaseolus vulgaris			
La Vega	Leaf	10	7
	Stem	4	8
Black Valentine	Leaf	7	5
	Stem	4	1

[a]Disease rating scale: 1 = no infection, and 10 = very severe response. Ratings of 2 to 9 indicate intermediate grades between 1 and 10.

[b]Culture 85 was isolated from P.I. 293474, and culture 86 was isolated from Black Valentine.

TABLE 2. Results of cultural, morphological, and physiological tests for classification of Puerto Rican *Xanthomonas* isolates 22, 85, and 86

Test	Reaction
Morphology	
Capsule stain	
Tyrosinase	Negative
Cellulose test	Negative
Physiology	
Gram stain	Negative
Indole	Negative
Methyl red	Negative
Voges-Proskauer	Negative
Nitrite	Negative
Ammonia	Weak-positive
Urea	Slow-positive
Gelatin	Liquefied
Yeast pectate medium	Liquefaction, alkaline
Litmus milk	Digestion of casein
Sodium malonate	Positive
Hydrogen sulfide	Positive
Hydrolysis of starch	Positive
Lipolytic activity	Positive
Triple sugar iron	
Alkaline and H_2S	Positive
Catalase test	Positive
Benedict test	Positive
Oxidase test	Positive
Hydrolysis of sodium hippurate	
Aesculin hydrolysis	Positive
Carbohydrates	
Lactose	Late positive
Pentoses and hexoses	Positive
Salicin and rhamnose	Negative
Dulcitol and inulin	Negative
Antisera	
X. phaseoli var. *sojensis* (Hedges) Starr & Burkh.	
1/2,560 titer	Positive
X. phaseoli (Smith) Dows.	
1/640 titer	Positive
X. campestris (Pam.) Dows.	
1/640 titer	Positive

pot label in the inoculum and lightly rubbing it over the leaf surface, or applying the inoculum with the thumb and forefinger, (ii) stem inoculation, which consisted of placing a droplet of bacterial suspension on the axis of a node and then piercing the stem repeatedly through the inoculum with a sterile needle. Re-isolations were made from both stem cankers and leaf lesions of bean and southern pea test seedlings. A second inoculation was performed with the re-isolated bacteria to confirm pathogenicity.

Grading seedling response to inoculation.—Responses to leaflet and stem inoculations were graded separately, and the average of the two grades was regarded as an expression of susceptibility. Leaf symptoms were graded from 1 to 10. Grade "1" indicated absence of disease, and "10" indicated wilted leaflets, petiole usually drooping and extension of colonization from the petiole into the stem. Grades 2 to 9 indicated intermediate responses. Stem symptoms were graded from 1 to 10 also, grade "1" indicating whitish callus tissue where the stem was pierced by the needle, and "10" indicating a vascular discoloration extending 3.5 cm or more, canker formation at the node, and abscission of the leaves at the infected node. Grades 2 to 9 referred to intermediate responses.

RESULTS.—Yellow bacterial colonies were isolated from diseased leaves of the southern pea, P.I. 293474, as well as from Black Valentine bean located in an adjacent row. Bacteria isolated from both host plants had the colony appearance of *Xanthomonas*. Culture 85 was isolated from P.I. 293474 and 86 from Black Valentine. Since only bean plants adjacent to the southern pea trial had become diseased, it was suspected that beans were also susceptible to the bacterium that causes blight and bacterial stem canker of southern pea. An inoculation test indicated that cultures 85 and 86 were pathogenic to both host species (Table 1). Culture 86 was less virulent than 85, but bacteria were re-isolated from the diseased tissues. Various cultural, morphological, and physiological tests were carried out for cultures 85 and 86, and *Xanthomonas* culture 22, which was isolated from the bean variety Contender (10). These cultures had yellow butyrous appearance on potato slant, and on tryptophane glucose agar. Also they caused turbidity in broth, forming both a ring and sediment. Morphologically, they were slightly motile and had thin walls. Because all three cultures had similar reactions, only one reaction is presented for all (Table 2).

As it is often difficult to distinguish taxonomically between various *Xanthomonas* isolates from cultural and physiological tests (4), it was decided to compare their pathogenicity with those of standard cultures from the American Type Culture Collection (ATCC).

Puerto Rican isolates also included in these inoculation tests were 113, a re-isolate from culture 85; and 253, an isolate from the bean variety Apollo. The ATCC standard cultures were *Xp* 9563, *Xpf* 13464, and *Xv* 11648. Table 3 gives the response of differential hosts to inoculation with these five bacterial cultures.

Puerto Rican isolate 113 was similar to ATCC *Xv* 11648 in its pathogenicity, while Puerto Rican isolate 253 was similar to ATCC *Xp* 9563 in its pathogenicity on the four differential hosts (Table 3).

TABLE 3. Comparison of pathogenicity of Puerto Rican isolates of *Xanthomonas* with those from the American Type Culture Collection (ATCC)

Culture[a] number	Host response[b,c]			
	Phaseolus vulgaris		*Vigna unguiculata*	
	La Vega	Bountiful	P.I. 293474	Early Ramshorn
Puerto Rico				
113	9	8	10	10
200	7	10	2	3
253	9	10	3	3
ATCC				
9563	9	9	2	3
11648	6	6	7	10
13464	10	10	2	4

[a]Culture number descriptions:
113 = local isolate, virulent on *P. vulgaris* and *V. unguiculata*.
200 = local isolate, virulent on *P. vulgaris*, brown pigment in culture.
253 = local isolate, virulent only on *P. vulgaris*.
9563 = *X. phaseoli* of American Type Culture Collection (ATCC).
13464 = *X. phaseoli* var. *fuscans* of ATCC.
11648 = *X. vignicola* of ATCC.
[b]1 = absence of response, and 10 = wilting and severe canker formation. Intermediate responses are represented by ratings of 2 to 9.
[c]Recorded seven days after inoculation.

Sixty-seven isolates obtained from diseased beans and southern peas in Puerto Rico were tested on the bacterial blight differential cultivars during 1972 and 1973 (11). Out of this total, 56 were pathogenic on both beans and southern peas, and 11 were pathogenic only on beans. Two of the latter group produced brown pigments on nutrient agar medium. All 11 cultivars pathogenic on *P. vulgaris* were isolated from beans; whereas, of the 56 isolates pathogenic on *P. vulgaris* and *V. unguiculata*, equal number were isolated from each host species. Also, of 23 *Xanthomonas* cultures isolated from diseased beans from 10 countries in tropical America, 11 cultures were pathogenic to both beans and southern peas.

DISCUSSION.—The problem of recognizing more than one pathogenic form of *Xanthomonas* on *P. vulgaris* has occupied bean pathologists for several decades (1, 4). Burkholder (1) stated, "Furthermore, the two pathogens being so similar in appearance, the cowpea organism already might be infecting the bean, and isolates when made might be mistaken for *Xanthomonas phaseoli*." The data on the pathogenicity of isolates collected in the tropics support Burkholder's contention that *Xanthomonas* isolates pathogenic on southern peas could also be pathogenic on beans. Actually, in tropical America, it seems that often *Xanthomonas* isolates from beans are also pathogenic on southern peas.

There were no physiological, morphological, or cultural differences between *Xanthomonas* isolates 85 from southern peas and 86 from beans, and the two isolates were pathogenic on both hosts. Culture 85 (or 113) has the same pathogenicity as ATCC *Xv* 11648. The two Puerto Rican *Xanthomonas* cultures, 200 and 253, also had the same pathogenicity as those of the ATCC *Xpf* 13464 and *Xp* 9563, respectively.

Because all yellow bacterial isolates from stem cankers and leaf lesions on southern peas were pathogenic on beans, it could be concluded that the known causal organism of this disease, *Xv*, is pathogenic to *P. vulgaris*. Conversely, because numerous yellow bacterial isolates from stem cankers and leaf lesions of beans were pathogenic to southern peas, it could be assumed that *Xp* is pathogenic to *V. unguiculata*. The latter assumption is not supported, because there were bacterial isolates from beans, such as 200 and 253, that were pathogenic only to beans. Therefore, in Puerto Rico, as well as in other countries in tropical America, there are at least two pathogenic types of *Xanthomonas* that cause bacterial blight of beans. One type, pathogenic only to beans, could be designated as *Xp* or *Xpf*, while the second type, which affects both beans and southern peas, could be designated as either *Xv* or as a widely virulent strain of *Xp*.

Puerto Rican isolates 22, 85, and 86 gave the same reactions to Gram stain, H_2S production, and casein hydrolysis tests as those presented by Saettler for *Xp* (7). They differed from *Xv*, which causes coagulation of casein (1). Whether this slight physiologic difference merits designating these cultures either as *Xp* or *Xv* is the concern of the systematic bacteriologist. From the point of view of pathogenicity, it is convenient to differentiate them as two pathogenic types. Hence, until proven otherwise, the *Xanthomonas* isolates that affect both bean and southern peas will be considered as *X. vignicola*, as originally suggested by Burkholder (1).

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