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Characteristics of Bacteriophages Attacking Psychrophilic and Mesophilic Pseudomonads[1]

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Ten *Pseudomonas* phage were isolated by sewage enrichment. Five psychrophilic and five mesophilic phage were selected for a description of some of their biological properties. In addition to growth on psychrophilic hosts, the psychrophilic phage studied were also able to grow on a mesophilic host within its growth temperature range. Latent periods for psychrophilic phage at 3.5 C were 6 to 12 hr and at 25 C were 30 to 60 min. Mesophilic phage had a latent period of 85 to 190 min at 25 C and 35 to 85 min at 37 C. Psychrophilic phage were significantly more heat-sensitive than the mesophilic phage. Of all the parameters studied, only thermal sensitivity correlated with growth at 3.5 C. Phage used in this study had a deoxyribonucleic acid base composition ranging from 39.6 to 68.2% guanine plus cytosine, deduced from melting temperature measurements.

There are several reports of phages growing near 0 C (17, 22). However, the biological properties of these phages have not been described nor has their composition been studied definitively. Furthermore, there are no reports of psychrophilic phages growing on mesophilic bacteria at temperatures within the growth temperature range of the mesophilic host. Previous studies on *Pseudomonas* phages have been concerned primarily with those attacking the mesophile *Pseudomonas aeruginosa*. These phages have been shown to possess single-stranded deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), as well as double-stranded DNA (8, 11, 24). The morphology of a few *Pseudomonas* phages has also been studied (4) and was found tc be as variable in form and size as that observed for phages of the coli-typhoid organisms.

There have been few definitive studies with phages attacking saprophytic or phytopathogenic *Pseudomonas* species. Investigations with phytopathogens have been concerned primarily with the identification of the host bacteria on the basis of their susceptibility to virulent phage or lack of it due to lysogeny (3, 10). The isolation and growth of phage attacking the soil saprophyte *P. fluorescens* has been described (13, 14), but other phages of this group have not been studied in this manner.

This report describes some of the biological properties of a group of psychrophilic and mesophilic phages. It was considered that, within the psychrophilic and mesophilic groups, variations in given biological properties would indicate the irrelevancy of these properties to growth at low temperatures. Furthermcre, we were particularly interested in phage characteristics correlating with psychrophily.

MATERIALS AND METHODS

Bacteriophage isolation and enumeration. Medium containing 0.5% tryptone (Difco), 0.1% glucose, and 0.25% yeast extract, in distilled water (TGE), was used for the routine cultivation and enumeration of bacterial and phage strains. Phage assays were made by use of the double agar-layer method of Adams (1). The base layer was solidified with 1.5% agar, and the soft agar layer contained 0.7% agar. $CaCl_2$ was also included in all media used for plating or adsorption of phage in one-step growth determinations. Phage was obtained from sewage effluent clarified by low-speed centrifugation. The resulting sewage supernatant fluid was added to an early logarithmic-phase culture of the enriching bacterial host which had been centrifuged and suspended in TGE broth. Sewage and propagating host suspensions were mixed at the ratio of 1:2, respectively. After 48 hr of incubation of a shallow

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enrichment under static conditions, the supernatant fluids obtained by low-speed centrifugation were freed from residual bacteria by the addition of chloroform to 4%. These chloroformed phage suspensions were diluted in TGE broth for use in single-plaque isolations. Stock phage suspensions were prepared by the picking and elution of single plaques after at least six serial single plaque isolations. These phage stocks were stored in TGE over chloroform at 3 C. High-titer bulk phage stocks were obtained by the elution of TGE plates which had been surface-inoculated with 4 ml of TGE broth containing approximately 5×10^8 bacteria and 5×10^4 phage. By use of this biphasic medium system, phage enriched and isolated at 37 C were propagated at 37 C, and those isolated at 20 to 25 C were propagated at 25 C. After elution, phage stocks were subjected to several cycles of low- and high-speed centrifugation to promote removal of debris. Final suspension was in TGE broth or TMN buffer [tris(hydroxymethyl)aminomethane, 0.01 M; NaCl, 0.15 M; $MgSO_4$, 0.01 M; *p*H 7.4] and storage was at 3 C.

Growth kinetics. The procedures used were essentially those of Adams (1). Logarithmically growing cultures were centrifuged and the cells were suspended in TGE broth to approximately 2×10^8 per ml. For 25 and 37 C trials, the bacteria were infected at a multiplicity of 0.01 phage per bacterium, and phage was allowed to adsorb for 10 min. Adsorption was stopped by dilution into TGE broth, and unadsorbed phage was determined by centrifugation or filtration. For growth at 3.5 C, the bacteria were infected at a multiplicity of 0.1, and adsorption took place for 60 min. Infected cultures were sampled for phage production at 60-min intervals at 3.5 C and at 5-min intervals for 25 and 37 C incubations.

RESULTS

Sewage effluent-TGE broth enrichment culture resulted in the isolation of fifty phages showing diverse biological properties. From these, 10 were selected for more detailed study because of their unique plaque morphologies, host range, or growth temperature range. The plaque morphologies are shown in Fig. 1 for the phages used in this study. These were obtained by use of the same bacterial strains and incubation temperatures as for primary isolation. Variation in plaque size regularly occurred for a given phage and was repeated when individual plaques of different size were picked and plated. When host range was studied, virulence of the mesophilic phages PX2, PX3, PX5, CB3, and PX7 was limited to *P. aeruginosa*, their mesophilic bacterial host. However, the psychrophilic phages PX1, PX4, PX10, PX12, and PX14 were capable of attacking both *P. aeruginosa* and the soil pseudomonads. This is of some interest since cross-susceptibility of *P. aeruginosa* and soil pseudomonads was not observed by others who tested for this possibility with virulent phages (13, 23). However, Patterson (18) has reported three temperate phage produced by *P. fluorescens* and attacking *P. aeruginosa*.

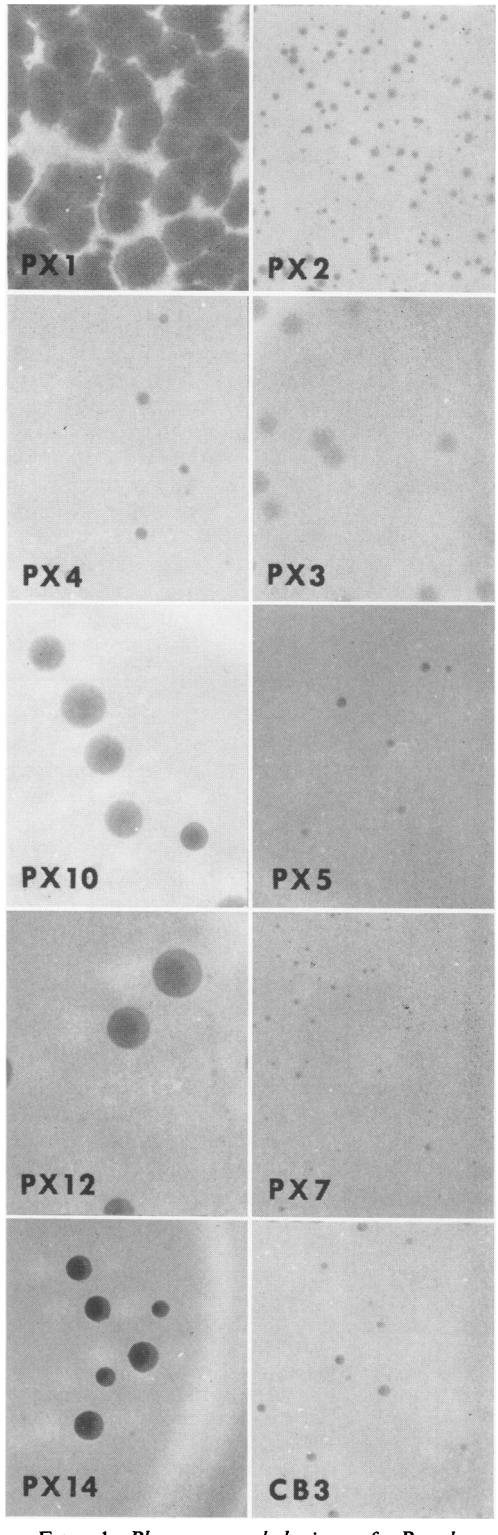

FIG. 1. *Plaque morphologies of Pseudomonas phages.*

The uniqueness of the phages selected for this study was supported by serological studies as well as by their differing plaque morphologies and host ranges. With the exception of one pair, the phages were serologically distinct. However, with PX7 and CB3, *K* values for the heterologous phage-antiserum combination were approximately 60 to 70% of the *K* value calculated for the homologous phage-antiserum determination. Plaque morphology differences for these were also minor.

Growth kinetics. The growth temperature ranges of the phages were determined by spotting bacterial lawns with approximately 10^4 phage and incubating the plates at various temperatures within the growth temperature range of the indicator bacteria. When this was done, the psychrophilic phage obtained by enrichment at 20 to 25 C grew on psychrophilic bacteria at or near 0 C, depending upon the bacterial strain. When plated on mesophilic bacterial lawns, the phages of this group were fully infective at the lowest incubation temperature resulting in indicator growth. A feature of these phages was their progressively larger plaque size as the incubation temperature was lowered to the minimal temperature which supported bacterial growth. A further distinguishing feature of these phages was their inability to propagate above 32 C. Although the psychrophilic indicators used did not grow above 32 C either, susceptible mesophilic bacteria were capable of growth up to 44 C. Thus, it was possible to establish the maximal growth temperature of the psychrophilic phage by use of mesophilic indicators.

The mesophilic phages varied in their growth temperature ranges. With the exception of PX5, the mesophilic phages PX2, PX3, PX7, and CB3 were isolated at 37 C on *P. aeruginosa* strains. However, the minimal growth temperature was observed to vary with the phage in question. In this regard, the growth of PX7 and CB3 was restricted at 20 C on some susceptible *P. aeruginosa* indicators and PX2 and PX3 did not form plaques on any of the mesophilic indicators at 20 C. On the other hand, PX5, which was isolated at 23 C, did not grow on any of the indicators at 37 C. Therefore, it would seem to resemble the behavior of psychrophilic phage plated on mesophilic indicators. However, psychrophilic bacterial indicators have not been found to test its ability to grow at 3.5 C. Furthermore, the plaque size of PX5 tends to decrease when the temperature of incubation is lowered from 20 C to the growth temperature minimum of the mesophilic indicator, whereas plaque size increased with psychrophilic phages.

Data representative of single-step growth experiments are shown in Table 1. For this work, at least five separate experiments were done, and the data shown are typical of the results obtained upon repeated trials. The duration of the latent period was constant, although the average burst size did vary considerably from experiment to experiment with some phage-propagating host combinations. The burst sizes shown here are representative of an experiment near the average for all experiments. With PX4, growth on *P. fluorescens* 14 was determined at 3.5 and 25 C because the mesophile *P. aeruginosa* 2 used for its isolation and routine propagation does not grow at 3.5 C nor does it support phage growth at this temperature. The five phage growing at 3.5 C showed a shorter latent period at 25 C than was observed for the mesophilic phages. In this respect, then, these phage growing at 3.5 C resemble psychrophilic bacteria whose optimal growth temperature is often less than that observed for related mesophilic species (12). Significantly, for PX4, this relationship was maintained even though the propagating host was a mesophile.

TABLE 1. *Growth kinetics of Pseudomonas phages*

Phage	Host	3.5 C		25 C		37 C	
		Latent	Burst	Latent	Burst	Latent	Burst
		hr		*min*		*min*	
PX1	*P. putida* A.3.12[a]	12	6	35	106		
PX4	*P. fluorescens* 14	11	16	60	23		
	P. aeruginosa 2	—	—	52	103		
PX10	*P. fluorescens* 22	8	6	35	80		
PX12	*P. fluorescens* 35	7	10	40	50		
PX14	*P. geniculata* 4[b]	6	25	30	120		
PX2	*P. aeruginosa* 1			190	35	85	162
PX3	*P. aeruginosa* 2			85	40	35	88
PX5	*P. aeruginosa* R629			110	155	—	—
PX7	*P. aeruginosa* R629			115	80	50	55
CB3	*P. aeruginosa* 1C			100	20	45	75

[a] Obtained from the American Type Culture Collection as *P. fluorescens* ATCC 12633.

[b] Obtained from Aaron Wasserman, Eastern Regional Research Laboratory. *P. aeruginosa* strains were provided by B. W. Holloway, Department of Bacteriology, University of Melbourne, Melbourne, Australia.

Characteristics of Pseudomonas phages. Some of the general biological characteristics of the psychrophilic and mesophilic phages were studied with the hope of establishing correlations between psychrophilic growth and particular phage properties. Those properties not related to growth temperature range are shown in Table 2. Plating in media containing citrate has been used as an indication of the calcium requirement for phage growth (9). In this instance, plating on citrate was compared to that on media containing calcium. The data indicate that the phage selected

TABLE 2. *Characteristics of Pseudomonas phages*

Phage	Reduction in plating efficiency on citrate[a]		Inactivation after dilution[b] from				Acid *p*H for 10% survival[c]	G + C[d]
			4 M NaCl		2 M sucrose			
	1.0%	0.1%	Fast	Slow	Fast	Slow		
								%
Psychrophilic								
PX1	96	0	0	—	20	0	3.0	52.4
PX4	90	14	0	—	100	100	2.0	44.4
PX10	69	0	0	—	2	0	4.0	53.0
PX12	100	100	24	0	38	0	4.0	55.8
PX14	90	15	0	—	28	0	4.0	53.8
Mesophilic								
PX2	100	66	0	—	91	0	3.0	68.2
PX3	73	2	0	—	0	—	2.0	45.0
PX5	100	94	67	100	3	0	4.0	39.6
PX7	0	0	0	—	0	—	<2.0	54.6
CB3	59	29	15	15	0	—	<2.0	60.4

[a] Plated in TGE-citrate-agar without added $CaCl_2$.

[b] Approximately 10^6 phage were held at 23 C for 30 min in TGE broth containing NaCl or sucrose. Slow dilutions were made over 90 min.

[c] Approximately 10^6 phage were held at 25 C for 30 min in TGE broth adjusted with HCl. Treatment was terminated by dilution into phosphate-buffered TGE broth (*p*H 7.0).

[d] Phage DNA was extracted with phenol as described by Thomas and Abelson (25). DNA was collected on glass rods and dissolved in saline-citrate or 5 M $NaClO_4$ (*p*H 7.2). Percentage G + C was determined from T_m values according to Marmur and Doty (15).

for this study vary considerably in their susceptibility to citrate inhibition of growth. However, by this means, PX7 and CB3, which were serologically related, could be distinguished. Results showing the inactivation of phage by high osmotic exposure are the average of three or more trials. The effect of dilution rate on survival was determined because it was previously shown that sucrose inactivation of coliphage may occur independently of the rupture of the phage usually associated with rapid alteration of the osmotic environment (2). This was observed with sucrose in the case of PX4. However, a similar effect was also noted for PX5 and CB3 with 4 M NaCl. With CB3, a constant fraction of the input phage was always inactivated by NaCl exposure. However, if resistant survivors were picked and tested, approximately the same proportion were again inactivated. Therefore, growth of this phage may result in the production of phenotypically mixed populations with regard to salt sensitivity. The other phages were not inactivated when slowly diluted from either salt or sucrose exposures. When survival of the various phages at *p*H 2.0 through 12.0 was determined, one of the phages, PX5, was inactivated by mere exposure to phosphate-buffered TGE broth. Therefore, *p*H treatment in this instance was terminated by immediate plating. All the phages were inactivated at *p*H 12.0; at *p*H 11.0, approximately 50% or greater survival occurred. Consequently, survival at acidic *p*H was more useful in distinguishing the phages. PX7 and CB3 were particularly refractive to inactivation at acidic *p*H. In this case, survival was complete at *p*H 2.0, whereas all the other phages tested showed 10% or less survival. Percentages of guanine plus cytosine (G + C) calculated from thermal denaturation profiles represent averages of two to seven separate T_m determinations on each of at least two extractions, with final suspension of the DNA in either standard saline citrate (SSC) or $NaClO_4$. Repeated trials varied by less than 1% from the averages shown in Table 2. These phages showed considerable variation in their composition: from 39.6 to 68.2% G + C. This variation in G + C was significantly greater than that indicated by two previous studies on *Pseudomonas* phage DNA where 54.5% G + C was reported for a *P. aeruginosa* phage (11), and 57.0% G + C for gh-1 phage attacking *P. putida* A.3.12 (14).

Thermal sensitivity was studied to point up possible structural differences occurring among these phages. For this procedure, we used 60 C because this temperature permitted grouping the phages into several categories. The results of these determinations are shown in Table 3. Phages PX1, PX10, PX12, and PX14 were significantly more thermolabile than the others. These phages showed approximately 50% inactivation at tem-

TABLE 3. *Percentage survival of Pseudomonas phages after heating at 60 C*

Phage	Exposure time[a]		
	10 min	30 min	60 min
Psychrophilic			
PX1	0	0	—
PX4	28	1	—
PX10	0	0	—
PX12	0	0	—
PX14	0	0	—
Mesophilic			
PX5	73	40	—
PX2	—	99	95
PX3	—	93	19
PX7	—	84	71
CB3	—	100	100

[a] TGE broth (1 ml) containing 10^8 phage was added to 99 ml of prewarmed broth and gently agitated during heating. Samples (1 ml) were taken and were added to 99 ml of broth held at 3 C to terminate heat treatment.

peratures of 50 to 55 C after 10-min exposure, although they were completely inactivated at 60 C as shown in Table 3. Phages PX2, PX3, PX7, and CB3 were more heat-resistant. After 30 min at 60 C, 80% or more of these phages survived. The heat resistance of PX4 and PX5 was intermediate to that observed for the preceding groups. Since PX4 was propagated on the mesophile, *P. aeruginosa* 2, it was considered that its greater heat resistance than that of the other psychrophilic phages may have occurred as a function of its growth on a more heat-resistant mesophilic host. Consequently, PX4 was grown with a psychrophile as the propagating host, and the heat resistance of this suspension was compared with that of phage grown on a mesophile. When phage suspensions were grown at the same temperature on a psychrophile or mesophile, the thermal sensitivity remained the same. Therefore, with PX4 the thermal sensitivity was not modified by growth on psychrophilic or mesophilic hosts. PX5, although more heat-sensitive than other mesophilic phages, was still more resistant than the psychrophilic group of phages. These data, then, indicate that heat sensitivity correlates with psychrophily.

Phage morphologies were determined by electron microscopy of preparations negatively stained with 1% neutral phosphotungstate (Fig. 2). The phages were of three types. First, phage were seen resembling coliphage T3 (6) with polygonal heads (six-sided) in plain view and short noncontractile tails without base plates. In this group were PX1, PX3, PX10, PX12, and PX14. The average head diameter of these phages was 50 mμ. However, they did show some variation in the size and shape of their tail structures. PX1 and PX3 tails were not tapered as were the others in this group, which showed wedge-shaped tail structures. Tail lengths varied from 27 mμ for PX3 and PX10 to 17 mμ for the others in this group. A second group which was isolated, including PX4, PX7, and CB3, resembled coliphage T2. These had contractile tails and tail fibers, although tail pins were not visible on base plates attached to the sheath of contracted specimens. The head diameters, averaging 63 mμ for PX4 and 52 mμ for PX7 and CB3, were smaller than those observed for T2 (6). They also differed from T2 in that cross striations were absent on uncontracted tails and their contracted tails showed longitudinal striations similar to those observed for typhoid phage 2 (6) and others (5). The third group, containing PX2 and PX5, had long noncontractile tails resembling coliphage T5 (6). PX2 appeared to have a bipyrimidal head like coliphage T2 of 50 × 70 mμ, with an average tail length of 110 mμ. PX5 head morphology resembled PX2, but it was larger, with average head dimensions of 60 × 80 mμ and an average tail length of 140 mμ. Also, it differed from PX2 in that a conspicuous base plate was evident at the tail tip.

DISCUSSION

One of the original objectives of this work was the isolation of phage capable of growth on psychrophilic bacteria near 0 C which could also attack mesophilic bacteria. This objective was achieved. We also were interested in determining whether any correlations could be observed between structure or compositional characteristics and low-temperature growth. In this regard, it was apparent that psychrophilic phages were more thermolabile than related mesophilic phages. This relationship also has been observed by others when the thermolability of psychrophilic and mesophilic bacteria or their enzymes were compared (12, 16, 19). In the case of phage, the target for heat inactivation was not associated with the presence or absence of morphological features such as contractile tails, since similar morphological features were found distributed among both psychrophilic and mesophilic phages. Also, the base composition (percentage G + C) clearly was not associated with the relative thermolability of the phage, as some of the more heat resistant mesophilic phage had a lower G + C content and hence a lower melting temperature than was observed for the psychrophilic phage. This observation also was supported by comparing PX4, a psychrophilic phage having 44.4%

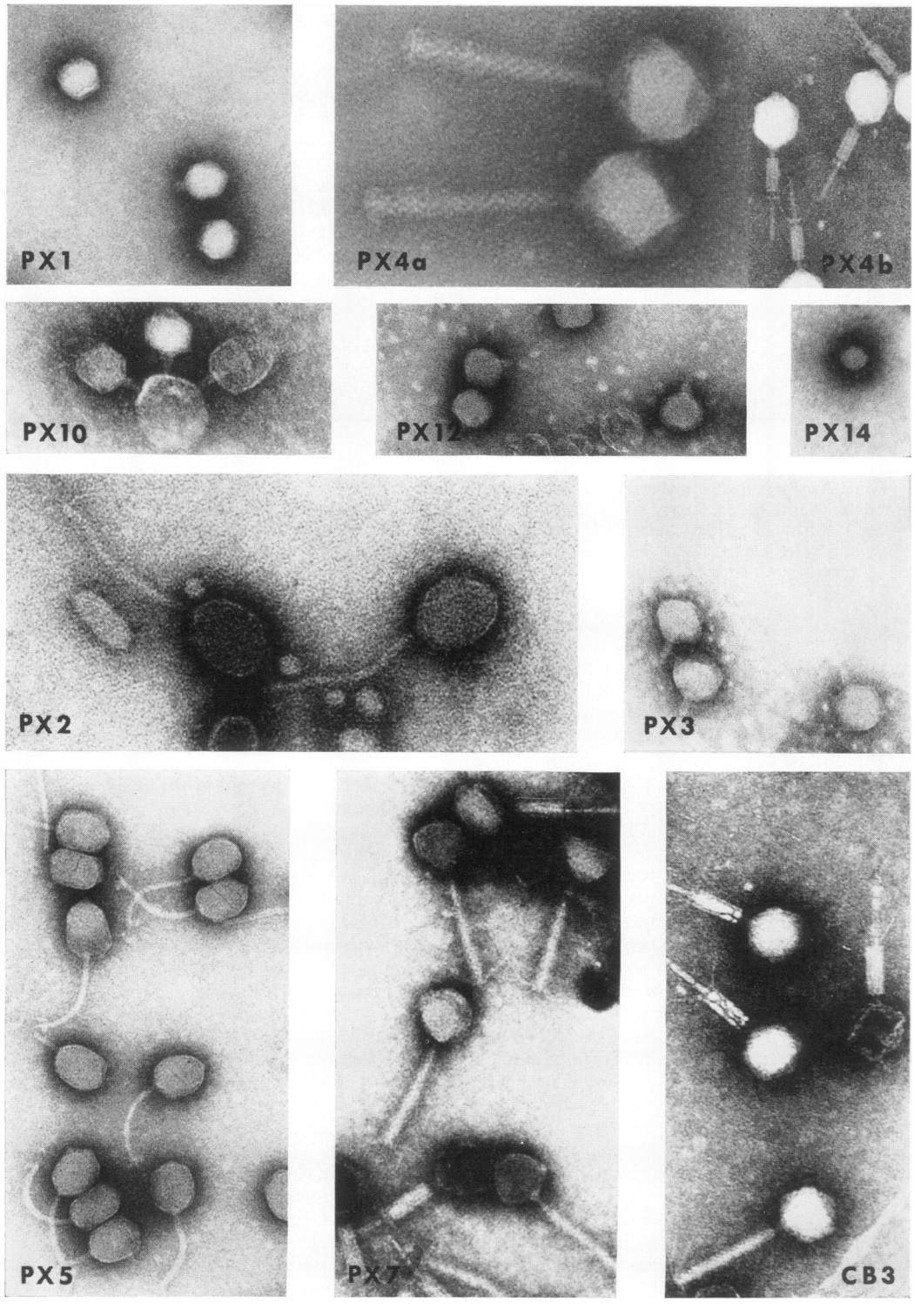

FIG. 2. *Electron micrographs of Pseudomonas phages. PX1, × 115,000; PX4(a), × 255,000; PX4(b), × 118,000; PX10, × 115,000; PX12, × 115,000; PX14, × 88,250; PX2, × 200,000; PX3, × 155,000; PX5, × 115,000; PX7, × 155,000; CB3, × 155,000.*

G + C with the other psychrophilic phages having 52.2 to 55.8% G + C. In this case, PX4, which had a lower G + C content, was more heat resistant. The irrelevancy of DNA percentage G + C composition to growth temperature range also has been supported by others in a study with thermophilic phages (21). Thus, the target for lower heat resistance by the psychrophilic phage may be a lytic enzyme or structural component associated with the entry of the phage genome into the host cell, and, hence, is another manifestation of similar observations made on some psychrophilic bacterial enzymes referred to previously.

Another interesting characteristic of the psychrophilic phages was their inability to grow on susceptible mesophiles at temperatures much above 32 C. Failure of the phage to adsorb to the host cell as a causal factor was eliminated by use of initial adsorption temperatures below 32 C and then shifting to an incubation temperature greater than 32 C (*unpublished data*).

Several mechanisms may be postulated for the maximal growth temperature determinant. In this regard, the heat stability of bacterial ribosomes has been suggested (20). However, in the case of psychrophilic phage-infected mesophilic bacteria, the pre-existing bacterial ribosomes which normally are functional above 32 C would be utilized for phage production. Hence, for these psychrophilic phage, restriction of phage production above 32 C may involve some mechanism other than the limitation of ribosome activity. Precedents from other phage systems suggest that the limitation of psychrophilic phage growth may reflect the inhibition of phage-directed enzyme synthesis, or, alternatively, the production of a thermolabile enzyme(s) associated with psychrophilic phage, as observed for psychrophilic bacteria.

Another aspect of this study pointing up the relationship of phage and host-cell growth-temperature range is the failure of the susceptible mesophilic bacterium *P. aeruginosa* to produce psychrophilic phage at 3.5 C. In this instance, the low temperature inhibition of a host-dependent function may be a contributing factor to the lack of phage production. However, limitation of phage synthesis at temperatures coincident to bacterial host minimal growth temperature may not occur in any precise manner. For example, *P. putida* A.3.12 supported phage production in broth culture at 3.5 C, although growth of this bacterium was severely inhibited at this temperature. Of particular interest in this regard is the fact that the latent period for PX1 on *P. putida* A.3.12 at 3.5 C was not significantly longer than that for PX4 growing on *P. fluorescens* 14, a psychrophilic bacterial strain having a generation time of less than 5 hr at 3.5 C. Another example of limitation of growth-temperature range by these *Pseudomonas* phage is seen with PX2 and PX3. In this instance, phage production was not observed at incubation temperatures much less than 32 C, although their host, *P. aeruginosa*, grew well as low as 13 C and was capable of supporting growth of other phages at 20 C.

A further example of the foregoing was seen in the behavior of PX7 and CB3 on several different strains of *P. aeruginosa* at 20 and 37 C. In this instance, phage growth on *P. aeruginosa* 2 did not occur at less than 32 C, whereas, with *P. aeruginosa* 1C, phage production occurred from 20 to 37 C. This phenomenon of restriction at low temperatures (20 to 32 C) resembles, in some of its obvious aspects, observations made by others for coliphage (7) and is the subject of another report (*in preparation*).

With all of the phages described in this report, the diversity of their biological properties was not altered by the host used for the preparation of phage lysates. Phage grown on psychrophilic or mesophilic hosts showed the same heat sensitivity, serological reaction, citrate sensitivity, etc. Thus, the biological properties of *Pseudomonas* phage, including psychrophily, were inherent in their genetic composition and did not indicate variations in phage properties primarily dependent on the characteristics of the propagating host.

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LITERATURE CITED

1. ADAMS, M. H. 1959. Bacteriophages. Interscience Publishing Co., Inc., New York.
2. ANDERSON, T. F., C. RAPPAPORT, AND N. A. MUSCATINE. 1953. On the structure and osmotic properties of phage particles. Ann. Inst. Pasteur **84**:5–14.
3. BILLING, E. 1963. The value of phage sensitivity tests for the identification of phytopathogenic *Pseudomonas* sp. J. Appl. Bacteriol. **26**:193–210.
4. BRADLEY, D. E. 1963. The structure of some *Staphylococcus* and *Pseudomonas* phages. J. Ultrastruct. Res. **8**:552–565.
5. BRADLEY, D. E. 1966. The structure and infective process of a *Pseudomonas aeruginosa* bacteriophage. J. Gen. Microbiol. **45**:83–96.
6. BRADLEY, D. E., AND D. KAY. 1960. The fine structure of bacteriophages. J. Gen. Microbiol. **23**:553–563.
7. CHRISTENSEN, J. R., AND S. H. SAUL. 1966. A cold-sensitive mutant of bacteriophage T1. Virology **29**:497–498.

8. FEARY, T. W., E. FISHER, JR., AND T. N. FISHER. 1963. A small RNA containing *Pseudomonas aeruginosa* bacteriophage. Biochem. Biophys. Res. Commun. **10**:359–365.
9. FRIEDMAN, M., AND P. B. COWLES. 1953. The bacteriophages of *Bacillus megaterium*. I. Serological, physical, and biological properties. J. Bacteriol. **66**:379–385.
10. GARRETT, C. M. E., AND J. E. CROSSE. 1963. Observations on lysogeny in the plant pathogens *Pseudomonas prunorum* and *Ps. syringae*. J. Appl. Bacteriol. **26**:27–34.
11. GROGAN, J. B., AND E. JOHNSON. 1964. Nucleic acid composition of a *Pseudomonas aeruginosa* bacteriophage. Virology **24**:235–236.
12. INGRAHAM, J. L., AND J. L. STOKES. 1959. Psychrophilic bacteria. Bacteriol. Rev. **23**:97–108.
13. KLINGE, K. 1959. *Pseudomonas fluorescens*, ein boden-und wasserkeim. III. Identifizierung von *P. fluorescens*—stammen durch Bakteriophagen. Arch. Mikrobiol. **34**:270–284.
14. LEE, L. F., AND J. A. BOEZI. 1967. Characterization of bacteriophage gh-1 for *Pseudomonas putida*. J. Bacteriol. **92**:1821–1827.
15. MARMUR, J., AND P. DOTY. 1962. Determination of base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
16. MORITA, R. Y. 1966. Marine psychrophilic bacteria. Ann. Rev. Oceanog. Marine Biol. **4**:105–121.
17. OLSEN, R. H. 1967. Isolation and growth of psychrophilic bacteriophage. J. Appl. Microbiol. **15**:198.
18. PATTERSON, A. C. 1965. Bacteriocinogeny and lysogeny in the genus *Pseudomonas*. J. Gen. Microbiol. **39**:295–303.
19. PUROHIT, K., AND J. L. STOKES. 1967. Heat-labile enzymes in a psychrophilic bacterium. J. Bacteriol. **93**:199–206.
20. SAUNDERS, G. F., AND L. L. CAMPBELL. 1966. Ribonucleic acid and ribosomes of *Bacillus stearothermophilus*. J. Bacteriol. **91**:332–349.
21. SAUNDERS, G. F., AND L. L. CAMPBELL. 1966. Characterization of a thermophilic bacteriophage for *Bacillus stearothermophilus*. J. Bacteriol. **92**:340–348.
22. SPENCER, R. 1955. A marine bacteriophage. Nature **175**:160–161.
23. STOLP, H. 1961. Neue Erkenntnisse über phytopathologene Bakterien und die von ihnen verursachten Krankheiten. I. Verwandtschaftsbeziehungen zwischen phytopathogenen *Pseudomonas*—"Arten" und saprophytischen Fluoreszenten auf der Gundlage von Phagenreaktionen. Phytopathol. Z. **42**:197–262.
24. TAKEYA, K., AND K. AMAKO. 1966. A rod-shaped *Pseudomonas* phage. Virology **28**:163.
25. THOMAS, C. A., JR., AND J. ABELSON. 1966. p. 557. *In* G. L. Cantoni and D. R. Davies [ed.], Procedures in nucleic acid research. Harper and Row Publishers, New York.