Serological Specificity of *Xanthomonas oryzae*, Incitant of Bacterial Blight of Rice

I. C. MAHANTA AND S. K. ADDY

Orissa University of Agriculture and Technology, Bhubaneswar, India

Agglutination and gel diffusion tests from 12 nomenclatures of the *Xanthomonas campestris* group, *Xanthomonas albilineans*, and individual strains of *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Agrobacterium tumefaciens*, *Erwinia amylovora*, *Erwinia stewartii*, and *Corynebacterium sepedonicum* indicated serological distinctiveness of *Xanthomonas oryzae*, a member of the *X. campestris* group. Heat-stable, species-specific antigens were demonstrated in *X. oryzae*. The serological tests also revealed that most of the *Xanthomonas* spp. and a few *Pseudomonas* and *Erwinia* spp. shared some of the common antigens with *X. oryzae*. *X. albilineans*, *A. tumefaciens*, and *C. sepedonicum* did not share any antigen with *X. oryzae*.

Although serology has been utilized in recent years for determining the specific taxonomic relationship among plant pathogens (1, 3, 4, 6, 8), no attempt has been made to elucidate the serological relationships between *Xanthomonas oryzae*, a member of the *Xanthomonas campestris* group, and other xanthomonads. In a study of segmental homology of deoxyribonucleic acid, it was reported that *X. oryzae* and *X. translucens* f. sp. *oryzicola*, the streak pathogen of rice, related only at the 10% level (7). It was also reported that the guanine-plus-cytosine content of the latter was 69 mol% as compared to 64.4 mol% for the former. Recently *X. oryzae* could be differentiated from *X. translucens* f. sp. *oryzicola* by its failure to produce certain enzymes, namely, phenylalanine deaminase, tyrosinase, and β -glucosidase (9). *X. albilineans* also differed from *X. campestris* in certain biochemical properties such as proteolysis of milk, production of H_2S from peptone, tolerance of sodium chloride, and acid production from arabinose, trehalose, and cellobiose (2). In the present investigation, both agglutination and gel diffusion techniques were employed to reveal specific antigenic differences between *X. oryzae* and a few selected species of *Xanthomonas* as well as some bacterial phytopathogens belonging to other genera.

Table 1 lists the strains of various phytopathogenic bacteria used as antigens. Although 12 other isolates of *X. oryzae* and 3 other isolates of *X. translucens* f. sp. *oryzicola* were also tested, they were not included in Table 1, as essentially the same results were obtained as with the ones included.

Antiserum against *X. oryzae* (H120) was raised in white rabbits that were immunized by

intravenous injections, after adjustment of protein concentration of the antigenic preparation to 675 μ g/ml (5).

Agglutination tests were performed at 37°C in small conical agglutination tubes using a two-fold dilution series of the antiserum.

Gel diffusion tests were performed in 10-cm Pyrex glass petri dishes containing 12 ml of 1% Ionagar no. 2 (Oxoid) in 0.05 M phosphate-buffered saline at pH 7.8.

The results of the agglutination test in the present study indicate that the highest titer of 5,120 was obtained with the homologous as well as the homologous-heated (100°C for 1 h) antigens (Table 1). The antisera to *X. oryzae* reacted to a high titer with *X. translucens* f. sp. *oryzicola*, *X. translucens* f. sp. *cerealis*, and *X. malvacearum*, indicating closer relationship. It, however, reacted to low titers with other *Xanthomonas*, *Pseudomonas*, and *Erwinia* spp., indicating the sharing of common antigens. *X. albilineans*, *Agrobacterium tumefaciens*, and *Corynebacterium sepedonicum* did not react to any titer.

The results of the gel diffusion tests indicated that the homologous antigen produced a greater number of precipitin bands than were produced by heterologous antigens. At least one sharp band showing identity was produced by antigens of *X. translucens* f. sp. *oryzicola*, *X. malvacearum*, *X. oryzae*, *X. vasculorum*, *X. translucens* f. sp. *cerealis*, and *X. phaseoli*. Similarly, another sharp band showing identity was also produced by antigens of *X. hyacinthi*, *X. campestris*, *X. vesicatoria*, *X. pruni*, and *X. citri*. Identity was also demonstrated in one of the bands produced by *X. juglandis* and *X. citri*. *X. albilineans* did not react serologically.

TABLE 1. *Antigenic reactions of some phytopathogenic bacteria against antisera to X. oryzae (H120)*

Antigen	Agglutinin titer	No. of precipitin bands	
		Sharp	Faint
<i>Xanthomonas oryzae</i> (H120)	5,120 ^a	4	2
<i>X. oryzae</i> (H120) (heated)	5,120	1	1
<i>X. tr. f. sp. oryzicola</i> (S3) ^b	2,560	3	0
<i>X. malvacearum</i> (XM104)	1,280	2	0
<i>X. tr. f. sp. cerealis</i> (XT12) ^b	1,280	2	1
<i>X. vasculorum</i> (XV106)	640	1	1
<i>X. campestris</i> (XC161)	640	4	1
<i>X. juglandis</i> (XJ102)	640	2	1
<i>X. pruni</i> (XP197)	640	3	1
<i>X. vesicatoria</i> (XV173)	640	1	2
<i>X. phaseoli</i> (XP205)	320	1	1
<i>X. citri</i> (XC1)	320	3	0
<i>X. hyacinthi</i> (XH111)	160	1	2
<i>X. albilineans</i> (XA135)	0	0	0
<i>Pseudomonas syringae</i> (PS200)	320	3	0
<i>P. solanacearum</i> (PS1)	160	4	0
<i>Erwinia stewartii</i> (SS12)	160	3	1
<i>E. amylovora</i> (E8)	80	1	0
<i>Agrobacterium tumefaciens</i> (TT136)	0	0	0
<i>Corynebacterium sepe-donicum</i> (CS118)	0	0	0

^a Number represents the denominator of the greatest dilution of the serum clearly showing agglutination.

^b *X. tr.*, *X. translucens*.

Of the phytopathogens belonging to other genera, some of the bands produced by antigens of *Pseudomonas syringae* crossed the bands produced by *P. solanacearum* and *Erwinia stewartii*, indicating nonidentity of these antigenic components. Similarly, the band produced by *E. amylovora* also crossed one of the bands produced by *E. stewartii*, again indicating nonidentity. *Corynebacterium sepe-donicum* and *Agrobacterium tumefaciens* produced no band, indicating their negative serological relationship with *X. oryzae*.

When heated antigens of all the xanthomonads and strains belonging to other genera were tested against antisera to *X. oryzae*, none of them produced any band except the homologous antigen. The heat-stable bands of the homologous antigen were apparently species specific. The process of heating the antigens at 100°C for 1 h has proved particularly advantageous in eliminating the common antigens and demonstrating the precipitation lines produced by heat-stable specific antigens of *X. oryzae*. The heat-labile common antigens are probably involved in the low levels of agglutinin titers obtained with the different species against antisera to *X. oryzae*. The heat-stable nature of the an-

tigen is also helpful in distinguishing *X. oryzae* from *X. translucens* f. sp. *oryzicola*, the streak pathogen in infected rice plants where the latter is commonly encountered as a mixture. This technique, therefore, should be considered as an additional tool in the recently developed method of differentiating *X. oryzae* from *X. translucens* f. sp. *oryzicola* by the enzymatic tests (9). Our result is also in good agreement with that of Murata and Starr (7) who reported only 10% DNA homology between *X. oryzae* and *X. translucens* f. sp. *oryzicola*, reflecting significant genetic divergence.

In the present study, the results of the agglutination as well as the gel diffusion tests further clearly establish that *Xanthomonas oryzae* is serologically distinct from other *Xanthomonas* nomenspecies. The high homologous agglutination titer was unequaled by any of the species of *Xanthomonas*. Similarly the characteristic precipitation band pattern produced in the gel diffusion test by the homologous antigen was not observed with antigens of any other species.

It is also apparent from the results of the foregoing experiments that most of the *Xanthomonas* spp. as well as a few *Pseudomonas* and *Erwinia* spp. share some antigenic components with *X. oryzae*. Therefore, it is quite logical that many of the species of *Xanthomonas* tested have some common basis to be placed under the genus *Xanthomonas*.

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REPRINT REQUESTS

Address reprint requests to: Dr. S. K. Addy, Department of Plant Pathology, Assam Agricultural University, Jorhat, 785013, Assam, India.

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