Xanthomonas citriのファージーのXanthomonas属細菌との比較について

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者名	後藤,正夫 Strr,M.P.
発行元	日本植物病理學會
巻/号	38巻3号
掲載ページ	p. 226-248
発行年月	1972年7月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

日植病報 38: 226–248 (1972)
Ann. Phytopath. Soc. Japan 38: 226–248 (1972)

Phage-host Relationships of *Xanthomonas citri* Compared with those of Other Xanthomonads

Masao GOTO* and M. P. STARR**

後藤正夫*・M. P. STARR**: *Xanthomonas citri* のファージ・細菌相互関係，とくに他の *Xanthomonas* 属細菌との比較について

Abstract

The temperate phages of *Xanthomonas citri* are completely inactivated by chloroform treatment, whereas the virulent phages are quite stable to the treatment. About 80 percent (but not all) of an extensive series of *X. citri* isolates is lysed by phage Cp1, Cp2, or both. Among 136 isolates of 43 *Xanthomonas* nomenspecies, only 9 isolates belonging to 7 nomenspecies [*X. begoniae* (XB8, XB111), *X. blepharidis* (XB115), *X. cucurbitae* (XC6), *X. phaseoli* (XP4, Xph), *X. phaseoli* var. *sojense* (XP21), *X. marantae* (XM126), and *X. translucens* f. sp. *phleum-pratense* (XT103)] showed susceptibility to any of the *X. citri* phages. One of the Stolp-Starr phages, ph25, showed lytic reactions on almost all isolates of *X. citri*, as well as on many other *Xanthomonas* nomenspecies. From the cross-susceptibility tests between *Xanthomonas* bacteria and various phages from different sources, it was concluded that, in general, the *Xanthomonas* phages which form tiny plaques of pinhead size could frequently be found in soil, and that they showed very broad host ranges, which extended not only over the different isolates of a homologous nomenspecies but also over strains of many heterologous nomenspecies. In contrast, the *Xanthomonas* phages which were isolated from diseased plant tissues usually showed very limited host ranges. (Received July 13, 1971)

The phage-host relationships in the genus *Xanthomonas* have been reported as highly specific with respect to the individual nomenspecies[3,6,13,15,16,17,18,24,25]. On the other hand, Stolp and Starr[23] found a great heterogeniety in the phage-host interactions of xanthomonads; that is, some of the twelve phage preparations they studied lysed only a few *Xanthomonas* nomenspecies and others lysed a great array of *Xanthomonas* nomenspecies. Furthermore, Stolp and Starr[23] showed that many *Xanthomonas* nomenspecies shared common lytic reactions with several different phage preparations. Left as an open question was the reason for such great discrepancies between the results obtained by Stolp and Starr[23] and by the other workers. One possible reason might be based on the rather limited number of bacterial strains used by most workers, compared with the enormous number of bacterial strains used by Stolp and Starr. It has been thought, however, more likely that a difference in the source from which the phages were isolated might also be responsible for the discrepancies; the Stolp-Starr phages has been isolated mainly from soils, whereas those used by other workers had usually been obtained from diseased plant materials. To check this possibility, we have compared the behavior of the twelve Stolp-Starr phages with that of other *Xanthomonas* phages which were isolated from diseased plant materials.

* Faculty of Agriculture, Shizuoka University, Iwata, Shizuoka, Japan. 静岡大学農学部
** Department of Bacteriology, University of California, Davis, California, U.S.A. カリフォルニア大学

The phage-host relationships in *Xanthomonas citri* also have been reported[18,26] to be highly specific. However, this bacterium was unfortunately not included in the Stolp-Starr investigation (because of U.S.A. quarantine regulations)[22] and the interrelationships between *X. citri* and the other xanthomonads from the viewpoint of phage-bacteria interaction was left for the present study in which the dangerous citrus canker pathogen was examined only in Japan where citrus canker is endemic. Our work on the lysogenization of xanthomonads by *X. citri* temperate phages is presented elsewhere[11].

Materials and methods

Bacterial cultures All *Xanthomonas* cultures were maintained on potato-glucose or yeast-glucose-$CaCO_3$ agar slants; stored at 5°C during this investigation. Eighty-seven isolates of *X. citri* (listed in Table 2) were collected in 1962 from the citrus growing areas in Shizuoka Prefecture, Japan. To indicate their original host plants, the letter N is included in the designation (Table 2) of each isolate from Natsudaidai orange; likewise, U for Unshu orange, B for navel orange, K for trifoliate orange, L for lemon, H for Hassaku orange, F for Fukuhara orange, S for Sampokan orange, T for Trovita orange, and P for Ponkan orange. Two *X. citri* isolates, 2–5 and 49–1, were provided by Dr. S. Wakimoto as the host bacteria for the phages Cp1 and Cp2, respectively[28]. Seven isolates of *X. oryzae* (B2, B10, B18, B20, B56, B69, B72), and five of *X. translucens* f. sp. *oryzicola*[2] (S1, S2, S3, S24, S27), were isolated by Goto[6,9,10] in the Philippines in 1963. The other isolates of *X. oryzae* were collected by Goto in Shizuoka Prefecture. The following cultures, as well as those listed in Table 7, were provided from the International Collection of Phytopathogenic Bacteria (ICPB), University of California, Davis (Starr[22]): *X. blepharidis* (XB115), *X. marantae* (XM126), *X. phaseoli* (XP104–1 and XP4), *X. begoniae* (XB8), *X. celebensis* (XC144), *X. corylina* (XC12), *X. cucurbitae* (XC6), *X. geranii* (XG10), *X. hyacinthi* (XH110), *X. malvacearum* (XM2 and XM106), *X. phaseoli* var. *sojense* (XP21), *X. ricinicola* (XO101 Sm^r), and *Pseudomonas* (?; see Stolp and Starr[23]) *rimaefaciens* (PR107). The cultures of other xanthomonads listed below were collected by Goto in Shizuoka Prefecture: *X.campestris* (1, 2), *X. nigromaculans* (1), *X. phaseoli* (Xph), *X. phaseoli* var. *sojense* (Xps), *X. physalidicola* (1, 2), *X. pisi* (1, 2), and *X. pruni* (1).

Culture media Peptone-sucrose agar medium: Distilled water 1,000 m*l*, peptone (Kyokuto brand) 10 g, sucrose (Kyokuto brand) 10 g, sodium glutamate (Ajinomoto brand) 0.5 g, and agar 15 g. Peptone-sucrose solution: The same composition as the above medium without agar. Potato-glucose agar: Potato decoction (200 g potato/1,000 m*l* distilled water) 1,000 m*l*, glucose (Daiichi Seiyaku Co., Ltd.) 10 g, and agar 15 g. Yeast-glucose-$CaCO_3$ agar: Distilled water 1,000 m*l*, yeast extract (Daigo brand) 10 g, glucose (Daiichi Seiyaku Co., Ltd.) 20 g, finely-divided $CaCO_3$ (Koso Chemical Co., Ltd.) 20 g, and agar 15 g.

Phage preparations To isolate virulent phages from diseased plants, about 10 g of the diseased plant materials (lesions) were collected, cut into small pieces, and added to about 100 m*l* tap water. An agar slant culture of a stock culture of a *Xanthomonas* strain corresponding to the causal bacterium of the particular disease was added to the sample for phage enrichment, at the rate of one slant per sample two to three times daily. The sample was kept at room temperature (about 25°C). A part of the liquid from the sample was removed one or two weeks later, centrifuged, filtered through a 0.22 μm Millipore filter, and plated with the host bacterium to test for plaque formation. When phage isolation was unsuccessful by this method, the causal bacterium itself was isolated on agar plates from the same lesions as those being used for phage isolation, before the plant materials were processed as mentioned above. The bacterial strain thus isolated was used for the phage enrichment

as well as for the phage isolation.

All phages were purified by the conventional single plaque technique and propagated in cultures of the host organism in peptone-sucrose solution from single plaques formed on agar plates with the host bacteria. The cultures were treated with chloroform after 24-hour incubation; 0.5 m*l* of chloroform was added to 10 m*l* of the phage-host preparation and the tubes were shaken vigorously so that tiny droplets of the chloroform were distributed uniformly throughout the medium. After the chloroform was completely settled, the supernatant was transferred to a sterillized tube. When the cultures were clouded by bacterial growth, they were clarified by centrifugation. Average titers of the phages were 10^8 to 10^9 plaque forming units (pfu) per m*l*. All phage samples were maintained in peptone-sucrose solution at 5°C.

The phages used in this study are listed in Table 1. Two virulent *X. citri* phages (Cp1 and Cp2) were provided by Dr. S. Wakimoto and another virulent phage (Ap) was isolated by Goto[5] from diseased citrus leaves. The temperate phages of *X. citri* were isolated from the respective bacterial cultures by the method of Hamon and Péron[12] with some modification. Bacteria, grown in 10 m*l* of peptone-sucrose solution for 24 hours, were irradiated with UV for 30 sec in Petri dishes, and one m*l* was transferred to 10 m*l* of the same medium and incubated for another 24 hours. Then the cultures were centrifuged at 6,000 rpm for 20 min and filtered through 0.22 μm Millipore filters, since the temperate phages were quite sensitive to chloroform. Average titres of these temperate phage

Table 1. Bacteriophage strains used in the study

Phage	Host bacterium (and strain) used for isolation, or propagation of phage	Source	Isolator
Cp1	*X. citri* (2-5)	citrus leaves infected by citrus canker	Wakimoto 1967
Cp2	*X. citri* (49-1)	citrus leaves infected by citrus canker	Wakimoto 1967
AP	*X. citri* (N4)	citrus leaves infected by citrus canker	Goto 1962
N23p[a)]	*X. citri* (N23)	culture of the bacterium	Goto 1967
N64p[a)]	*X. citri* (N64)	culture of the bacterium	Goto 1967
N79p[a)]	*X. citri* (N79)	culture of the bacterium	Goto 1967
U6p[a)]	*X. citri* (U6)	culture of the bacterium	Goto 1967
U7p[a)]	*X. citri* (U7)	culture of the bacterium	Goto 1967
U76p[a)]	*X. citri* (U76)	culture of the bacterium	Goto 1967
U85p[a)]	*X. citri* (U85)	culture of the bacterium	Goto 1967
L21p[a)]	*X. citri* (L21)	culture of the bacterium	Goto 1967
L22p[a)]	*X. citri* (L22)	culture of the bacterium	Goto 1967
K2p[a)]	*X. citri* (K2)	culture of the bacterium	Goto 1967
2-5p[a)]	*X. citri* (2-5)	culture of the bacterium	Goto 1967
49-1ap[a)]	*X. citri* (49-1a)	culture of the bacterium	Goto 1967
49-1bp[a)]	*X. citri* (49-1b)	culture of the bacterium	Goto 1967
Op1	*X. oryzae* (XOd)	rice leaves infected by bacterial leaf blight	Yoshii et al. 1953
Op1h	*X. oryzae* (XOa)	rice leaves infected by bacterial leaf blight	Ikari et al. 1958
Op1h2	*X. oryzae* (XOa)	rice leaves infected by bacterial leaf blight	Wakimoto 1960
Op2	*X. oryzae* (XOa)	rice leaves infected by bacterial leaf blight	Yoshimura et al. 1960

Bp1	*X. oryzae* (B2)	rice leaves infected by bacterial leaf blight	Goto 1965
Bp2	*X. oryzae* (B20)	rice leaves infected by bacterial leaf blight	Goto 1965
Bp3	*X. oryzae* (B18)	rice leaves infected by bacterial leaf blight	Goto 1965
Bp4	*X. oryzae* (B69)	rice leaves infected by bacterial leaf blight	Goto 1965
Bp5	*X. oryzae* (B56)	rice leaves infected by bacterial leaf blight	Goto 1965
Bp6	*X. oryzae* (B2)	rice leaves infected by bacterial leaf blight	Goto 1965
Bp7	*X. oryzae* (B2)	rice leaves infected by bacterial leaf blight	Goto 1965
Sp1	*X. translucens* f. sp. *oryzicola* (S3)	rice leaves infected by bacterial leaf streak	Goto 1965
Sp2	*X. translucens* f. sp. *oryzicola* (S1)	rice leaves infected by bacterial leaf streak	Goto 1965
Sp3	*X. translucens* f. sp. *oryzicola* (S24)	rice leaves infected by bacterial leaf streak	Goto 1965
Sp4	*X. translucens* f. sp. *oryzicola* (S3)	rice leaves infected by bacterial leaf streak	Goto 1965
Sp5	*X. translucens* f. sp. *oryzicola* (S24)	rice leaves infected by bacterial leaf streak	Goto 1965
Sp6	*X. translucens* f. sp. *oryzicola* (S24)	rice leaves infected by bacterial leaf streak	Goto 1965
Cmp	*X. campestris* (2)	cabbage leaves infected by black rot	Goto (unpublished)
ph1	*X. begoniae* (XB8)	clover field	Stolp and Starr 1964
ph5	*X. celebensis* (XC144)	plowed field	Stolp and Starr 1964
ph6	*X. corylina* (XC12)	sunflower field	Stolp and Starr 1964
ph7	*X. cucurbitae* (XC6)	leek bed	Stolp and Starr 1964
ph9	*X. geranii* (XG10)	soil under almond	Stolp and Starr 1964
ph11	*X. hyacinthi* (XH110)	soil under plum tree	Stolp and Starr 1964
ph15	*X. malvacearum* (XM2)	peach orchard	Stolp and Starr 1964
ph17	*X. oryzae* (XO101)	alfalfa field	Stolp and Starr 1964
ph20	*X. phaseoli* (XP104)	grass land	Stolp and Starr 1964
ph22	*X. phaseoli* var. *sojense* (XP21)	soil under plum tree	Stolp and Starr 1964
ph25	*X. ricinicola* (XR101Sm^r)	soil under peach tree	Stolp and Starr 1964
ph219	*P. rimaefaciens* (PR107)		Stolp and Starr 1964
XM106p	*X. malvacearum* (XM106)		El Sharkawy

a) Temperate phage.

preparations were between 10^7 and 10^8 pfu/m*l*.

The virulent phages of *X. oryzae* and *X. translucens* f. sp. *oryzicola* (Bp1 to Bp7 and Sp1 to Sp6, respectively) were isolated by Goto[9,10] in the Philippines. Another four strains of *X. oryzae* phages (Op1, Op1h, Op1h2, and Op2) were provided by Dr. S. Wakimoto. A virulent phage of *X. campestris* (Cmp) was isolated by Goto (unpublished) from diseased cabbage leaves in 1968. The twelve Stolp-Starr[23] phages were also used (Table 1).

Phage susceptibility tests The qualitative sensitivity tests were done by spotting the phage suspensions with a platinum loop on the surface of an agar layer inoculated with the bacteria in Petri dishes. All positive reactions were confirmed to be the lytic reaction of the phages by forming separate plaques on plates (using the double-layer technique) which were seeded with the mixture of

properly diluted phage suspension (about 100 to 300 pfu per plate) and different doses of bacterial inocula (approximately 10^9 to 10^7 cells per plate).

Results and discussion

Susceptibility of the isolates of *X. citri* to the virulent and temperate phages

As far as the virulent phage reactions are concerned, phage Ap was almost identical to Cp1 in host range patterns, whereas phage Cp2 was inverse in the patterns with respect to the individual isolates (Table 2). Among the six temperate *X. citri* phages[11] used in our study, phages N64p and U7p resembled each other in their wider host range and seemed to be rather close to the virulent phage Cp2 in their patterns. Although the other four temperate phages (N79p, U6p, N23p, and 2-5p) were narrower in their host ranges, most reactions fell in the general patterns of phages N64p and U7p. Fifteen isolates of *X. citri* out of 87 were resistant to all phages of *X. citri* used in our study.

Table 2. Reactions[a] of *Xanthomonas citri* isolates to *X. citri* phages

Xanthomonas citri isolate	Phage preparations								
	Virulent phages			Temperate phages					
	Ap	Cp1	Cp2	N64p	N79p	U6p	N23p	2-5p	U7p
U1	−	3+s	4+	3+	2+	2+	3+	2+	4+
U3	−	1+	4+	3+	2+	2+	2+	2+s	4+
U6	−	−	−	−	−	−	−	−	−
U7	−	−	2+	−	−	−	−	−	−
U10	4+	4+	2+	3+	3+	2+	2+	2+	2+
U21	−	2+	4+	2+	2+	2+	3+	2+s	4+
U23	4+	4+	3+	−	−	2+	−	−	2+
U25	4+	4+	2+	3+	3+	2+	2+	2+s	3+
U30	−	2+	4+	3+	1+	2+	2+	2+s	3+
U37	−	2+	3+	3+	3+	−	3+	−	−
U59	4+	4+	−	−	−	−	−	−	−
U64	−	−	−	−	−	−	−	−	−
U67	4+	4+	2+	−	−	−	−	−	−
U76	−	2+	4+	−	−	−	−	−	−
U78	−	2+	4+	−	−	−	−	−	−
U79	−	2+	4+	1+	−	−	−	−	−
U80	−	−	−	−	−	−	−	−	−
U85	3+	2+	−	−	−	−	−	−	−
U89	−	2+	4+	4+	−	2+	−	−	4+
U103	−	2+	4+	3+	−	−	−	−	−
U104	−	2+	4+	2+	−	2+	2+	−	2+
U106	4+	4+	−	−	−	−	−	−	−
U107	−	1+	4+	2+	−	−	−	−	2+
U108	1+	2+	4+	3+	2+	1+	1+	−	2+
U109	−	2+	4+	4+	−	2+	−	−	2+
U105	4+	4+	2+	2+	2+	2+	2+	2+s	3+

N4	4+	4+	2+	4+	2+s	2+	2+	3+	3+
N22	2+s	2+	−	−	−	−	−	−	−
N23w[b)]	−	−	−	−	−	−	−	−	−
N23t[b)]	−	2+	2+	3+	2+	2+	−	−	2+
N28w[b)]	−	2+	4+	4+	−	−	−	−	3+
N28t[b)]	2+	2+	4+	3+	2+	2+	−	−	3+
N32w[b)]	−	2+	4+	4+	−	2+s	2+s	2+s	2+
N32t[b)]	−	2+	3+	2+	−	−	−	−	2+
N31	−	2+	4+	4+	−	2+	−	−	3+
N33	−	2+	4+	3+	−	2+	−	−	3+
N36	4+	4+	−	−	−	−	−	−	−
N40	−	−	−	−	−	−	−	−	−
N41	−	2+	4+	2+	−	−	2+	2+	2+
N43	4+	4+	2+	2+	2+	2+	2+	2+	3+
N49w[b)]	4+	4+	−	−	−	−	−	−	−
N49t[b)]	4+	4+	3+s	3+	2+	2+	−	−	3+
N53	−	−	4+	−	−	−	2+	2+s	1+
N54	4+	4+	−	2+	1+	−	−	−	2+
N59	4+	4+	2+s	2+	2+s	−	−	−	−
N62	4+	4+	2+	−	−	2+	2+	2+s	2+
N64	−	−	−	−	−	−	−	−	−
N67	−	−	−	−	−	−	−	−	−
N68	−	−	−	−	−	−	−	−	−
N76	−	−	−	−	−	−	−	−	−
N79	4+	4+	−	−	−	−	−	−	−
N80	3+	2+	−	−	−	−	−	−	−
N83	−	−	−	−	−	−	−	−	−
N88	3+	3+	−	−	−	−	−	−	−
N91	4+	4+	2+	3+	3+	2+	2+	1+	3+
N100w[b)]	2+	2+	2+	3+	2+	−	−	−	−
N100t[b)]	2+	2+	−	−	−	−	−	−	−
N108	−	3+	4+	2+	2+s	1+	3+	2+s	1+
N110	4+	4+	2+	3+	2+	2+	2+	−	2+
N113	4+	4+	2+	3+	2+	3+	−	−	4+
N117	4+	4+	−	−	−	−	2+	−	−
B5	2+	2+	3+	−	−	−	−	−	−
B9	−	−	−	−	−	−	−	−	−
B11	4+	4+	−	−	−	−	−	−	−
B12	4+	4+	−	−	−	−	−	−	−
B16	4+	4+	−	−	−	−	−	−	−
B18	4+	4+	2+	3+	2+	2+	3+	3+s	3+
B20	2+	2+	−	−	−	−	−	−	2+
B21	−	−	−	−	−	−	−	−	−
B23	4+	4+	−	−	−	−	−	−	−

B25w[b]	4+	4+	2+	3+	2+s	2+	−	−	2+
B25t[b]	4+	4+	−	−	−	−	−	−	−
K1	−	2+	4+	3+	2+s	2+	2+	2+s	3+
K2	−	−	−	−	−	−	−	−	−
K5sw[b]	−	−	−	−	−	−	−	−	−
K5t[b]	−	2+	4+	2+	−	−	1+	−	−
L4	4+	4+	2+	3+	2+	2+	2+	−	3+
L6	4+	4+	2+	3+	2+	2+	2+	2+s	3+
L14	4+	4+	1+	2+	−	−	−	−	2+
L21	4+	4+	−	−	−	−	−	−	−
L22	4+	4+	−	−	−	−	−	−	−
L26	4+	4+	−	−	−	−	−	−	−
H7	−	2+	4+	3+	2+s	2+	2+	2+	2+
F1	−	2+	4+	2+	−	−	2+	−	−
F2	4+	4+	2+	3+	3+	2+	−	−	2+
F3	4+	4+	−	−	−	−	−	−	−
S1	4+	4+	2+	2+	2+	2+	2+	2+	3+
S4	4+	4+	2+	3+	2+	3+	−	−	3+
P1w[b]	4+	4+	2+	4+	2+	−	1+	−	1+
P1t[b]	4+	4+	2+	4+	2+	2+	3+	2+s	2+
T3	4+	4+	2+	3+	3+	2+	2+	−	2+
T5	4+	4+	2+	3+	2+	2+	2+	2+	2+
T6	4+	4+	2+	2+	2+	2+	2+	2+	3+
2-5	4+	4+	2+	−	−	−	−	−	−
49-1a	−	−	4+	2+	−	−	−	−	−
49-1b	−	−	−	−	−	−	−	−	−

[a] The reactions are scored as follows: 4+, clear plaques with no trace of turbidity; 3+, plaques with a trace of turbidity; 2+, plaques with moderate turbidity; 1+, very turbid plaques; −, no plaque; s, isolated, scattered plaques; *underscored* reactions are those which changed to "−" after treatment of phage preparations Cp1 and Cp2 with chloroform.

[b] w, opaque, wild-type colony; sw, small, wild-type colony; t, transparent, mutant type colony (Goto, 1970).

Since we had not set out to determine the frequency of occurrence in Japan of phage-resistant strains of *X. citri* (and there may well have been inadvertent selection for phage resistance in the strains we did examine), we might mention here other, possibly more representative, findings on this point. Serizawa[21] has reported that 16 (2.7 percent) out of 602 strains of *X. citri* collected in Shizuoka Pre-

fecture were resistant to both phages Cp1 and Cp2. Mr. T. Obata (personal communication, 1972) has informed us that he has surveyed the ability of phages Cp1 and Cp2 to lyse a large number of *X. citri* strains collected from most of the citrus-growing regions of Japan. Approximately 2 percent of the 876 *X. citri* strains tested by him were resistant to both of these phages; resistance to both phages in the individual series examined by Obata ranged from 0.0 to 6.7 percent. Although the findings of Serizawa and of Obata are quantitatively different from ours for as yet undetermined reasons, this difference in frequency should not obscure the fundamental qualitative similarity. There are indeed strains of *X. citri* in Japan which are not lysed by any of the presently known phages (including Cp1 and Cp2).

The negative reactions to the temperate phages recorded in Table 2 might be explained in several ways such as immunity to phages (due to the identity between the temperate phages tested and the prophages of each isolate), non-lysogenicity of bacteria, or absence of affinity between the phages and bacteria. Which of the above-mentioned possibilities is actually concerned in each reaction remains to be deterimined, isolate by isolate.

Phage Cp2 formed turbid plaques on the propagating isolate, 49-1. The bacterium was then plated and found to be a mixture of two clones different in phage susceptibility (49-1a) and (49-1b). Variations in phage susceptibility were also noticed between the original wild-type and the transparent colony mutants, the patterns of the latter being either broader or narrower than that of the wild-type depending on the individual isolates. This phenomenon is not unusual in cultures of xanthomonads which originated from single-cell isolations, particularly when colony variants are involved[7,8].

As shown in Table 2, the phage preparations of Cp1 and Cp2 propagated on the host isolates

Table 3. Effect of chloroform treatment of the temperate phages of *Xanthomonas citri*

Phage	Lytic reaction on isolate			
	N4		U30	
	Original	Chloroformed	Original	Chloroformed
N64p	4+	−	3+	−
N79p	2+s	−	1+	−
U6p	2+	−	2+	−
N23p	2+	−	2+	−
2-5p	3+	−	2+s	−
U7p	3+	−	3+	−
L21p	3+	−	2+	−
L22p	2+s	−	2+	−
U85p	2+s	−	2+s	−
K2p	3+	−	3+	−
U76p	2+s	−	2+s	−
49-1a	3+	−	3+	−
49-1b	3+	−	3+	−
Cp1 (virulent)[a]	4+	4+	2+	−
Cp2 (virulent)[b]	2+	−	4+	4+
Ap (virulent)[c]	4+	4+	−	−

a) propagated on isolate 2-5, lysogenic strain.
b) propagated on isolate 49-1a, lysogenic strain.
c) propagated on isolate N4, non-lysogenic strain.

Table 4. Effect of chloroform treatment on the titer (plaque-forming units) of virulent phages of *Xanthomonas citri*

Phage	Original	Filtered through Millipore	Chloroformed
Cp1	1.8×10^9	5.0×10^8	1.7×10^9
Cp2	1.3×10^9	5.0×10^8	1.3×10^9

designated by Wakimoto (2–5 and 49–1, respectively) showed (underscored in Table 2) weak reactions which became completely negative reactions after chloroform treatment. The virulent phage, Ap, propagated on a non-lysogenic *X. citri* strain, N4, however, showed no lytic reaction corresponding to that due to the temperate phages. The other temperate phages of *X. citri* were also completely inactivated when exposed to chloroform, whereas the virulent ones were quite stable to the treatment (Table 3 and Table 4). Thus, the host range patterns in *X. citri* phages must be carefully checked for the probable reactions due to temperate phages derived from the host bacteria used for phage propagation. This was true also in the case of temperate phages isolated from the cultures of *X. campestris* (unpublished). On the other hand, no virulent *Xanthomonas* phages used in our investigation (including the Stolp-Starr phages) suffered the slightest damage due to the chloroform treatment. Thus, as far as the phage preparations which were free from bacterial cells were concerned, the difference in behavior upon treatment with chloroform seems to provide a simple and useful way to differentiate the virulent and temperate phages of xanthomonads. Consequently, this technique may be useful to purify virulent phages which are mixed with temperate phages derived from the host bacterium used for the propagation of the former. It is uncertain, however, whether the procedure is generally applicable to the phages attacking bacteria of other genera, since there are some counter-examples reported, such as isolation and purification of the temperate phages for *Pseudomonas morsprunorum* and *P. syringae* by treating the bacterial cultures with chloroform[4].

Susceptibility of *X. citri* isolates of the Stolp-Starr phages

Twelve *Xanthomonas* phages used in this phase of our study were isolated by Stolp and Starr[23] from California soils with the following species as the host bacteria: *X. begoniae* (XB8) for the phage ph1, *X. celebensis* (XC144) for ph5, *X. corylina* (XC12) for ph6, *X. cucurbitae* (XC6) for ph7, *X. geranii* (XG10) for ph9, *X. hyacinthi* (XH110) for ph11, *X. phaseoli* var. *sojense* (XP21) for ph22, *X. ricinicola* (XR101SM^r) for ph25, and *P.* (?) *rimaefaciens* (PR107) for ph219.

These 12 phages could be tentatively clustered into three groups based on the data in Table 2 of their paper[23]: the first group characterized by a very broad host range covering more than half of 80 *Xanthomonas* nomenspecies (ph1, ph17, ph25, and ph219), the second group with very narrow host ranges attacking only a few nomenspecies (ph5, ph11, and ph20), and the third intermediate group with lytic reaction on bacteria belonging to around 20 nomenspecies (ph6, ph7, ph9, ph15, and ph22). Out of 7 phages which showed lytic action on the isolates of *X. citri* in Table 5 of this paper, four phages (ph1, ph17, ph25, and ph219) were included in the first group and two phages (ph11, ph22) in the second and third groups, respectively. It must be mentioned that the positive reactions between the isolates of *X. citri* and these phages are not surprising in view of the wide-spectrum behavior of these phages against various other xanthomonads reported by Stolp and Starr[23]. Even so, phage ph25 very characteristically showed a positive lysis on almost all isolates of *X. citri*. Phage ph25 had already been shown to give a similar response on the isolates of *X. begoniae*, *X. corylina*, *X. juglandis*, *X. malvacearum*, *X. pruni*, etc.; the majority of isolates belonging to each of these species

Table 5. Phage reactions[a] of *Xanthomonas citri* isolates against the 12 Stolp-Starr phages and phage XM106

Bacterial isolates	Phage preparations												
	ph1	ph5	ph6	ph7	ph9	ph11	ph15	ph17	ph20	ph22	ph25	ph219	XM106
U1	−	−	−	−	−	−	−	−	−	−	3+	4+	−
U3w[b]	2+	−	−	−	−	−	−	−	−	−	2+	−	−
U3t	2+	−	−	−	−	−	−	−	−	−	2+	3+	−
U6	−	−	−	−	−	−	−	−	−	−	2+	−	−
U7	−	−	−	−	−	−	−	−	−	−	2+	−	−
U10w	4+	−	−	−	−	−	−	−	−	−	3+	4+	−
U10t	4+	−	−	−	−	−	−	−	−	−	2+	3+	−
U21	−	−	−	−	−	−	−	−	−	−	3+	3+	−
U23	−	−	−	−	−	−	−	3+	−	−	2+	−	−
U25	4+	−	−	−	−	4+s	−	−	−	−	3+	3+	−
U30	−	−	−	−	−	−	−	3+s	−	−	2+	3+	−
U37	−	−	−	−	−	−	−	−	−	−	3+	−	−
U59	−	−	−	−	−	−	−	−	−	−	3+	−	−
U64	−	−	−	−	−	−	−	−	−	−	2+	−	−
U67	−	−	−	−	−	−	−	−	−	−	−	−	−
U76	−	−	−	−	−	−	−	3+s	−	−	2+	3+	−
U78	−	−	−	−	−	−	−	−	−	−	2+	−	−
U79	−	−	−	−	−	−	−	−	−	−	2+	−	−
U80	−	−	−	−	−	−	−	−	−	−	2+	−	−
U85	−	−	−	−	−	−	−	−	−	−	2+	−	−
U89	−	−	−	−	−	−	−	−	−	−	2+	−	−
U103	−	−	−	−	−	−	−	−	−	−	2+	2+	−
U104	−	−	−	−	−	−	−	−	−	−	2+	2+	−
U105	−	−	−	−	−	−	−	−	−	−	3+	3+	2+
U106	−	−	−	−	−	−	−	−	−	−	2+	−	−
U107	−	−	−	−	−	−	−	−	−	−	2+	−	−
N4	4+	−	−	−	−	−	−	−	−	3+	2+	3+	−
N22	−	−	−	−	−	−	−	−	−	−	3+	−	−
N23w	−	−	−	−	−	−	−	−	−	−	2+	−	−
N23t	2+	−	−	−	−	−	−	−	−	−	3+	2+	−
N28w, t	−	−	−	−	−	−	−	−	−	−	2+	−	−
N31	−	−	−	−	−	−	−	−	−	−	3+	4+	−
N32	−	−	−	−	−	−	−	−	−	−	4+	−	−
N33	−	−	−	−	−	−	−	−	−	−	2+	3+	−
N36	−	−	−	−	−	−	−	−	−	−	3+	−	−
N40	−	−	−	−	−	−	−	−	−	−	2+	−	−
N41w	−	−	−	−	−	−	−	−	−	−	2+	4+	−
N41t	−	−	−	−	−	−	−	−	−	−	2+	−	−
N43w, t	4+	−	−	−	−	2+s	−	−	−	−	4+	4+	−

N49w	−	−	−	−	−	−	−	−	−	−	2+	−	−
N49t	4+	−	−	−	−	−	−	−	−	3+	3+	4+	−
N53	−	−	−	−	−	−	−	−	−	−	3+	3+	−
N54	−	−	−	−	−	−	−	−	−	−	2+	−	−
N59	4+	−	−	−	−	−	−	−	−	−	2+	3+	−
N62	−	−	−	−	−	−	−	−	−	−	3+	−	−
N64	−	−	−	−	−	−	−	−	−	−	2+	−	−
N67	−	−	−	−	−	−	−	−	−	−	2+	−	−
N58	−	−	−	−	−	−	−	−	−	−	2+	−	−
N76	−	−	−	−	−	−	−	−	−	−	2+	−	−
N79	−	−	−	−	−	−	−	−	−	−	3+	−	−
N80	−	−	−	−	−	−	−	−	−	−	3+	−	−
N83	−	−	−	−	−	−	−	−	−	−	2+	−	−
N91w	4+	−	−	−	−	−	−	−	−	2+	2+	4+	−
N91t	4+	−	−	−	−	−	−	−	−	3+	2+	4+	−
N100w	−	−	−	−	−	−	−	−	−	−	3+	−	−
N100t	−	−	−	−	−	−	−	−	−	−	3+	−	−
N108	−	−	−	−	−	−	−	−	−	−	2+	3+	−
N110w	4+	−	−	−	−	2+s	−	−	−	3+	3+	3+	−
N110t	4+	−	−	−	−	2+s	−	−	−	4+	3+	4+	−
N113	4+	−	−	−	−	−	−	−	−	−	2+	3+	−
N117	4+	−	−	−	−	−	−	−	−	−	2+	4+	−
B5	−	−	−	−	−	−	−	−	−	−	2+	−	−
B9	−	−	−	−	−	−	−	−	−	−	2+	−	−
B11	−	−	−	−	−	−	−	−	−	−	4+	−	−
B12	−	−	−	−	−	−	−	−	−	−	3+	−	−
B16	−	−	−	−	−	−	−	−	−	−	3+	−	−
B18	4+	−	−	−	−	−	−	−	−	2+	2+	2+	−
B20	−	−	−	−	−	−	−	−	−	−	2+	−	−
B21	−	−	−	−	−	−	−	−	−	−	2+	−	−
B23	−	−	−	−	−	−	−	−	−	−	3+	−	−
B25w	3+	−	−	−	−	−	−	−	−	−	2+	2+	−
B25t	−	−	−	−	−	−	−	−	−	−	4+	−	−
K1w	−	−	−	−	−	−	−	−	−	−	2+	−	−
K1t	−	−	−	−	−	−	−	−	−	−	2+	3+	−
K2	−	−	−	−	−	−	−	−	−	−	2+	−	−
K5sw	−	−	−	−	−	−	−	−	−	−	2+	−	−
K5t	−	−	−	−	−	−	−	−	−	−	4+	−	−
L4w	3+	−	−	−	−	−	−	−	−	4+	2+	3+	−
L4t	4+	−	−	−	−	−	−	−	−	4+	4+	4+	−
L6	4+	−	−	−	−	−	−	−	−	4+	3+	4+	−
L14	−	−	−	−	−	−	−	−	−	−	2+	−	−

L21	−	−	−	−	−	−	−	−	−	−	4+	−	−
L22w, t	−	−	−	−	−	−	−	−	−	−	4+	−	−
L26w, t	−	−	−	−	−	−	−	−	−	−	3+	−	−
H7	−	−	−	−	−	−	−	−	−	−	3+	−	−
F1	−	−	−	−	−	−	−	−	−	−	3+	−	−
F2	−	−	−	−	−	−	−	−	−	−	2+	4+	−
F3	−	−	−	−	−	−	−	−	−	−	4+	−	−
S1	4+	−	−	−	−	−	−	−	−	−	3+	4+	−
S4	4+	−	−	−	−	−	−	−	−	−	2+	−	−
P1	3+	−	−	−	−	−	−	−	−	−	3+	2+	−
T3w, t	4+	−	−	−	−	2+s	−	−	−	−	3+	2+	−
T5	4+	−	−	−	−	−	−	−	−	−	2+	−	−
T6	4+	−	−	−	−	−	−	−	−	−	2+	3+	−
2-5	−	−	−	−	−	−	−	−	−	−	3+	−	−
49-1a	−	−	−	−	−	−	−	−	−	−	2+	−	−
49-1b	−	−	−	−	−	−	−	−	−	−	2+	−	−

a) The reactions are scored as follows: 4+, clear plaques with no trace of turbidity; 3+, plaques with a trace of turbidity; 2+, plaques with moderate turbidity; 1+, very turbid plaques; −, no plaque; s, isolated, scattered plaques.

b) w, opaque, wild-type colony; sw, small, wild-type colony; t, transparent, mutant type colony (Goto, 1970).

was lysed[23]. This was also true with phages ph1 and ph219. On the contrary, phage ph17 showed lysis on a limited number of isolates of *X. citri*, whereas a very common reaction over most isolates of *X. oryzae* is shown in Table 11. This phage ph17 had shown a common lysis on most isolates of *X. campestris*, *X. corylina*, etc[23]. These facts suggest that the reactions of phages ph1, ph25, and ph219 on most isolates of *X. citri* do not necessarily indicate any special phage-bacteria relationship between only those phages and bacteria, but rather the common ones seen among certain xanthomonads.

Table 6. Susceptibility to phage ph25 propagated on different isolates of *Xanthomonas citri*, using *X. citri* cultures of different ages

X. citri isolate	Age of culture	Phage ph25 propagated on					
		K2	N68	B9	U6	N4 (1st)	N4 (2nd)
B9w	aged[a]	−	−	−	−	−	−
	fresh[b]	2+	2+	2+	2+	2+	2+
N76	aged	2+	2+	2+	2+	2+	2+
	fresh	3+	3+	3+	3+	3+	3+
B21	aged	−	−	−	−	−	−
	fresh	3+	3+	3+	3+	3+	3+

N67	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	2+	2+	2+
U6	aged	−	−	−	2+	−	−
	fresh	3+	3+	3+	3+	3+	3+
N23w	aged	2+	−	−	2+	2+	2+
	fresh	4+	4+	4+	4+	4+	4+
K2	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	2+	2+	2+
U64	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	2+	2+	2+
N83	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	2+	2+	2+
U80	aged	2+	−	−	−	2+	2+
	fresh	2+	2+	2+	2+	2+	2+
N64	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	2+	2+	2+
49-1b	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	3+	2+	2+
N40	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	2+	2+	2+
N68	aged	−	1+	−	1+	1+	1+
	fresh	2+	2+	2+	2+	2+	2+
K5w	aged	−	−	−	−	−	−
	fresh	2+	2+	2+	2+	2+	2+

a) 7 day-old potato-sucrose slant culture.
b) 24 hour-old potato-sucrose slant culture.

The plaques of the phage ph25 were tiny dots on any susceptible xanthomonads. On the majority of isolates, the plaques were clearly recognized irrespective of the age of the cultures (at least within 7 days). However, the reactions often were indistinct or even negative on some isolates of *X. citri* but, in these cases, depended on the age of the cultures as is shown in Table 6. In such isolates, a rather low inoculum dose of the indicator bacteria led to successful plaque formation (in comparison with the reaction to high doses); very careful examination of the plate was needed to find the very small plaques.

Susceptibility of xanthomonads to *X. citri* phages

Among 136 isolates belonging to 43 nomenspecies of the genus *Xanthomonas*, only 9 isolates of 7 nomenspecies showed susceptibility at various degrees to *X. citri* phages (Table 7). They were *X. begoniae* (XB8, XB111), *X. blepharidis* (XB115), *X. cucurbitae* (XC6), *X. phaseoli* (XP4, Xph), *X. phaseoli* var. *sojense* (XP21), *X. marantae* (XM126), and *X. translucens* f. sp. *phleum-pratense* (XT103). Compared with the results obtained by Stolp and Starr[23], it could be said, in general, that the host ranges of the *X. citri* phages are rather narrow regardless of whether they are virulent or temperate phages. *X. phaseoli* (XP4) and *X. begoniae* (XB8) showed a characteristically wide susceptibility to many strains of *X. citri* phages used in our investigation.

Table 7. Susceptibility of xanthomonads to *Xanthomonas citri* phages

Bacteria	Phage preparations								
	Ap	Cp1	Cp2	N64p	N79p	U6p	N23p	2-5p	U7p
X. begoniae (XB8)	−	2+	2+s	2+s	2+s	−	3+	2+s	−
X. begoniae (XB111)	−	−	−	−	−	−	−	+[a)]	−
X. blepharidis (XB115)	−	−	4+	−	−	−	−	−	−
X. cucurbitae (XC6)	−	−	−	−	−	−	−	+[a)]	−
X. marantae (XM126)	−	−	4+	−	−	−	−	−	−
X. phaseoli (XP4)	−	2+	4+	3+	3+	3+	3+	3+	3+
X. phaseoli (Xph)	−	−	−	−	3+	−	−	3+	2+
X. phaseoli var. *sojense* (XP21)	−	−	2+s	−	−	−	−	−	−
X. translucens f. sp. *phleum-pratense* (XT103)	−	−	−	2+	3+	−	−	2+	3+

a) Degree of lysis was not recorded.

The xanthomonads which were free from any lysis by these phage preparations were: *X. alfalfae* (XA121), *X. campestris* (XC3, XC135, XC153, XC160, XC161, and two other isolates), *X. corylina* (XC5, XC12), *X. carotae* (XC11), *X. cassavae* (XC110), *X. celebensis* (XC144, XC145), *X. dieffenbachiae* (XD101), *X. fragariae* (XF102, XF106), *X. geranii* (XG1, XG10), *X. hederae* (XH1), *X. hyacinthi* (XH110), *X. incanae* (XI3), *X. juglandis* (XJ3, XJ103, XJ107), *X. lespedezae* (XL1), *X. lactucae* (XL4), *X. malvacearum* (XM2, XM106), *X. manihotis* (XM4), *X. maculifoliagardeniae* (XML6), *X. mangiferae-indicae* (XM127, XM129), *X. nigromaculans* (XN103 and one other isolate), *X. oryzae* (XO101, XO103, XO110, and 42 other isolates), *X. translucens* f. sp. *oryzicola* (XO105, XO109, XO113, and 5 other isolates), *X. phaseoli* (XP2, XP14, XP104, XP205, XP206), *X. papavericola* (XP5), *X. pruni* (XP10, XP13, XP165, and one other isolate), *X. pelargonii* (XP15, XP118, XP121), *X. phaseoli* var. *sojense* (Xps), *X. poinsettiaecola* (XP137), *X. pisi* (XP171 and two other isolates), *X. phaseoli* var. *fuscans* (XP173, XP207), *X. plantaginis* (XP195), *X. physalidicola* (1, 2), *X. ricinicola* (XR101Sm^r), *X. translucens* f. sp. (XT2, XT4, XT7, XT9, XT13, XT16), *X. taraxaci* (XT11), *X. tamarindi* (XT20), *X. vasculorum* (XV1), *X. vesicatoria* (XV3, XV135, XV151, XV173), *X. vesicatoria* var. *raphani* (XV174), "*Pseudomonas*" *rimaefaciens* (PR107).

Susceptibility of *X. citri* to some other *Xanthomonas* phages

Susceptibility of the isolates of *X. citri* was tested against 11 *X. oryzae* phages, 6 *X. translucens* f. sp. *oryzicola* phages, and a *X. campestris* phage, all of which were isolated from diseased leaves of rice and cabbage, respectively (Table 8). Among the *X. oryzae* phages, only phage Bp3 showed moderate lysis on several isolates of *X. citri*, and the other *X. oryzae* phages did not infect *X. citri* at all. In contrast to the above, all of the *X. translucens* phages showed positive reactions on some isolates of *X. citri*, phage Sp3 being particulary interesting because of its wide host range encompassing over 70 percent of the *X. citri* isolates used in these experiments. Their positive reactions were mostly found on such isolates in common as U25, N113, N88, and S4. The *X. campestris* phage also formed scattered plaques on a few isolates of *X. citri*.

Infectivity was tested of these phages, as well as the Stolp-Starr phages, to the isolates of *X. oryzae*, *X. translucens* f. sp. *oryzicola*, and some other xanthomonads. The *X. oryzae* phages, which infected no isolate of *X. citri*, showed various degrees of host specificity over the isolates of the homologous nomenspecies (Table 9). Also, phages Sp3, Sp6, ph1, ph7, and ph17 showed very wide host ranges on the isolates of *X. oryzae* (Table 11), corresponding to those on the homologous combination of phages and bacteria shown in Table 9. As presented in Table 11, phages ph1, ph7, ph11,

Table 8. Phage susceptibility patterns of *Xanthomonas citri* isolates against some *Xanthomonas translucens*, *X. oryzae*, and *X. campestris* phages

X. citri isolate	Phage preparations								
	Sp1	Sp2	Sp3	Sp4	Sp5	Sp6	Bp3	Bp1, Bp2, Bp4, Bp5, Bp6, Bp7, Op1, Op1h, Op1h2, Op2	Cmp
U1	−	−	4+	−	−	−	−	−	−
U3	−	−	3+	−	−	−	−	−	−
U6	−	−	2+	−	−	−	−	−	−
U7	−	−	−	−	−	−	−	−	−
U10	−	−	3+	−	−	−	−	−	−
U21	−	−	4+	−	−	−	−	−	−
U23	−	−	2+	−	−	−	−	−	−
U25	3+s	3+s	4+	4+s	4+s	3+s	−	−	−
U30	−	−	4+	−	−	−	−	−	−
U37	−	−	3+	−	−	−	−	−	−
U59	−	−	3+	−	−	−	−	−	−
U64	−	−	−	−	−	−	−	−	−
U67	−	−	3+	−	−	−	−	−	−
U76	−	−	4+	−	−	−	−	−	−
U78	−	−	−	−	−	−	−	−	−
U79	−	−	−	−	−	−	−	−	−
U80	−	−	2+	−	−	−	−	−	−
U85	−	−	−	−	−	−	−	−	−
U89	2+s	−	2+	−	−	−	−	−	−
U103	−	−	4+	−	−	−	−	−	−
U104	−	−	3+	−	−	−	−	−	−
U105	−	−	3+	−	−	−	−	−	−
U106	−	−	−	−	−	−	−	−	−
U107	−	−	3+	−	−	−	−	−	−
U108	−	−	4+	−	−	−	−	−	−
U109	−	−	4+	−	−	−	−	−	−
N4	−	−	3+	−	−	−	−	−	−
N22	−	−	4+	−	−	−	−	−	−
N23w	−	−	3+	−	−	−	−	−	−
N23t	−	−	4+	−	−	−	−	−	3+s
N28w	−	−	4+	−	−	−	−	−	−
N28t	−	−	2+	−	−	−	−	−	−
N31	−	−	4+	−	−	−	−	−	−
N23w,t	−	−	2+	−	−	−	−	−	−
N33	−	−	4+	−	−	−	−	−	−
N36	−	−	−	−	−	−	−	−	−
N40	−	−	3+	−	−	−	2+	−	−
N41	−	−	4+	−	−	−	−	−	−

N43	−	−	3+	−	−	−	−	−	−
N49w	−	−	−	−	−	−	−	−	−
N49t	−	−	−	−	−	−	−	−	−
N53	−	−	4+	−	−	−	−	−	−
N54	−	−	2+	−	−	−	−	−	−
N59	−	−	2+	−	−	−	−	−	−
N62	−	−	2+	−	−	−	−	−	−
N64	−	−	−	−	−	−	−	−	−
N67	−	−	−	−	−	−	−	−	−
N68	−	−	4+	−	−	−	−	−	−
N76	−	−	2+	−	−	−	−	−	−
N79	−	−	−	−	−	−	−	−	−
N80	−	−	−	−	−	−	−	−	−
N83	−	−	−	−	−	−	−	−	−
N88	−	−	4+	2+s	2+s	2+s	−	−	−
N91w, t	−	−	2+	−	−	−	−	−	−
N100w, t	−	−	3+	−	−	−	−	−	−
N108	−	−	2+	−	−	−	−	−	−
N110w, t	−	−	3+	−	−	−	−	−	−
N113	3+s	3+	4+	3+s	3+	3+	−	−	3+s
N117	−	−	4+	−	−	−	2+s	−	−
B5	−	−	−	−	−	−	−	−	−
B9	−	−	−	−	−	−	−	−	−
B11	−	−	−	−	−	−	−	−	−
B12	−	−	−	−	−	−	−	−	−
B16	−	−	−	−	−	−	−	−	−
B18	−	−	3+	−	−	−	−	−	−
B20	−	−	−	−	−	−	−	−	−
B21	−	−	−	−	−	−	−	−	−
B23	−	3+	3+	−	−	−	−	−	−
B25w, t	−	−	4+	−	−	−	−	−	−
K1w, t	−	−	3+	−	−	−	−	−	−
K2	−	−	−	−	−	−	−	−	−
K5sw, t	−	−	4+	−	−	−	−	−	−
L4w	−	−	3+	−	−	−	2+	−	−
L4t	−	−	3+	−	−	−	2+	−	−
L6	−	−	2+	−	−	−	−	−	−
L14	−	−	2+	−	−	−	−	−	−
L21	−	−	3+	−	−	−	−	−	−
L22w, t	−	−	−	−	−	−	−	−	−
L26w, t	−	−	−	−	−	−	−	−	−

H7	−	−	4+	−	−	−	−	−	−
F1	−	−	3+	−	−	−	−	−	−
F2	−	−	2+	−	−	−	−	−	−
F3	−	−	3+	−	−	−	−	−	−
S1	−	−	3+	−	−	−	−	−	−
S4	−	2+s	2+s	2+s	−	−	−	−	−
P1	−	−	3+	−	−	−	−	−	−
T3w	−	−	2+	−	−	−	−	−	3+s
T3t	−	−	3+	−	−	−	2+	−	−
T5	−	−	3+	−	−	−	−	−	−
T6	−	−	4+	−	−	−	−	−	−
2-5	−	−	2+	−	−	−	−	−	−
49-1a	−	−	−	−	−	−	−	−	−
49-1b	−	−	4+	−	−	−	−	−	−

Sp1-6 are *X. translucens* f. sp. *oryzicola* phages ; Bp1-7 and Op1-Op2 are *X. oryzae* phages ; Cmp is a *X. campestris* phage.

Symbols are the same as in Table 5.

Table 9. Phage susceptibility patterns of *Xanthomonas oryzae* isolates to their homologous phages

X. oryzae isolate	Phage preparations										
	Bp1	Bp2	Bp3	Bp4	Bp5	Bp6	Bp7	Op1	Op1h	Op1h2	Op2
B2	4+	4+	−	4+	4+	4+	4+	2+	3+	4+	4+
B10	4+	4+	4+	4+	4+	4+	4+	2+	3+	4+	4+
B18	−	−	4+	−	−	−	−	−	−	−	−
B20	−	3+	−	−	−	−	−	−	−	−	−
B56	−	−	−	4+	4+	−	−	2+	−	−	−
B69	4+	4+	4+	4+	−	−	−	−	−	−	4+
B72	−	−	−	−	−	−	−	−	−	−	−
XOa	4+	4+	4+	4+	4+	4+	4+	2+	4+	4+	4+
XOb	4+	4+	4+	4+	4+	4+	4+	2+	4+	4+	4+
XOd	4+	4+	−	−	−	−	−	4+	−	−	4+
XOe	4+	4+	−	−	−	−	−	4+	−	−	4+
XOf	4+	4+	−	−	−	−	−	4+	−	−	4+
XOg	4+	4+	−	−	−	−	−	4+	−	−	4+
XOi	4+	4+	−	−	−	−	−	4+	−	−	4+
XOj	4+	4+	−	−	−	−	−	4+	−	4+s	4+
XOk	4+	4+	−	−	−	−	−	4+	−	2+	4+

XOl	4+	4+	−	−	−	−	−	4+	−	−	4+
XOm	4+	4+	4+	4+	4+	4+	4+	2+	4+	4+	4+
XOn	4+	4+	4+	4+	4+	4+	4+	−	4+	4+	4+
XOp	4+	4+	−	−	−	−	−	4+	−	−	4+
XOq	4+	4+	−	−	−	−	−	4+	−	−	4+
XOr	4+	4+	−	−	−	−	−	4+	−	−	4+
XOs	4+	4+	−	−	−	−	−	4+	−	−	4+
XO19-1	4+	4+	2+	4+	4+	4+	4+	2+	4+	4+	4+
XO20-6	4+	4+	3+	4+	4+	4+	4+	2+	2+	4+	4+
XO20-8	4+	4+	3+	4+	4+	4+	4+	2+	2+	4+	4+
XO20-9	4+	4+	4+	−	−	−	−	−	−	−	4+
XO22-2	4+	4+	−	4+	4+	4+	4+	3+	3+	4+	4+
XO22-6	−	2+	3+	−	−	−	3+	2+	−	−	−
XO23-1	4+	4+	2+	4+	4+	−	−	−	−	4+	4+
XO24-1	−	−	4+	−	−	2+	2+	−	−	−	−
XO24-2	4+	4+	4+	4+	4+	4+	4+	2+	2+	4+	4+
XOA-1	−	−	4+	−	−	−	−	−	−	4+	−
XOB-1	4+	4+	4+	−	−	4+	4+	−	−	4+	4+
XOC-2	4+	4+	4+	4+	4+	4+	4+	−	3+	4+	4+
XOD-1	4+	4+	4+	4+	4+	4+	4+	−	−	4+	4+
XOD-2	4+	4+	2+	4+	4+	4+	4+	−	−	4+	4+
XOE-1	−	−	2+	−	−	−	4+	−	−	−	−
XOE-2	4+	4+	4+	4+	4+	4+	4+	−	−	4+	4+
XOF-1	4+	4+	4+	4+	−	−	−	−	−	4+	4+
XOF-2	4+	4+	4+	4+	−	−	−	−	−	4+	4+
XOH-2	−	−	4+	4+	4+	4+	4+	−	−	−	−

Symbols are the same as in Table 5.

Table 10. Phage susceptibility patterns of *Xanthomonas translucens* f. sp. *oryzicola* isolates to their homologous phages

X. translucens f. sp. *oryzicola* isolate	Phage preparations					
	Sp1	Sp2	Sp3	Sp4	Sp5	Sp6
S1	−	4+	−	−	−	−
S2	−	−	4+	−	−	−
S3	4+	−	4+	4+	−	4+
S24	−	−	4+	−	4+	4+
S27	−	−	−	−	−	−

Symbols are the same as in Table 5.

and ph17 showed wide host ranges extending over the isolates *X. translucens* f. sp. *oryzicola*, the host ranges of the *X. translucens* f. sp. *oryzicola* phages (Table 10) generally being more strain-specific than those of phages ph1, ph7, ph11, and ph17. Phages Sp3 and Bp3 attacked the isolates of many nomenspecies of the genus *Xanthomonas* as shown in Table 12. The other *X. translucens* f. sp. *oryzicola*

Table 11. Phage susceptibility patterns of the Stolp-Starr phages and the other *Xanthomonas* phages against *Xanthomonas oryzae* and *X. translucens* f. sp. *oryzicola* isolates[a)]

Isolates	Phage preparations							
	Sp3	Sp5	Sp6	ph1	ph7	ph11	ph15	ph17
X. oryzae								
B2	−	−	−	−	−	−	−	−
B10	4+	−	−	4+	−	4+	4+s	4+
B18	4+	−	2+	3+	−	−	−	4+
B20	−	−	−	−	−	−	−	−
B56	−	−	−	−	−	−	−	−
B69	2+	2+	2+	−	−	−	−	3+
B72	−	−	−	−	−	−	−	−
XOa	4+	−	3+	−	−	−	−	4+
XOb	4+	−	2+	−	2+	−	−	4+
XOd	4+	−	2+	3+	3+	−	−	4+
XOe	4+	−	−	−	2+	−	−	4+
XOf	4+	−	−	−	−	−	−	4+
XOg	4+	−	−	4+s	−	−	−	4+
XOi	4+	−	−	−	−	−	−	4+
XOj	3+	−	−	2+	−	−	−	4+
XOk	4+	−	3+	2+	3+	−	−	4+
XOl	4+	−	3+	4+	3+	−	−	4+
XOm	−	−	−	−	−	−	−	−
XOn	−	−	−	−	−	−	−	−
XOo	4+	−	2+	−	3+	−	−	3+
XOp	4+	−	2+	−	−	−	−	4+
XOq	4+	−	−	−	2+	−	−	4+
XOr	3+	−	2+	−	2+	−	−	4+
XOs	4+	−	2+	−	3+	−	−	4+
XO19-1	−	−	−	−	−	−	−	3+
XO20-6	4+	−	−	−	−	−	−	4+
XO20-8	−	−	−	−	−	−	−	−
XO20-9	−	−	−	−	−	−	−	4+
XO22-2	−	−	−	−	−	−	−	−
XO22-6	4+	−	2+	−	3+	−	−	3+
XO23-1	3+	−	−	4+	2+	−	−	4+
XO24-1	−	−	−	−	−	−	−	4+
XO24-2	4+	−	2+	−	3+	−	−	4+
XOA-1	4+	−	2+	−	−	−	−	4+
XOB-1	−	−	−	−	−	−	−	−
XOC-2	2+	−	−	−	−	−	−	3+
XOD-1	2+	−	−	−	2+	−	−	−
XOD-2	−	−	−	−	−	−	−	4+
XOE-1	3+	−	3+	−	−	−	−	4+
XOE-2	3+	−	2+	−	4+	−	−	4+
XOF-1	3+	−	3+	−	2+	−	−	4+

XOF-2	3+	−	2+	−	2+	−	−	4+
XOH-2	4+	−	2+	2+	4+	−	−	−
X. translucens f. sp. *oryzicola*								
S1	−	−	−	−	−	2+	−	−
S2	−	−	−	2+	2+	2+	−	3+
S3	−	−	−	4+	3+	2+s	−	4+
S24	−	−	−	4+	4+	4+s	−	4+
S27	−	−	−	−	−	−	−	−

a) Only those Stolp-Starr phage preparations and other *Xanthomonas* phages having infectivity on some isolates of *X. oryzae* or *X. translucens* f. sp. *oryzicola* are listed; all others (see Table 1) were not infective.

Symbols are the same as in Table 5.

Table 12. Phage susceptibility patterns of xanthomonads to some *Xanthomonas* phages (excluding the Stolp-Starr phages) which, although isolated and propagated on other than *X. citri*, can lyse *X. citri*[a)]

Bacterial species	Phage preparations							
	Sp1	Sp2	Sp3	Sp4	Sp5	Sp6	Bp3	Cmp
X. begoniae (XB8)	−	−	−	−	−	−	−	−
X. blepharidis (XB115)	−	−	−	−	−	−	4+	4+s
X. campestris (1)	−	−	−	−	−	−	−	−
X. campestris (2)	−	−	3+	−	−	−	4+	4+
X. celebensis (XC144)	−	−	−	−	−	−	−	−
X. corylina (XC12)	2+	2+	4+	2+	2+	−	3+	−
X. cucurbitae (XC6)	−	−	−	−	−	−	−	−
X. geranii (XG10)	−	−	3+	2+	3+	−	−	−
X. hyacinthi (XH110)	−	−	−	−	−	−	−	−
X. malvacearum (XM2)	−	−	4+	−	−	−	3+	−
X. malvacearum (XM106)	−	−	4+	−	−	−	4+	−
X. marantae (XM126)	−	−	−	−	−	−	−	−
X. nigromaculans (1)	−	−	4+	−	−	−	−	−
X. phaseoli (XP104)	−	−	−	−	−	−	−	4+s
X. phaseoli (XP4)	−	−	−	−	−	−	−	−
X. phaseoli (1)	−	−	4+	−	−	−	−	−
X. phaseoli var. *sojense* (XP21)	−	−	4+	−	−	−	3+	−
X. phaseoli var. *sojense* (1)	−	−	−	−	−	−	−	−
X. physalidicola (1, 2)	−	−	−	−	−	−	−	−
X. pisi (1, 2)	−	−	−	−	−	−	−	−
X. pruni (1)	−	−	−	−	−	−	−	−
X. ricinicola (XR101 Sm^r)	−	−	−	−	−	−	−	−
P. (?) rimaefaciens (PR107)	−	−	4+	−	−	−	4+	−

a) Only phages not originating on *X. citri* and having infectivity on some of these isolates are listed; except for the Stolp-Starr phages, all other phages (see Table 1) were not infective on these xanthomonads. See Table 7 for reactions of some of these xanthomonads to *X. citri* phages.

Symbols are the same as in Table 5.

phages (Sp1, Sp2, Sp4, and Sp5) also caused lysis of the isolates of *X. corylina* and *X. geranii*, and a *X. campestris* phages lysed the isolates of *X. blepharidis* and *X. phaseoli.*

However, there were clear differences in plaque morphology. The size of the plaques was generally large measuring 2 to 4 mm in diameter in the homologous combination of the phages for *X. oryzae* and *X. translucens* (except Sp3 and Bp3). On the other hand, the plaques of phages ph1, ph7, and ph17, as well as Sp3 and Bp3, were quite small, almost dots even in the homologous combination; this was probably due to the extended latent period of these infectious phages[9,10]. Those small plaque formers with broad host ranges were tested for inactivation by anti-Sp3 serum. None of the phages except Sp3 was inactivated, indicating no serological relationship among them.

Generally speaking, therefore, it can be said that the *Xanthomonas* phages with broad host ranges extending over heterologous bacteria are characterized by the formation of tiny plaques. Since they are usually soil-borne and can be detected quite easily from the ordinary soil samples, it is not formally correct to give them specific names relating them to the host strain used for isolation and propagation, for instance, "*X. ricinicola* phage". However, such usage is common and we will lapse into it for convenience in presentation and thus avoid such cumbersome locutions as "the phage which was isolated and/or propagated on *X. ricinicola.*" The broad host range phages might be thought of as a sort of soil-inhabiting phage which may—possibly with genetic modification of host-range—propagate through various soil bacteria inclucing phytopathogenic xanthomonads. (The present study, of course, gives practically no experimental evidence bearing on this conjecture.) In contrast, the majority of the *Xanthomonas* phages isolated from diseased plant tissues are usually different from those isolated from soil in that they have very narrow host ranges and large plaques. Such phages might somewhat more correctly be called by referring to the name of the propagating host bacterium, because of their rather specific relationship to that individual bacterial taxon.

Thus, there seems to be two distinct categories of xanthomonad phages. The conclusions of Stolp and Starr[23] concerning phage-bacteria relationship (from the viewpoint of the taxonomy of the genus *Xanthomonas*) should be carefully limited only to the broad host range kind of phage. It is left as an open question whether the phage-bacteria relationships of the genus *Xanthomonas* described here can be applied to any other groups of phytopathogenic bacteria. As far as the phages of the pseudomonads and the *Erwinia* soft rot group are concerned, relationships quite similar to those described here have been observed by us (unpublished) in the host range patterns, with similar distinctions between phages isolated from diseased plant tissues, on the one hand, and those isolated from soil and sewage, on the other hand—the former being strictly limited in specificity. An essentially identical conclusion has been presented in a recent paper by Billing[1] on phytopathogenic pseudomonads. When the phages of phytopathogenic bacteria are discussed, therefore, it is essential to note precisely their sources and the specificity or broadness of host range, otherwise errors may be made in understanding the important relationships between phages and bacteria in nature.

Acknowledgements

Supported by a research grant (AI-08426) from the National Institute of Allergy and Infectious Diseases and by an International Postdoctoral Fellowship (TW-01031), both awarded by the United States Public Health Service. We are grateful to Dr. Arun K. Chatterjee for critical readings of the manuscript.

Literature cited

1) Billing, E. (1970). J. appl. Bact. 33: 478–491.
2) Bradbury, J. F. (1971). Int. J. Syst. Bact. 21: 72.
3) Eisenstark, A. and Bernstein, L. B. (1955). Phytopathology 45: 596–598.
4) Garrett, C. M. E. and Crosse, J. E. (1963). J. appl. Bact. 26: 27–34.
5) Goto, M. (1962). Bull. Fac. Agr. Shizuoka Univ. 12: 3–72.
6) Goto, M. (1965). Ann. Phytopath. Soc. Japan 30: 253–257.
7) Goto, M. (1970). *Ibid.* 36: 171 (abstr.).
8) Goto, M., Frank, P. G. and Ou, S. H. (1971). *Ibid.* 37: 249–258.
9) Goto, M. and Okabe N. (1965 a). Bull. Fac. Agr. Shizuoka Univ. 15: 31–38.
10) Goto, M. and Okabe, N. (1965 b). *Ibid.* 15: 39–44.
11) Goto, M. and Starr, M. P. (1972). Ann. Phytopath. Soc. Japan 38 (4): (in press)
12) Hamon, Y. and Péron, Y. (1961). Ann. Inst. Pasteur, Paris, 100: 818–821.
13) Hayward, A. C. (1964). J. gen. Microbiol. 35: 287–298.
14) Ikari, H. and Wakimoto, S. (1958). Proc. Assoc. Plant Protect., Kyushu, 4: 38–40.
15) Katznelson, H. and Sutton, M. D. (1953). Can. J. Bot. 31: 725–729.
16) Klement, Z. (1959). Nature 184: 1248–1249.
17) Klement, Z. and Lovrekovich, L. (1960). Conference on Scientific Problems of Plant Protection (Budapest), Proc. 1: 187–192.
18) Matsumoto, T. and Okabe, N. (1937). Nogyo oyobi Engei 12: 2055–2059.
19) Okabe, N. (1961). *In* Commemoration of Dr. T. Matsumoto. pp. 61–73.
20) Okabe, N. and Goto, M. (1963). Ann. Rev. Phytopath. 1: 397–418.
21) Serizawa, S. (1971). Bull. Shizuoka Pref. Citrus Exp. Sta. 9: 89–96.
22) Starr, M. P. (1970). Curatorial problems peculiar to an international collection of phytopathogenic bacteria. *In* Culture Collections of Microorganisms (Iizuka, H. and Hasegawa, T. eds.). University of Tokyo Press. pp. 69–71.
23) Stolp, H. and Starr, M. P. (1964). Phytopath. Zeits. 51: 442–478.
24) Sutton, M. D. and Katznelson, H. (1953). Canad. J. Bot. 31: 201–205.
25) Thornberry, H. H., Braun, A. C. and Elrod. R. P. (1949). Phytopathology 39: 152–154.
26) Vasudeva, R. S. (1960). Sci. Rep. Agric. Res. Inst., New Delhi, 1957–1958, pp. 111–130. (R. A. M. 40, 652).
27) Wakimoto, S. (1960). Ann. Phytopath. Soc. Japan 25: 193–198.
28) Wakimoto, S. (1967). *Ibid.* 33: 301–310.
29) Yoshii, H., Yoshida, T. and Matsui, C. (1953). *Ibid.*. 17: 177. (abstr.)
30) Yoshimura, S., Saito, T., Yoshino, M., and Morihashi, T. (1960). Proc. Assoc. Plant Protect., Hokuriku, 8: 15–20.

和文摘要

Xanthomonas citri のファージ・細菌相互関係，とくに他の *Xanthomonas* 属細菌との比較について

後藤正夫・M. P. STARR

Xanthomonas citri のテンペレート・ファージはクロロホルム処理によって完全に不活化されたが，ビルーレント・ファージは同処理に安定であった。供試した *X. citri* 87菌株96培養のうち81培養が Cp1 か Cp2，または両者によって溶菌された。また *Xanthomonas* 属細菌43種136菌株中，7種9菌株 (*X. begoniae*, *X. blepharidis*, *X. cucurbitae*, *X. phaseoli*, *X. phaseoli* var. *sojense*, *X. marantae*, *X. translucens* f. sp. *phleum-pratense*) が *X. citri* ファージの少なくとも一つに感受性を示した。Stolp-Starr (1964) が分離したファージのうち，ファージ ph25 は他の xanthomonads に対するのと同様に，*X. citri* の大部分の菌株に対しても溶菌作用を示す点で特徴があった。一般に土壌から分離されたファージには，寄主範囲がきわめて広く，かつ小型溶菌斑を形成するものが多いのに対し，病斑組織から分離されたファージには寄主特異性が高く，溶菌斑の大型のものが多かった。両者はまったく異なった範ちゅうに属するものと考えられ，*Xanthomonas* 属細菌のファージ・細菌相互関係を検討する場合，両者を区別して考察しなければならないことを示唆した。