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Characterization of *Xanthomonas phaseoli* Bacteriophages

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Ten bacteriophages for *Xanthomonas phaseoli* were characterized. On the basis of adsorption rates, latent period and burst size, plaque morphology, host range, efficiency of plating, ultrastructure, sensitivity to osmotic shock, streptomycin sensitivity, temperature effects on plating efficiency, and serology, the phages were separated into at least three groups. Some of the phages were infectious for *Pseudomonas phaseolicola* (four strains) and *P. syringae* (one strain) in a narrow temperature range. The taxonomic and ecological significance of this finding is discussed.

Little is known about bacteriophages of phytopathogenic bacteria including *Xanthomonas* species (10), although phage typing of particular *Xanthomonas* pathogens has been done (6, 12, 14). Some data are available on epidemiology of phages of *Xanthomonas* species (14) and on their host range, latent period and burst size, and morphology (8, 9, 11, 13, 14). Only one phage originally isolated for *X. phaseoli*, cause of common blight of beans, has been characterized (8). Comparative data on *X. phaseoli* phages have not been available.

The present study was undertaken to obtain basic information about the phages necessary for further work on the interaction of *X. phaseoli* phages with their hosts and on the effect of this interaction on the plant host.

MATERIALS AND METHODS

Media. All *Xanthomonas* strains were maintained on yeast-glucose-carbonate-agar (2). All other bacteria were maintained on a nutrient broth-yeast extract-glucose (NBY) agar (15). NBY broth was used for propagation of bacteriophages and their hosts, whereas 1.5% NBY agar and 0.7% agar were used for the hard- and soft-agar layers, respectively, in phage plating (1).

Bacterial cultures. *X. phaseoli* strains SB7-244, 2ESB, XPS, K4-T, K4-Op, K3-A, SB-1, SB7-191, C-T, and C-Op were isolated in this laboratory from infected bean leaves or pods. All cultures were streaked out repeatedly until one colony type was obtained. The original isolates produced large, opaque, mucoid colonies on NBY agar. The designations T or Op indicate cultures which, after three to five repeated single-colony isolations, grew as translucent or opaque colonies, respectively. Translucent colonies retained the mucoid character but were smaller than the opaque type. Some of the T cultures reverted to the initial opaque type but retained the phage sensitivity patterns found in the T state. Pathogenicity of T and Op isolates appeared indistinguishable.

X. phaseoli strain XP6022 is a derivative of strain P60, kindly provided by M. D. Sutton, Plant Research Institute, Ottawa, Canada. Strains XP4 (from which XP4-T and XP4-Op were derived) and XP104 sm^r, a rough streptomycin-resistant strain, were provided by M. P. Starr, from the International Collection of Phytopathogenic Bacteria, University of California, Davis.

Other bacteria tested for phage sensitivity were isolated in this laboratory or obtained elsewhere. The bacteria included: *X. phaseoli* var. *fuscans* (4 isolates), *X. phaseoli* var. *sojense* (1), *X. vesicatoria* (3), *X. ricinicola* (1), *Agrobacterium tumefaciens* (1), *Corynebacterium flaccumfaciens* (1), *C. flaccumfaciens* var. *aurantiacum* (1), *Escherichia coli* (3), *Pseudomonas aeruginosa* (1), *P. fluorescens* (2), *P. phaseolicola* (21), *P. syringae* (5), *P. tabaci* (1), *Serratia marcescens* (3), *Serratia* species (2), and *Streptococcus faecium* (1).

Bacteriophage isolation and purification. Methods of isolation, purification, and propagation were essentially as described by Adams (1). Phage sources are shown in Table 1. For phages isolated in this laboratory, samples of soil (about 5 g), seed or bean straw (about 2 g), or sewage (10 ml of a sample centrifuged at 2,000 × *g* for 10 min) were added to 30 ml of exponentially growing XP6022 in 250-ml DeLong flasks. The cultures were incubated for 20 to 22 hr at 25 C on a rotary shaker at 150 rev/min. After allowing debris to settle, the supernatant fluid was decanted and centrifuged at low speed for 10 min (12,100 × *g*, Servall RC2-B). The supernatant fluids were tested for phage both directly and after shaking with approximately 10% (v/v) chloroform.

All phage isolates, including those obtained elsewhere, were purified by three successive single-plaque isolations on their original host (Table 1). A single plaque was then stabbed and used to inoculate 10 to

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20 ml of log-phase cultures in 250-ml DeLong flasks. These cultures were incubated as before. Moderately large lysates were then prepared by adding the phage to six flasks at a ratio of phage to bacteria of 0.1 to 0.2 to 150 ml of log-phase cells at 10^8 to 2×10^8 bacteria per ml, in 500-ml DeLong flasks. After incubation at 25 C for 6 to 10 hr, the flask contents were pooled and stored at 2 C for 1 week or longer. Initial titers ranged from 10^{10} to 10×10^{10} plaque-forming units (PFU)/ml. The lysates were differentially centrifuged by alternating three to four cycles at 12,100 $\times$ *g* for 10 min with two to three cycles at 27,000 $\times$ *g* for 2 hr (Spinco, model L). After each high-speed centrifugation, the pellets were resuspended for 24 to 48 hr on an inclined rotator (Scientific Industries) at 1 rev/min in 1 ml of phosphate-magnesium buffer (0.01 M phosphate, 10^{-3} M $MgSO_4$, *p*H 7.2). Before the second high-speed centrifugation, the pooled pellets for each phage (about 30 ml of each suspension) were treated with ribonuclease (Worthington Biochemical Corp., Freehold, N.J.) at 1 μg/ml and deoxyribonuclease (Worthington) at 10 μg/ml for 30 min at 37 C and then were treated with chloroform (5%, v/v) for about 5 min on a rotary shaker. After the final low-speed centrifugation, the purified lysates were kept at 2 C over 1 to 2% (v/v) chloroform.

Adsorption rates and one-step growth experiments. For determining the adsorption rate, 0.1 ml of phage at 1.5×10^7 to 3.0×10^7 PFU/ml was added to 0.9 ml of cells at 10^8 to 2×10^8 bacteria per ml, kept at 25 C (water bath). Samples were diluted at intervals into cold NBY containing chloroform (5%, v/v) and were assayed for unadsorbed phage. The adsorption rate constant was determined as described by Adams (1). The one-step growth experiment was essentially as described by Adams (1). Phage were added at a ratio of phage to bacteria of 0.01 to 0.02 to 0.9 ml of bacteria at 10^8 to 2×10^8 bacteria per ml in a water bath at 25 C. The phage were allowed to adsorb for 20 min (50 to 90% adsorption, depending upon the phage), diluted 10^{-3} and 10^{-5} into NBY, and assayed for unadsorbed phage. The diluted cultures were incubated at 25 C on a rotary shaker (150 rev/min) and were assayed at intervals up to 5.5 hr.

TABLE 1. *Source of isolation of X. phaseoli bacteriophages*

Code no.	Bacterium code no.	Origin of sample	
		Material	Geographic source
1[a]	XP4	Soil	California
20[a]	XP104sm[r]	Soil	California
22[a]	XP4	Soil	California
ϕSD	XP6022	Seed infected with *P. phaseolicola*	New York
ϕSL	XP6022	Soil, infested with *P. phaseolicola*	New York
ϕ56	XP6022	Dry bean straw, infected with *X. phaseoli* var. *fuscans*	Nebraska
ϕ112	XP6022	Dry bean straw, infected with *X. phaseoli*	Nebraska
ϕRS	XP6022	Raw sewage	Nebraska
ϕPS	XP6022	Primary sewage	Nebraska
Pg60[b]	XP6022	Seed, compost, sewage mixture	Canada

[a] Provided by M. P. Starr, University of California, Davis.

[b] Provided by M. D. Sutton, Plant Research Institute, Ottawa, Canada.

Host range and efficiency of plating (EOP). Both spot tests with concentrated phage suspensions and quantitative tests were used. A glass multiple inoculator, designed to fit Disposo-trays (Linbro Chemical Co.), was used in the spot tests, in which 0.5×10^4 to 10^4 PFU were delivered to the inoculated soft-agar layer. All phages were tested against a given host on a single plate.

In determining the relative EOP, the titer on the best host was used as the standard (1). In determining the effect of temperature on EOP, the plaque count at the optimal temperature was taken as the standard.

Plates incubated at 24, 28, and 32 C were read after 24 hr (*X. phaseoli* plates) or 48 hr (*P. phaseolicola* and *P. syringae* plates). Plates incubated at 20 C were read after 2 to 3 days; those incubated at 13 and 8 C were read after 4 days; and those incubated at 2 C were read after 5 to 7 days at 2 C followed by 24 hr at 24 C. The incubation at 24 C was used because no visible lawn appeared at 2 C after 5 to 7 days. The EOP values at 2 C are therefore not strictly comparable to the values at the higher temperatures; however, the values at 2 C are consistent with the trends of the higher temperature values.

Serology. Bacteriophages ϕPS and ϕ56 at titers of 5×10^{11} PFU/ml and 2×10^{12} PFU/ml, respectively, were used for preparation of antiphage sera. Each antigen was injected into two rabbits intramuscularly. The first injection consisted of 2 ml of a 1:1 mixture of phage and Freund adjuvant emulsified together. After 5 days, 0.5-ml quantities of the phages were injected intravenously, followed by two more intramuscular injections, 2 and 7 days later (0.5 ml and 0.125 ml, respectively, with equal volumes of Freund adjuvant). One month after the final injections, after positive test bleedings, the rabbits were bled by cardiac puncture. Antisera were kept frozen at -20 C until required.

Neutralization experiments were essentially as described by Adams (1). Antisera were diluted in 10^{-3} M NaCl. All tests were at 25 C (water bath). Samples of 0.1 ml of the test phage in NBY at 10^6 or 10^7 PFU/ml were added to 0.9 ml of antiserum, diluted at intervals, and assayed for survivors. Control serum and 10^{-3} M NaCl had no effect on any phage.

Electron microscopy. A drop of phage suspension was placed on a carbon-backed collodion membrane on a specimen grid, followed by a drop of fixative, 5% glutaraldehyde in 0.1 M PO_4 (*p*H 7). The drop of phage and fixative was allowed to stand for 5 to 30 min and blotted dry; then the grid was washed in a gentle

stream of water for about 5 sec. The samples were stained several ways. For uranyl acetate staining, 2% uranyl acetate was then added, and the excess was removed immediately with filter paper. Alternatively, phage were stained with neutral 2% potassium phosphotungstate, or with a mixture of 2% phosphotungstic acid-2% potassium phosphotungstate-1% vanadatomolybdate (1:1:0.6), or with a mixture of 2% potassium phosphotungstate and 1% vanadatomolybdate (3:1). The vanadatomolybdate was prepared according to Callahan and Horner (4). The integrity of the head structure was best preserved in the uranyl acetate. Extensive head structure disintegration, even after fixation, occurred in neutral phosphotungstate mixtures, except for phages 22 and ϕSL.

RESULTS

Plaque morphology. In the usual incubation period (18 to 24 hr at 24 C), phage virulent for the propagating *Xanthomonas* host strain produced clear plaques with sharp boundaries, except ϕSL which produced plaques with irregular borders. The plaque diameter of Pg60 on XP6022 was 4 to 5 mm, that of ϕSL was 1 mm, and that of the remainder was 2 to 3 mm. Plaque development of phages on *Pseudomonas* species was slow, so that few plaques were visible by 18 to 24 hr, although lawn development was excellent. By 36 to 48 hr, however, plaques were readily seen. All plaques were turbid.

Host range and EOP. Most *X. phaseoli* strains were susceptible to one or more phages, although only Pg60 affected all *X. phaseoli* strains tested (Table 2). All T strains were derived from Op strains, and each of these differed in phage sensitivity spectrum from the Op strain of origin. Strains K4-T and C-T reverted to the Op state but retained the phage sensitivity spectra of the T state. All other strains were stable to storage and transfer. Of the four isolates of *X. phaseoli* var. *fuscans* tested, only D-Op was susceptible to phage. *X. phaseoli* var. *sojense* was not susceptible to any phage, nor was a single strain of *X. ricinicola* or two strains of *X. vesicatoria*. The L-2 strain of *X. vesicatoria* was susceptible to a number of phages, but such nonspecificity of phages for nomen species (nomenclatural units) of *Xanthomonas* has been observed before (12, 13). White mutants of XP104 sm^r and *X. phaseoli* var. *fuscans* responded as the parent strain to the phages.

Strains of the fluorescent phytopathogenic pseudomonads *P. phaseolicola* and *P. syringae* were susceptible to a number of phages originally isolated for *X. phaseoli* strains (Table 2). However, the sensitive strains were susceptible only in a limited temperature range (*see below*). All other bacteria tested were insensitive to the phages.

Adsorption, latent period, and burst size. Since XP6022 was susceptible to all of the phages, it was chosen as the host bacterium. Table 3 shows that all of the phages adsorb at a reasonably rapid rate and generally have long latent periods (corresponding approximately to the generation time of the host, 110 min) and moderate to good burst sizes. When "old cells" (10^9 to 2×10^9 bacteria per ml) were used, adsorption rates remained about the same, except for Pg60, for which adsorption decreased by a factor of 10. The latent and rise periods remained the same, whereas burst sizes generally decreased slightly.

Cross-resistance of phage-resistant mutants. Mutants of XP6022 were selected for resistance to each of the 10 phages. Two or three independent isolations were made of each resistant type. The Pg60-resistant mutants were susceptible to all of the other nine phages, whereas strains resistant to any one of the remaining nine phages were resistant to all of the phages except Pg60 (Table 3).

Temperature effect on EOP. The EOP at different temperatures was determined, with EOP at the optimal temperature taken as 1. *X. phaseoli* phages, except phage 22, had a wide temperature range where plating efficiency was near optimal, encompassing the temperature range of good bacterial growth (Fig. 1). Phage 22 had an optimal EOP at a lower temperature than the other phages. The decrease in plaque counts at high temperature (32 C) on XP6022 could be mitigated for all phages, except 22 and Pg60, by prior incubation of plated phage for 30 to 60 min at 24 C. Phages 22 and Pg60 produced no plaques at 32 C, whether incubation was immediate or delayed. The sensitivity of *P. phaseolicola* and *P. syringae* to certain phages (Table 2) occurred in a narrow

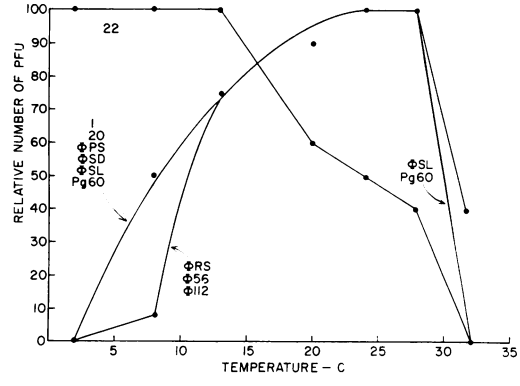

FIG. 1. *Effect of temperature on the EOP of X. phaseoli phages on XP6022. EOP was determined as described.*

TABLE 2. *Host range and EOP of X. phaseoli bacteriophages*[a]

Bacterial strain	Bacteriophage									
	1	20	22	φPS	φRS	φ56	φ112	φSD	φSL	Pg60
X. phaseoli										
XP6022	1	1	0.2	1	1	1	1	1	1	1
XPS	1[b]	1[b]	0.2[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1[b]	1
SB7-244	1	1	0.2	1	1	1	1	1	1	0.3
K3-A	1	1	0.02[c]	1	1	1	1	1	1	0.05[c]
SB7-191	1[b]	1[b]	0.04[b]	1[b]	1[b]	1[b]	1[b]	1[b]	0.3[b]	1[b]
2ESB	0.1[b]	0.5[b]	2×10^{-5}[b]	0.5[b]	0.5[b]	0.5[b]	0.5[b]	0.5[b]	0.1[b]	0.5
K4-T	1	1	0.04	1	1	1	1	1	1	1
K4-Op	0.2[b]	1[b]	2×10^{-4}[b]	1[b]	1[b]	1[b]	1[b]	1[b]	10^{-3}[b]	1
XP4-T	0.1	0.1	1	0.1	0.1	0.1	0.1	0.1	1	1
XP4-Op	0[d]	0	0	0	0	0	0	0	0	1
C-T	0	0	0	0	0	0	0	0	0	0.5[c]
C-Op	0	5×10^{-3}[b]	0	5×10^{-3}[b]	5×10^{-3}[b]	5×10^{-3}[b]	5×10^{-3}[b]	5×10^{-3}[b]	0	0.5
Xp104	0	0.5	0	0	0	0	0	0	0	1
SGN	0	0	0	0	0	0	0	0	0	1
X. phaseoli var. *fuscans*										
D-T	0	0	0	0	0	0	0	0	0	0
D-Op	0	10^{-3}[b]	0	10^{-3}[b]	10^{-3}[b]	10^{-3}[b]	10^{-3}[b]	10^{-3}[b]	0	0
X. vesicatoria										
L2	0.5[b]	1[b]	2×10^{-3}[b]	1[b]	1[b]	1[b]	1[b]	1[b]	10^{-5}[b]	5×10^{-5}[b]
P. phaseolicola[e]										
HB11	4×10^{-4}	6×10^{-2}	0	5×10^{-2}	2×10^{-2}	10^{-2}	10^{-2}	6×10^{-2}	0	0
HBW	6×10^{-4}	3×10^{-2}	0	6×10^{-2}	3×10^{-2}	6×10^{-2}	3×10^{-2}	7×10^{-2}	0	0
R1-B	2×10^{3}	5×10^{-2}	0	5×10^{-2}	2×10^{-2}	2×10^{-2}	2×10^{-2}	3×10^{-2}	0	0
P33	0	10^{-2}	0	10^{-2}	10^{-2}	6×10^{-3}	10^{-2}	10^{-2}	0	0
P. syringae[f]										
PsC-1B	0	4×10^{-2}	0	7×10^{-2}	4×10^{-2}	3×10^{-2}	3×10^{-2}	8×10^{-2}	0	0

[a] In determining the relative EOP, the titer on the best host was used as the standard = 1; plates were incubated for 18 to 24 hr at 24 C.
[b] Turbid plaques.
[c] Semiturbid plaques.
[d] Not susceptible.
[e] *P. phaseolicola* plates were incubated for 36 to 48 hr at 28 C; all plaques were turbid.
[f] *P. syringae* plates were incubated for 36 to 48 hr at 32 C; all plaques were turbid.

TABLE 3. *Characteristics of X. phaseoli bacteriophages*

Phage	Adsorption rate constant K[a]	Latent period[a]	Rise period[a]	Burst size (relative increase in PFU/ml)[a]	Cross resistance pattern[b]	Temp of optimal EOP[a]	Susceptibility of *P. phaseolicola* or *P. syringae*[c]	Serological group[d]	K values[e]		Streptomycin sensitivity[f]	Osmotic shock sensitivity[f]	Morphology[g]
									Anti-φ56 serum	Anti-φPS serum			
	ml/min	*min*	*min*			*C*							
1	2×10^{-10} 3×10^{-10}	110–120	90–120	90–130	I	24–28	a, b, C	1	29	12	R	R	E, C
20	3×10^{-10} 4×10^{-10}	100–140	70	130–170	I	24–28	A, B, C	1	6	11	R	R	H, T
φPS	3×10^{-10} 4×10^{-10}	120–125	60–80	90–190	I	24–28	A, B, C	1	5	212	R	S	E, C
φSD	3×10^{-10} 4×10^{-10}	115–120	60–65	240–260	I	24–28	A, B, C	1	5	212	R	S	E, C
φRS	2×10^{-10} 3×10^{-10}	110–120	60–90	110–180	I	24–84	A, B, C	1	165	127	R	R	E, C
φ56	3×10^{-10}	120–125	75–85	95–110	I	24–28	A, B, C	1	110	10	R	R	E, C
φ112	3×10^{-10} 4×10^{-10}	120–135	80–100	80–190	I	24–28	A, B, C	1	110	10	R	R	E, C
φSL	10^{-10}	150–165	75–100	50–110	I	24–28	a, b, c	1	1.5	2.5	R	R	E, C, T
22	2×10^{-10} 3×10^{-10}	170–200	70–100	35–65	I	2–13	a, b, c	2	0	0	S	R	H
Pg60	10^{-9}	35–40	15–25	40–60	II	24–28	a, b, c	2	0	0	R	R	H

[a] Host strain XP6022; three to five experiments.

[b] I, a bacterial mutant resistant to any phage in this group is resistant to all phages of the group. II, no cross-resistance to any group I mutants.

[c] A, *P. syringae* PsC-1B, susceptible; a, not susceptible, B, *P. phaseolicola* P33, susceptible; b = not susceptible. C, *P. phaseolicola* R1-B, HBW, susceptible; c = not susceptible.

[d] Group 1 is related; group 2 may be unrelated to each other.

[e] See text.

[f] R, resistant; S, sensitive.

[g] E, elongated, hexagonal head; H, hexagonal head; C, cross-bars on tail; T, tail fibers or appendages.

temperature range (Fig. 2). The optimal EOP of phages on sensitive *P. phaseolicola* strains was at 24 or 28 C, depending on the particular host strain and phage (Fig. 2B to 2D). *P. phaseolicola* strain P33, not shown, was sensitive to the phages φPS, φSD, φRS, φ56, φ112, and 20 at 24 C, with no plaque development at 20 or 28 C. The optimal EOP of four phages on *P. syringae* was near the temperature limit for growth (about 34 C); the other two phages had an optimal EOP at the optimal growth temperature (28 C; Fig. 2A).

Serology. All phages were tested with φPS and φ56 antisera. Phages φ56 and φ112 were indistinguishable by both antisera, as were φPS and φSD (Table 3). In 8 of 12 experiments, the heterologous phage φRS reacted more strongly with the φ56 antiserum than with φ56 itself (Fig. 3), with two independently produced antisera. In 4 of 12 experiments, φRS inactivation was indistinguishable from φ56 inactivation. Phages 22 and Pg60 showed no reaction in either antiserum. Either antiserum neutralized the remaining phages, but more slowly than they neutralized their homologous phages. Therefore, there are at least two serological groups, one of which comprises most of the phages, the other which comprises phages 22 and Pg60.

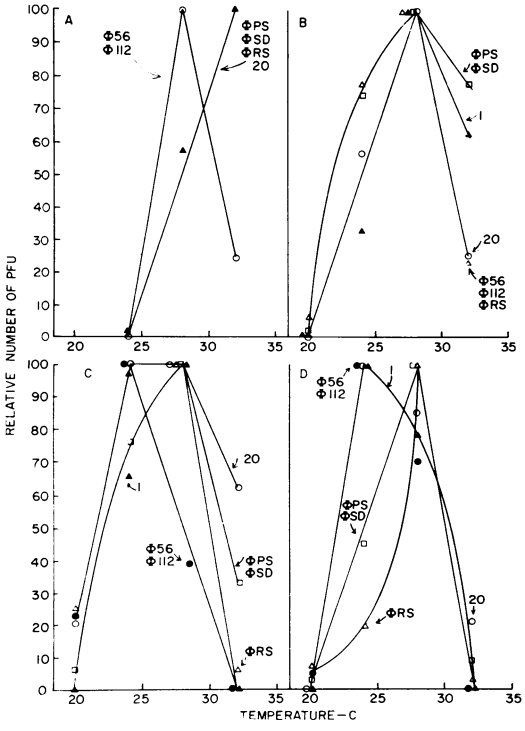

FIG. 2. *Effect of temperature on the EOP of X. phaseoli phages on P. syringae and P. phaseolicola. (A) P. syringae, PsC-1B; (B) P. phaseolicola, R1-B; (C) P. phaseolicola, HBW; (D) P. phaseolicola, HB11.*

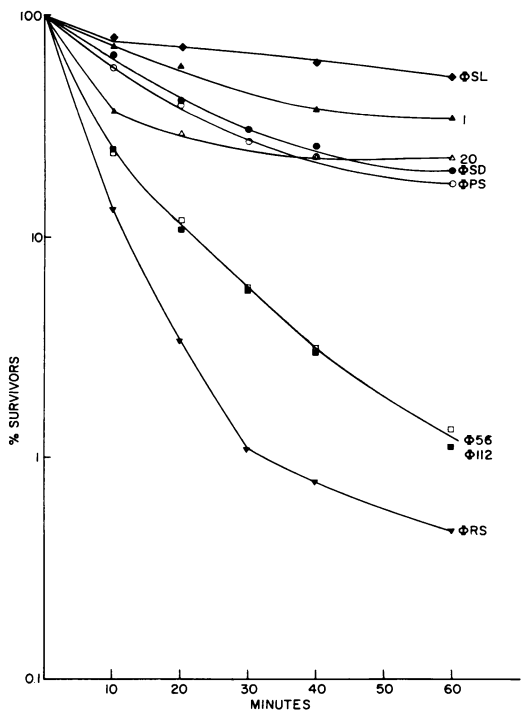

FIG. 3. *Antiserum action on X. phaseoli phages. Phage φ56 antiserum was diluted 1:1,000 in 10⁻³ M NaCl to test phages 1, φ56, φ112, and φRS, and 1:100 to test φPS, φSD, φSL, and 20.*

Neutralization rate constants or the *K* value [*see* Adams (1)] are not applicable to reactions which are not first-order. The neutralization curves obtained approached first-order kinetics after an initial fast reaction. As an approximation and for comparative purposes, the *K* values were calculated for the 20-min neutralization point (Table 3). Heating the antisera at 56 C for 30 min had no effect on the reaction kinetics.

Streptomycin sensitivity. When plated on streptomycin (500 μg/ml)-NBY agar with a streptomycin-resistant mutant of XP6022, all phages, except 22, were resistant to the antibiotic (Table 3).

Osmotic shock. Phages φPS and φSD (which are serologically identical) were inactivated by exposure to 0.9 M NaCl for 30 sec or more, followed by 100-fold dilution into NBY. Inactivation was greater than 99%. None of the other phages was sensitive to salt exposure (Table 3), even for as long as 120 min.

Electron microscopy. The gross similarity in morphology of these phages is shown in Fig. 4. Phages 1, φPS (Fig. 4a), φSD, φ56, φ112, and

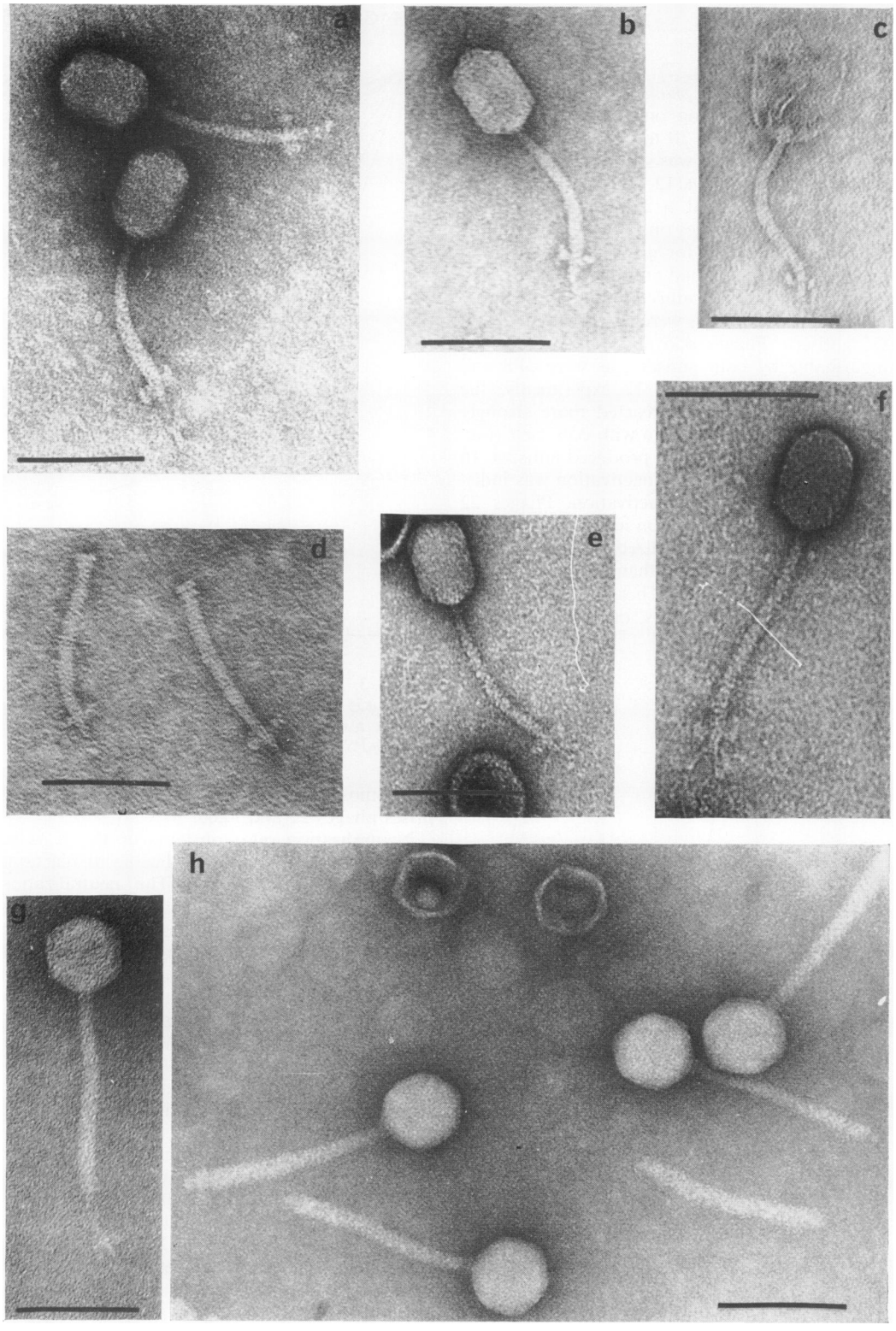

FIG. 4. *Electron micrographs of X. phaseoli phages. (a) Phage φPS, (b) φRS, (c) φRS empty virion, (d) φRS tails, (e and f) φSL, (g) phage 20, (h) phage 22. (a to e, h) × 200,000; (f) × 250,000; (g) × 190,000. Markers, 0.1 μm*

ϕRS (Fig. 4b) are similar to *P. aeruginosa* phage PB2 (3). The elongated hexagonal head size averages 75 by 50 nm, and the tapered tail averages about 130 nm. The tail wings or cross bars are prominent when seen at all but are not seen on all particles. Ghosts and isolated tails (Fig. 4c and d) show a distinct disc at the presumed head attachment end. Phage ϕSL, although similar to the above phages, has tail fibers in addition (Fig. 4e and f). The phages Pg60, 20, and 22 (Fig. 4h) have hexagonal heads, with an average head diameter of 60 nm, and tails averaging 160 nm. Phage 20 appears to have a tail appendage (Fig. 4g). No phages with contracted tails were observed. These morphological features are listed in Table 3.

DISCUSSION

The wide distribution of apparently identical phages, e.g., ϕSD from New York and ϕPS from Nebraska, is curious, since a short generation time would tend to augment genetic drift. Alternatively, the apparent identity of these phages may be due to some very fast transport system. Since bacteria in plant epidemics can travel 500 miles in 6 days or less (16), phages may also. That 8 of 10 phages fall in one serological group also suggests wide distribution of a restricted number of phage types for *X. phaseoli*.

The phages appear to fall into three groups. Group 1 comprises all of the serologically related phages, which are similar in several, but not in all, other properties. Phage 22 (group 2) is not serologically related to the group 1 phages but is related to these phages on the basis of similar rereactions towards the same phage-resistant mutants. Phage 22 and phage Pg60 (group 3) are probably unrelated to each other since they differ markedly in almost all characters: latent period, burst size, EOP at different temperatures, plaque morphology, and reaction towards the same phage-resistant mutants. Phage 20 appears to belong to group 1 on serological grounds (Table 3) and in group 2 or 3 on morphological grounds.

The serological neutralization of homologous and heterologous phages resulted in complex inactivation curves. Similar inactivation curves have been observed for other phages and may result from several factors (1). The faster reaction of the heterologous phage, ϕRS than ϕ56, in ϕ56 antiserum may result from a greater quantity of the reacting antigen(s) present in ϕRS, greater accessibility of the reacting antigen(s), or differences in the critical antigens. ϕRS differs from ϕ56 on the basis of reaction to ϕPS antiserum and on the basis of greater susceptibility to inactivation during the purification process of lysates (1% recovery compared with 70 to 80%).

The close relationship between *Xanthomonas* and *Pseudomonas*, determined by deoxyribonucleic acid hybridization tests (5), is supported by the crossover of *X. phaseoli* phages to *Pseudomonas* species reported here.

Temperature may affect the ecological balance between phages and their usual hosts. Thus in hot weather, *X. phaseoli* phages may attack not only *Xanthomonas* but also *Pseudomonas* species which would be immune at lower temperatures. This hypothesis suggests that halo blight of bean (caused by *P. phaseolicola*) and brown spot of bean (caused by *P. syringae*) might be more prevalent at or below 22 C because of phage resistance. Both diseases are well known as cool-temperature diseases (17). Of course, other factors may also affect disease prevalence; e.g., toxin formation by *P. phaseolicola* is also favored by low temperatures (7).

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