Phage Typing and Lysotype Distribution of *Xanthomonas campestris*

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ABSTRACT

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Eleven phages of *Xanthomonas campestris* were tested and found to be species specific. An assembled group of 145 *X. campestris* strains also was spot-tested against the phages at titers of 100 × routine-test-dilution. Based on differences in lytic patterns, a phage-typing scheme was proposed that utilizes five phages for differentiation of the bacteria into two major lysotypes designated A and B. Subtypes were distinguished by their differential lytic responses to the typing phages used to identify the major lysotypes. A group of 26 bacterial strains could not be typed because they were resistant to the phages. The geographical distribution of lysotypes based on bacterial origins also was presented.

The host-specificity of bacteriophages (hereafter called "phages") of plant pathogens forms the basis of many useful techniques (10); for example, phages have been used for rapid identification of bacterial pathogens (16) and to resolve problems in speciation (2–4,12). Bacterial strains can be typed by differential patterns of sensitivity to phages. Phage-typing schemes have been used extensively in epidemiological studies of human pathogens (1,11) and for studying the occurrence and distribution of lysotypes of phytopathogens (5–7,9,15,17).

Phages that can attack *Xanthomonas campestris* have been reported previously. A host-specific phage was reported by Hayward (6) and Sutton and Quadling (14) were able to isolate a highly specific temperate phage. However, polyvirulent phages that also lyse *X. campestris* have been reported (5,12,13).

We recently have studied other phages of *X. campestris* and this paper reports the results of testing the host-specificity and differential lytic capability of those phages.

MATERIALS AND METHODS

Propagation and maintenance of phage stocks. The 11 *X. campestris* phages and their propagating hosts used in the present study have been previously described (8). High-titer stocks prepared by the agar plate method were purified after one cycle of differential centrifugation and stored over chloroform (~1%) at 4 C. Phosphate-buffered tryptone water (PBT) was used as phage diluent, and samples of phage stocks were aerated to remove the chloroform prior to making a 10-fold dilution series for determination of the routine-test-dilution (RTD). RTD is defined as the highest dilution just giving confluent lysis of the propagating host. Tryptone-yeast-glucose (TYG) medium was used in spot-tests and consists of tryptone (5 g), glucose (5 g), yeast extract (3 g) and 10^{-3} M $MgSO_4 \cdot 7H_2O$ in 1 L of water.

Bacterial strains. *X. campestris* strains were kindly provided by many donors and the original bacterial designations are listed: E. Echandi (North Carolina State University, Raleigh) B-24, XC43, XC47, XC49, XC57, XC61, XC70, XC81–84, XC93, XC97–98, XC107, XC112–115, XC118, XC131 and XC133; M. P. Starr (International Collection of Phytopathogenic Bacteria, University of California, Davis), (ICPB) XC1, XC16, XC106, XC122, XC128, XC130–132, XC135, XC149, XC153, XC154, XC157, XC163, and XC169; S. T. Hsu (National Chung Hsing University, Taichung, Taiwan), XC6–11; N. W. Schaad (University of Georgia, Experiment Station), B-2, B-6, B-8, B-12, and B-14; A. C. Hayward (University of Queensland, Brisbane, Australia), 030, 0530, 0523, and MM0239; P. H. Williams (University of Wisconsin, Madison), PHW-42-Wisconsin, PHW-46-Florida and PHW-109-Australia; M. Goto (Shizuoka University, Shizuoka, Japan), OH_2 and OK_2; S. Yoshimura (National Institute of Agricultural Sciences, Tokyo, Japan), X1-1-1 and 76F-1; K. Masabayashi (T. Sakata & Co., Yokohama, Japan), two undesignated cultures; and J. C. Sutton (University of Guelph, Ontario, Canada), two undesignated cultures.

Other bacterial strains used in the study either were obtained from the culture collection maintained at the Department of Plant Pathology of the University of Hawaii, or were isolated from collected diseased materials and specimens sent to the Plant Disease Clinic, Cooperative Extension Service. The number of isolates tested for each species include: *Xanthomonas campestris,* 82; *X. ampelina,* one; *X. begoniae,* three; *X. dieffenbachiae,* one; *X. hederae,* one; *X. manihotis,* one; *X. phaseoli,* four; *X. pisi,* one; *X. vesicatoria,* seven; *X. vitians,* one; *Agrobacterium tumefacins,* one; *Corynebacterium michiganense,* one; *C. flaccumfaciens,* one; *Erwinia amylovora,* one; *E. atroseptica,* one; *E. carotovora,* one; *E. herbicola,* one; *Pseudomonas cepacia,* one; *P. fluorescens,* one; *P. solanacearum,* one; *P. syringae,* one; and *Escherichia coli,* one. In addition, some xanthomonads isolated from *Polyscias* spp., five; *Allium* spp., five; and *Cordyline terminalis,* two; and three unidentified yellow saprophytes isolated from cabbage seeds also were tested. Prior to use all bacterial cultures were grown on TYG

TABLE 1. Lysotypes of *Xanthomonas campestris* differentiated by phage reaction

Lysotype	Reactions[a] of bacteriophage: HT_{3h}	HP_3	OH_2	HXX	P_{1-3a}	No. of isolates
A 1	+	+	+	−	−	62
A 2	+	+	−	−	−	1
A 3	+	−	+	−	−	6
A 4	+	−	−	−	−	14
B 1	−	−	−	+	+	25
B 2	−	−	−	+	−	5
B 3	−	−	−	−	+	6
NT[b]	−	−	−	−	−	26

[a] Symbols: + = sensitive; − = resistant. Spot tests with phage titers at RTD × 100. RTD = routine test dilution = the highest dilution that causes confluent lysis of the propagating host.

[b] Not typeable.

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TABLE 2. Distribution of *Xanthomonas campestris* lysotypes

Location[a]	Number of isolates per lysotype								
	A1	A2	A3	A4	B1	B2	B3	NT	Total
United States									
Hawaii	53	0	1	6	8	2	0	8	78
N. Carolina	0	0	0	3	6	0	3	3	15
Georgia	2	0	0	1	2	0	0	0	5
Wisconsin	2	0	2	2	0	0	0	1	7
Florida	0	0	0	1	1	0	0	1	3
Oregon	1	0	0	0	0	0	0	0	1
Others[b]	1	0	0	0	0	0	0	3	4
Japan	1	1	1	0	2	0	0	3	8
Taiwan	0	0	0	0	3	3	0	0	6
Australia	0	0	1	0	3	0	1	0	5
Canada	0	0	0	0	0	0	2	0	2
Mauritius	0	0	1	0	0	0	0	0	1
Germany	1	0	0	0	0	0	0	0	1
S. Africa	1	0	0	0	0	0	0	0	1
Malawi[c]	0	0	0	1	0	0	0	0	1
Others[d]	0	0	0	0	0	0	0	7	7

[a] Location of original isolation or of source material.
[b] Origins not identified.
[c] Originally listed as Nyasaland.
[d] ICPB isolates from U.K. (2); India (2), and one each from New Zealand, Rhodesia, and Tanzania (Tanganyika). Four ICPB isolates of U.S. origin also were not typeable.

agar except for *X. ampelina* which was cultured on yeast-glucose-calcium carbonate agar.

Phage typing. Spot-tests were performed by using the double-agar layer method where bacterial suspensions were seeded into 2.5 ml of water agar (0.8%) and poured over TYG bottom agar. Phage suspensions were spotted with a 4-mm-diameter platinum loop, and after absorption of the phage droplets, the plates were incubated at 28 C. Earlier tests using phage dilutions at RTD often gave inconsistent results. Consequently, phage titers were raised to RTD × 100 in all subsequent tests. The potency of each phage test suspension was checked at each typing by the inclusion of the complete set of propagating hosts. Except for phages A342 and HT_{3h}, the titers of the phages remained stable. Test phage suspensions of phages A342 and HT_{3h} were freshly diluted before each typing.

RESULTS

The 11 phages were host-specific since only *X. campestris* strains were lysed in the spot-tests. Moreover, specificity within the species was further restricted since no phage could lyse all 145 isolates that were tested. Conversely, no bacterial isolate was lysed by all the phages. Twenty-six or 18% of the isolates were completely resistant.

Based on differences in the lytic responses of the sensitive *X. campestris* strains to the phages, it was possible to differentiate lysotypes or bacterial strains with similar lytic patterns. Some of the phages had similar host ranges. By eliminating some of these, and phages RR68 and A342 which could lyse only their propagating hosts, a simplified typing scheme with five phages was assembled (Table 1). Two main lysotypes designated A and B can be recognized by differential responses to HT_{3h}. Within each major lysotype, subtypes can be distinguished by differences in reactions to the typing phages of each group. Twenty-six resistant isolates are listed as nontypable in the present scheme. These include 11 strains (from diverse geographic origins) that were received from the International Collection of Phytopathogenic Bacteria.

Subtypes within group A may be differentiated by their reaction to phages HP_3 and OH_2. Four subtypes were recognized, with the most bacterial strains identified as lysotype A1 (Table 1). Phages HXX and P_{1-3a} were used to identify B group in which three subtypes were encountered. The phage type distribution which is listed in Table 2 identifies the *X. campestris* strains by the location of original isolation or by origin of diseased material from which the bacteria were isolated.

DISCUSSION

The typing scheme proposed for *X. campestris* is relatively simple, and the two major lysotypes with several subtypes may be identified by differential lytic patterns. Interestingly, the morphologies of the typing phages within each group are similar. Phages HT_{3h}, HP_3, and OH_2 used for differentiating A subtypes have tails with contractile tail sheaths while phages HXX and P_{1-3a} used for identifying B subtypes have long flexuous noncontractile tails.

Lysotypes of both A and B groups were found in strains originating from Hawaii, Georgia, Japan, and Australia. In Hawaii, all the *X. campestris* strains from commercial cabbage farms have the A lysotype except for two strains. Among those with A lysotype, A1 lysotype was predominant. Bacteria with the other lysotypes were either isolated from packeted seeds sold in garden shops or were from materials submitted to the Plant Disease Clinic by the public.

Modifications in the proposed typing scheme can be made to accommodate additional subtypes. Highly specific phages like RR68 and A342 are to be avoided since they add to the phage list without adding any significant information to the scheme as a whole. Their values are not discounted, however, since their extreme specificities may be exploited as markers for comparative studies. Extensive tests should be made with phages and bacterial strains of different geographic origins before a comprehensive typing scheme can be developed for *X. campestris*. Other major lysotypes probably do exist and an indication of this is the fact that, of 15 strains received from the International Collection of Phytopathogenic Bacteria, 11 of these diverse bacterial isolates could not be typed by the phages of the present scheme.

Phage typing done with care and consistency is useful in epidemiological studies because it is a relatively simple and reliable technique for differentiating numerous strains. With this typing scheme, it is now possible to study the dissemination of bacterial inocula using lysotypes as stable markers, or even to relate the occurrence and distribution of black rot of crucifers to particular inoculum sources like infected seeds and diseased crop debris.

The phages and their bacterial hosts have been deposited in the American Type Culture Collection, Rockville MD 20852.

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