JOURNAL OF VIROLOGY, Sept. 1974, p. 689-699
Vol. 14, No. 3
Printed in U.S.A.
Copyright © 1974 American Society for Microbiology

# Characteristics of PRD1, a Plasmid-Dependent Broad Host Range DNA Bacteriophage

RONALD H. OLSEN, JUNE-SANG SIAK, AND ROBERT H. GRAY

*The University of Michigan, Department of Microbiology, The Medical School, and Department of Environmental and Industrial Health, Cellular Chemistry Laboratory, The School of Public Health, Ann Arbor, Michigan 48104*

Received for publication 27 March 1974

Several distinctive properties of PRD1, an icosahedral plasmid-dependent phage, are described. The drug-resistance plasmid-dependent host range of PRD1 extends beyond the P incompatibility group and includes gram-negative bacteria containing plasmids of incompatibility groups N and W. PRD1 phage will infect pseudomonads and *Enterobacteriaceae* containing either a P or W incompatibility group plasmid. PRD1 adsorbs to the cell wall of $R^+$ bacteria and thus its infectivity indicates cell wall alterations by these drug-resistance plasmid groups. PRD1 nucleic acid is duplex DNA with an estimated molecular weight of $24 \times 10^6$. The appearance of PRD1 in electron micrographs is suggestive of lipid content in addition to its buoyant density of 1.348 in CsCl and its sensitivity to chloroform. The latent period of PRD1 varies with the $R^+$ host bacterial strain used for growth of the phage.

We have recently reported the isolation and characteristics of PRR1, an RNA phage specific for the broad host range *Pseudomonas* drug-resistance plasmid R1822 (21, 23). Since that time, we have sent our strains to others working with RP1, a plasmid whose origin is the same clinical isolate which served as the source of our R1822 (15). When the percent guanine plus cytosine (G+C), molecular weight, and contour length of R1822 and RP1 DNA were compared, identical results for RP1 and R1822 were obtained (M. Richmond, personal communication). Accordingly, we are redesignating R1822 as RP1 since it is indistinguishable from RP1 by any of its known biological or physical properties.

Bacterial drug-resistance factors have been classified on the basis of their incompatibility (2, 19), i.e., their inability to coexist stably in the same host. This classification scheme previously has been a convenient criterion, based on biological assay, to determine the relationship of a new plasmid to known groups. The efficacy of groupings based on the incompatibility of related plasmids is reinforced by the specificity of several phages for bacteria possessing plasmids of a given compatibility group (13, 14, 20). The recent description of the RP1 plasmid specific phage, PRR1 (21), increases the number of known plasmid compatibility groups having specific phages to include F, I, N, and P group plasmids. When PRR1 was isolated from Kalamazoo, Mich., sewage, another apparently P group-dependent phage was obtained which we designated PRD1. This phage differs significantly from other plasmid-dependent phages previously described with respect to its receptor sites, morphology, host range, and composition. Some of the salient features of this phage and its purification are presented in this report. (A preliminary report of some of this work was presented at the Annual Meeting of the American Society for Microbiology, 23-28 April 1972, Philadelphia, Pa.)

## MATERIALS AND METHODS

**Bacterial strains and growth medium.** Most bacterial strains and growth media routinely used in this study have been described (21, 23). Additional strains and their drug-resistance plasmids used in the present study are described in the tables. PRD1 host range determinations on bacteria containing drug-resistance plasmids considered representative of various compatibility groups were done by using tryptone-glucose-yeast extract medium (TGE) or TGE containing NaCl (8.5 g/liter) (TGEN). Indicator bacteria for phage enumeration were grown overnight on TGE agar medium containing an antibiotic whose resistance was specified by the particular drug-resistance plasmid the bacteria contained (if any). Cells containing RP1 were grown on medium with carbenicillin at 500 $\mu$g/ml. The concentration of other antibiotics used was at least four times the minimal inhibitory concentration observed for the $R^-$ parent. A turbid suspension was made from overnight growth on solid me-

dium and was inoculated into TGE broth medium. These cultures were incubated with agitation for 2 to 3 h, or until approximately $10^8$ cells/ml was achieved. All cultures were grown at 37 C with the exception of *Pseudomonas putida* PPO13.12 and the psychrophile, *P. fluorescens* PFO15.4, which were grown at 30 C. All media used for phage growth or adsorption contained $CaCl_2$ at 0.015% (wt/vol).

Preliminary studies had shown that *Salmonella typhimurium* LT2(RP1) was the best plating host for PRD1, and this strain was used for all estimates of phage titer unless otherwise indicated in the text.

**Reference phage strains and growth of phage stocks.** Several well-studied phages were used as a point of reference for physical characterization studies on PRD1. They included lambda and P22c received from D. Friedman, University of Michigan, and $\phi$X174 received from C. Kulpa, University of Notre Dame. $R^-$ hosts for growth and assay of these phages were *Escherichia coli* C80 grown on tryptone-yeast extract agar medium with maltose (0.1%) for lambda; *S. typhimurium* LT2 for P22c; and *E. coli* for $\phi$X174. Phage stocks were prepared by harvesting confluently lysed plates and clarification of the homogenized soft agar overlays by several 4 C centrifugations at 8,000 $\times$ *g*. These lysates were stored over chloroform at 3 C in TGE broth medium.

**Electron microscopy.** A bacterial culture growing in TGEN broth medium (approximately $10^8$ cells/ml) was added to a $CaCl_2$-containing broth suspension of phage resulting in a multiplicity of approximately 20 phage per bacterium. Phage were allowed to adsorb 20 min at ambient temperature followed by immersion of the mixture in an ice bath. From this, drops of the suspension were deposited on carbon-coated Formvar grids. Excess fluid was removed from this grid with filter paper and a drop of 1% phosphotungstic acid (pH 6.8) was placed on the grid for 1 min. Several drops of 1% aqueous ammonium acetate were used to wash excess phototungstic acid from the grid. The ammonium acetate was removed with filter paper. The grids were examined in an AEI Corinth 275 electron microscope.

**Preparation of PRD1 high-titer lysates.** *P. aeruginosa* PAO67(RP1) was used as the host bacterium for the growth of PRD1. All Tris-based buffers contained tris(hydroxymethyl)aminomethane. Other procedures used for PRD1 growth, precipitation, and concentration were as reported previously for PRR1 (21), except that the buffer (pH 7.0) used for suspension of the concentrated phage contained Tris (0.01 M, pH 7.0); NaCl (5%); $CaCl_2$ (0.001 M); and tryptone (Difco) (0.5%) (TNCT). The phage suspension from the ammonium sulfate precipitate was stored overnight at 4 C. This was followed by centrifugation at 10,000 $\times$ *g* for 20 min at 4 C in the Sorvall SS34 rotor (low-speed centrifugation). The supernatant was then decanted and centrifuged at 100,000 $\times$ *g* for 2 h at 4 C in the Spinco type 30 rotor (high-speed centrifugation). The pellets from each tube were suspended in 5 ml of TNCT and pooled. Two more cycles of low- and high-speed centrifugation were then used to promote further purification. This phage lysate was further processed by centrifugation through a 5 to 20% sucrose gradient in the Spinco SW27 rotor at 17,000 rpm for 150 min at 4 C. The sucrose gradients were prepared in TNCT buffer. The gradient tube showed a visible band containing phage particles confirmed by plating and which adsorbed strongly at 260 nm, indicating the presence of nucleic acid. The phage band was collected through the bottom of the tube by a Beckman fraction recovery system, followed by dialysis against 2 liters of TNCT buffer at 4 C. The phage was then treated as described previously (22) with RNase and DNase, followed by another high-speed centrifugation and suspension of the phage pellet in TNCT. Table 1 indicates the phage recovery at each step. DNA and RNA contents of the purified phage lysate were determined by the diphenylamine and orcinol methods, respectively. RNA content was negligible and DNA content was $8 \times 10^{-11}$ $\mu$g/PFU. When the composition and molecular weight of PRD1 DNA were subsequently estimated, we calculated that PRD1 lysates purified by this method had 50% noninfective DNA containing particles in the total phage population. All media or buffers used were at pH 7.0 unless otherwise specified.

**Phage DNA extraction.** PRD1 and lambda phage DNA were extracted essentially as described by Sobieski and Olsen (26). Phage lysates were in SSC (0.15 M NaCl; 0.02 M sodium citrate, pH 7.0). The phage lysate was treated 2 h at 37 C with Pronase (10 mg/ml) before extraction with SSC-saturated phenol. PRD1 DNA was extracted by using six serial phenol extractions; three were used for lambda. After exhaustive dialysis against SSC at 4 C, PRD1 DNA usually had a 260/280 nm abosrption ratio of 1.4 to 1.5, and lambda DNA had an absorption ratio of 1.8 to 1.9.

**RP1 DNA.** Tritiated RP1 DNA was prepared from *E. coli* JC1553(RP1) by the method of Guerry et al. (8). DNA was labeled with [$^3$H]thymine having a specific activity of 15 Ci/mmol (Schwarz/Mann). The supercoiled plasmid DNA prepared by the above procedure was nicked during dialysis against SSC overnight at 4 C. The majority of the plasmid DNA became open circular molecules with a sedimentation value of 43*S*, as previously reported for RP1 by Grinsted et al. (7).

**PRD1 DNA.** PRD1 DNA was readioactively labeled using *P. aeruginosa* PAO67(RP1) as the host bacterium. *P. aeruginosa* PAO67(RP1) was grown

TABLE 1. *Purification of PRD1 phage*

| Purification step | Vol (ml) | PFU/ml | Total PFU | % recovery |
|---|---|---|---|---|
| Crude lysate | 7,200 | $2.7 \times 10^{10}$ | $1.9 \times 10^{14}$ | 100 |
| Ammonium sulfate precipitate | 70 | $1.6 \times 10^{12}$ | $1.1 \times 10^{14}$ | 57 |
| High-speed centrifugation | 15 | $7.4 \times 10^{12}$ | $1.1 \times 10^{14}$ | 57 |
| 5-20% sucrose gradient and dialysis | 20 | $1.3 \times 10^{12}$ | $2.6 \times 10^{13}$ | 13 |
| DNase and RNase treatment and high-speed centrifugation | 4 | $6 \times 10^{12}$ | $2.4 \times 10^{13}$ | 12.3 |

overnight at 37 C on TGE agar medium containing 500 $\mu$g of carbenicillin per ml. This culture was then inoculated into TGE broth medium containing 20 $\mu$g of uracil (non-radioactive) per ml and grown at 37 C to mid-logarithmic phase ($A_{425}$ = 0.5). The culture was centrifuged at 13,000 $\times$ $g$ at 25 C for 10 min. The pellets were suspended in TGE broth medium to $A_{425}$ = 1.0 and warmed to 37 C. A 2.5-ml suspension of PRD1 phage ($10^7$ PFU/ml) at 37 C was added to 2.5 ml of cell suspension, to which $CaCl_2$ was then added (final concentration of 0.015%). [2-$^{14}$C]uracil, having a specific activity of 60 mCi/mmol (Schwarz/Mann), was added 20 min after infection, and the culture was incubated statically at 37 C for 6 h. After this preliminary infection, an additional 5 ml of *P. aeruginosa* PAO67(RP1) ($A_{425}$ = 1.0) growing exponentially in TGE broth medium containing $CaCl_2$, and more [2-$^{14}$C]uracil were added to the culture. Uracil labeling was used since pseudomonads do not take up thymine. Phage growth was continued for an additional 16 h. Cell debris was removed by centrifugation at 13,000 $\times$ $g$ for 10 min at 25 C. The $^{14}$C-labeled phage was copurified with 10 ml of previously purified unlabeled PRD1 ($6 \times 10^{12}$ PFU/ml) using three successive cycles of low- and high-speed centrifugations. The labeled high-titer lysate was treated with RNase and DNase before DNA extraction.

**Lambda DNA.** A high-titer lysate of lambda phage labeled with [$^3$H]thymidine was prepared using the method described by De Czekala et al. (5). It was labeled with [$^3$H]thymine having a specific activity of 15 Ci/mmol (Schwarz/Mann). The lambda lysate was purified by the method described above for PRD1.

## RESULTS

**Host range of PRD1.** Not all RP1-containing strains are able to propagate PRD1. This result resembles our previous report for RP1 plasmid-dependent RNA phage, PRR1 (21). The plaque-forming ability of PRD1 on RP1-containing strains shown previously to act as conjugal donors (23) is shown in Table 2. These strains have been grouped according to their ability to show plaques when phage was plated either by the overlay technique or the spotting of phage dilutions on surface lawns of indicator bacteria. None of these strains plated PRD1 in the absence of the RP1 plasmid. Of the strains in group A, the plaque morphology was variable, ranging from 3- to 4-mm (diameter) clear plaques on *S. typhimurium* LT2(RP1) to 1-mm turbid plaques with poorly defined peripheries on *Acinetobacter calcoaceticus* ACJ1(RP1). The strains in group B, after repeated testing, never showed evidence of plaque formation even when spotting surface lawns. We have found the latter method to be a more sensitive test than either plating for PFU or the commonly used cross-streak test on the surface of solid medium. However, when any of the strains included in

TABLE 2. *Host range of PRD1 on RP1-containing bacteria*

| $R^+$ indicator | $R^+$ strain designation[a] | Lytic reaction[b] |
|---|---|---|
| Strains forming plaques | | |
| *Pseudomonas aeruginosa* | PA067(RP1) $FP^-$ | Plaque morphology variable depending upon the $R^+$ clonal isolate tested |
| | PA067(RP1) $FP^+$ | |
| | PAT904(RP1) FP* | |
| | PAT2(RP1) $FP^+$ | |
| | PT013(RP1) $FP^+$ | |
| | PAL1822 | |
| *P. fluorescens* | PF015.4SmR(RP1) | |
| *P. putida* | PP013.12(RP1) | |
| *E. coli* | K12 W1177(RP1) | |
| | Hfr H(RP1) | |
| | Hfr C(RP1) | |
| | B WR3951(RP1) | |
| | C(RP1) | |
| *Salmonella typhimurium* | LT2(RP1) | |
| *Proteus mirabilis* | PM17(RP1) | |
| *Vibrio cholera* | VCF2(RP1) | |
| *Acinetobacter calcoaceticus* | ACJ1(RP1) | |
| Strains not forming plaques | | |
| *Azotobacter vinelandii* | AVM(RP1) | No plaque-forming ability or growth inhibition by spotting surface lawns |
| *Neisseria perflava* | NPM(RP1) | |
| *Shigella boydii* | SBM(RP1) | |

[a] For source and other characteristics see previous reports (21, 23).

[b] Determinations were done at 37 C with the exception of RP1 containing PF015.4SmR and PP013.12, which were performed at 30 C.

group B were used as RP1 donors for mating with R$^-$ recipient strains shown in group A, the resulting R$^+$ exconjugants plated PRD1. Consequently, it would seem that the group B strains lack the ability to express PRD1 susceptibility, although the genetic potential of the plasmid is maintained and subsequently expressed in appropriate group A hosts. However, even with R$^+$ strains in group A, purified exconjugants from mating experiments varied somewhat with regard to PRD1 plating. For example, when *Vibrio cholera* VCF2(RP1) clones derived from a mating experiment were tested, some of the isolates showed no evidence of PRD1 susceptibility, although all the resistance determinants as well as plasmid donor ability were expressed. As was seen for group B isolates, when these PRD1-insensitive isolates were subsequently mated with other strains, PRD1 susceptibility again was expressed in these exconjugants. Thus, PRD1 susceptibility may relate to the particular exconjugant tested among the group A isolates. However, *S. typhimurium* LT2(RP1) and most R$^+$ *Pseudomonas* strains tested uniformly demonstrated phage susceptibility when exconjugant clones of these strains were purified and tested. When clones identified as PRD1 susceptible were serially restreaked and isolates tested, PRD1 susceptibility was maintained in all progeny, indicating the stability of the plasmid as well as the uniform expression of PRD1 susceptibility.

**Salt effect and PRD1 plating on other drug-resistance plasmid compatibility groups.** Several bacterial strains containing plasmids unrelated to RP1 were tested during the course of our initial studies on PRD1 host range. The susceptibility of some strains was subsequently confirmed. This was followed by testing more representatives of previously described plasmid compatibility groups for PRD1 susceptibility on salt- and non-salt-containing medium. The results of our testing these R$^+$ bacteria on non-salt medium (TGE) were uniformly negative both by the criteria of plating for PFUs in soft-agar overlays and spotting on bacterial lawns. However, different results obtained with a salt-containing medium (TGEN) and the results of this survey conducted with salt-containing medium are shown in Table 3. Of the compatibility groups tested, representatives of groups N and W indicated susceptibility. However, one plasmid-containing strain of group W, J53(R7K), showed no PRD1 susceptibility. This result has been confirmed by S. Falkow (personal communication). Therefore, within a recognized compatibility group, the expression or presence of plasmid dependent genetic information for the PRD1 receptor may

TABLE 3. *Drug-resistance plasmid host range of PRD1*

| *E. coli* R$^+$ strain[a] | Compatibility group[b] | PRD1 susceptibility[c] |
|---|---|---|
| J53(R192-7) | Fi$^+$ | – |
| J53(R538.1) | | – |
| J53(R64-11) | I | – |
| J53(R163-7) | | – |
| J53(R15) | N | + |
| J53(RN3) | | + |
| J53(R46) | | + |
| J53($R_{s-a}$) | W | + |
| J53(R388) | | + |
| J53(R7K) | | – |
| J53(RA1) | A | – |
| J53(RA1-16) | | – |
| J53(R391) | J | – |
| J53(R402) | T | – |
| J53(R6K) | X | – |
| J53(R576.1) | C | – |
| J53(R64) | C or 6 | – |
| J5(R135) | 7 | – |
| J5(R111i) | 8 | – |
| J5(R71a) | 9 | – |

[a] Strains received from N. Datta, Royal Postgraduate Medical School, London, England, and G. Jacoby, Harvard University Medical School, Boston, Mass.
[b] For description of compatibility groups see previous reports of Datta and Hedges (2) and others (19).
[c] Performed by spotting surface inoculated lawns of bacteria on TGEN agar medium with a loop of phage suspension. Susceptibility indicated by the formation of cloudy plaques at appropriate phage concentrations.

vary. However, these results, when considered with those in Table 2, show that the host range of PRD1 extends beyond a single compatibility group and that those plasmids that confer PRD1 sensitivity contain functionally equivalent or identical genetic information for the specification of the PRD1 receptors, although differing in other respects.

**Morphology of PRD1.** In view of the specificity of PRR1 for pili (21), we next determined if pili were the adsorption site for PRD1. The electron micrographs in Fig. 1 clearly indicate PRD1 adsorbed to the cell wall. Our survey of PRD1 phage RP1-containing bacteria never showed PRD1 adsorbed to RP1 pili clearly in view. Thus, among plasmid-dependent phages, PRD1 is unique since it apparently does not require pili for adsorption. PRD1 was never seen adsorbed to R$^-$ bacteria, and this confirms broth culture adsorption studies. During the examination of these and other electron micro-

graphs of RP1, RN3, and R388 plasmid-containing strains, PRD1 phage was always seen adsorbed to the cell surface. In many instances, pili associated with the plasmid were also evident but they were free of adsorbed phage. Thus, we conclude on the basis of our electron microscopic observations that PRD1 is cell-wall specific. The morphological features of PRD1 resemble in several respects several other phages associated with *Pseudomonas* bacteria. For example, PM2, a lipid-containing phage specific for a marine pseudomonad, has similar icosahedral morphology and a well-defined membrane surrounding a central particle (6). Brushlike projections occurring at the vertices of the phage coat are also seen with PRD1 (Fig. 1A) as with the PM2 phage envelope, although less well-defined here. The average diameter of PRD1 is 62 nm and thus is similar to the 60-nm diameter reported for PM2 (6). PRD1 also resembles $\phi$6, a *P. phaseolicola*-dependent lipid-containing phage which has been recently described (28). The head diameter reported for $\phi$6 is 60 to 70 nm and it also possesses a membranous-like head structure. However, unlike $\phi$6, no amorphous saclike head structures or adsorption to pili has been observed for PRD1.

**Growth of PRD1.** One-step growth kinetics of PRD1 are shown in Fig. 2. Phage growth on the four hosts shown here has been corrected for unadsorbed phage. It is apparent from the differing profiles that the identity of the host influences the growth behavior of PRD1. For example, the phage latent period was observed to vary from 35 min for *S. typhimurium* LT2(RP1) to 60 min for *P. aeruginosa* PAO67(RP1), and the order of these differences was reproducible on repeated trials. However, as indicated here, the burst size of approximately 100 is similar for the four hosts tested.

When growth (measured as plaque-forming ability) is tested on a psychrophilic *P. fluorescens* strain, PFO15.4(RP1), no plaques are produced at 3.5 C, although plaques are clearly visible at 30 C. Thus, based on its growth at 37 C, and not at 3.5 C, PRD1 is a mesophilic phage capable of infecting a psychrophile. Although we have previously reported psychrophilic phages infecting mesophilic bacteria (22), this is the first instance of a mesophilic phage infecting a psychrophile. In this case, as with psychrophilic phages, the phage growth temperature range is phage dependent.

**Phage adsorption to $R^+$ bacteria.** Data in Tables 2 and 3 indicate variations in the occurrence of PRD1 phage susceptibility which may reflect the occurrence of phage receptors and/or the expression of phage determinants during the infective process. Accordingly, to gain information concerning the variance cited above, we determined the relationship between cell lethality and phage adsorption. This was done by using several RP1-containing bacterial strains (Table 4). We also compared differences in the adsorption process as shown by the profiles in Fig. 3.

The data in Table 4 show that *S. typhimurium* LT2(RP1) adsorb more phage resulting in greater bacterial lethality from the infective process than observed for the $R^+$ *Pseudomonas* strains. This conclusion is also supported by efficiency of plating studies to be discussed later. The data in Table 4, however, do not unequivocally distinguish whether the *Pseudomonas* strains have fewer receptor sites per cell or malfunctioning phage receptors. However, the adsorption profile in Fig. 3 provides some information concerning this consideration. It is unusual that adsorption sites on the $R^+$ pseudomonads should be saturated in view of the low (1.0) multiplicity of phage input used here. This result may indicate poor expression of PRD1 receptors in these strains. Thus, the data here point to quantitative considerations concerning the incidence of PRD1 receptors as, alternatively, poorly functioning receptors would show less steep adsorption profiles. Therefore, the primary difference among the results for bacteria examined here seems to reflect the density of functional phage receptors. Phage receptors may be constantly and uniformly present but not fully or adequately exposed. This could reflect the partial presence of capsule or slime layers associated with these bacteria. Occlusion of phage receptors by mucoid surface layers has been demonstrated for *E. coli* phages (24).

**Restriction and modification of PRD1.** The effect of growth host on the subsequent plating efficiency of PRD1 is shown in Table 5. As will be shown later, PRD1 contains double-stranded DNA and hence we considered the possibility that the variance in PRD1 susceptibility among RP1-containing bacteria (Tables 2 and 4) may partially reflect the expression of modification and restriction mechanisms known to function in *E. coli* (18) and *Pseudomonas* (12) species. The data in Table 5 clearly indicate that some bacterial strains are better plating hosts than others, and this occurs independently of the host used for growth of the phage. For example, *E. coli* B is a poorer plating host than *E. coli* K-12 for all phage lysates including *E. coli* B-grown phage. These data indicate the possibility that PRD1 lacks the appropriate nucleotide sequence(s) allowing restriction of its DNA. This is consistent with a similar lack of modifi-

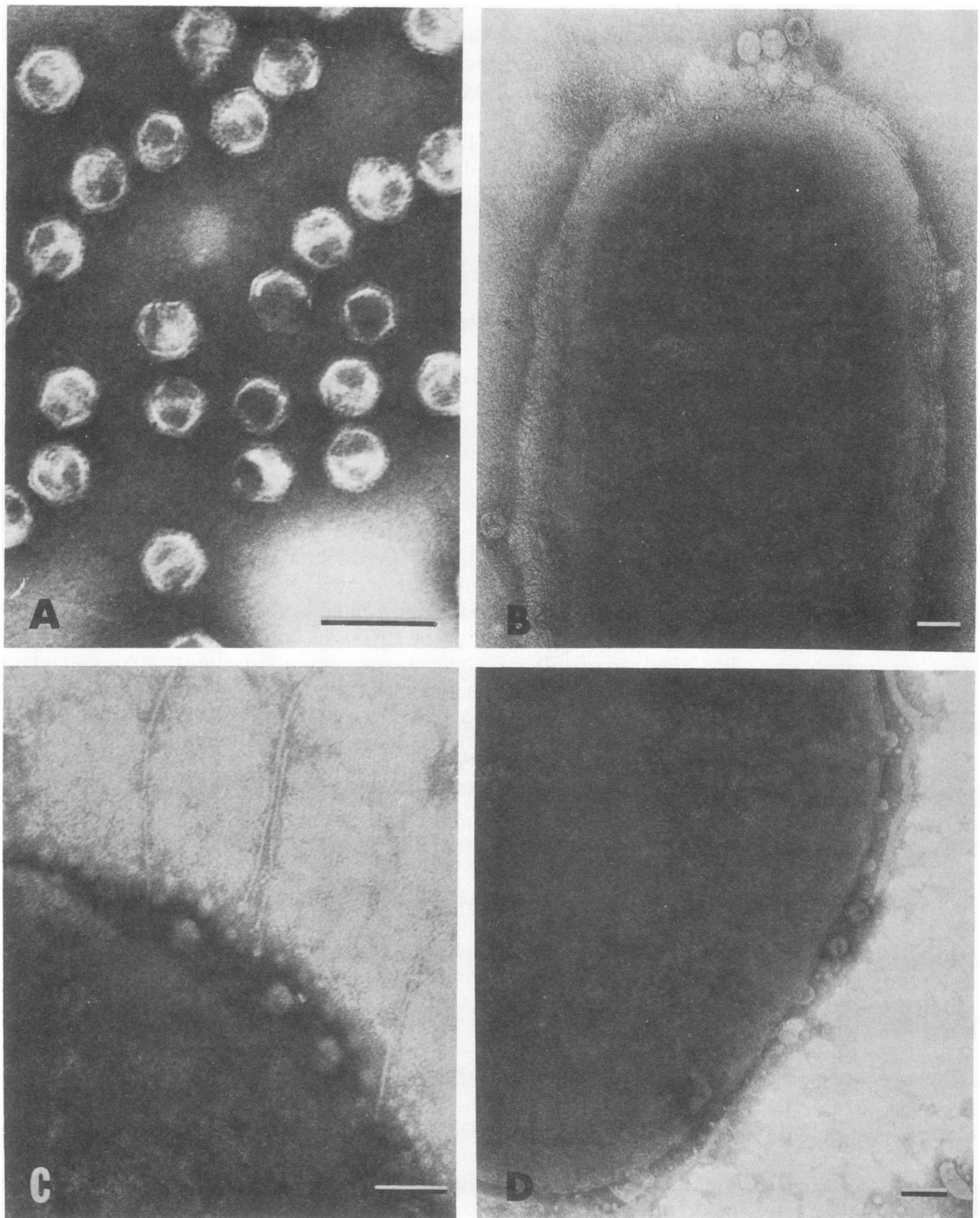


FIG. 1. *Morphology and adsorption of PRD1 to $R^+$ bacteria. (A) PRD1 morphology, ×160,000; (B) PRD1 adsorbed to the cell wall of S. typhimurium LT2(RP1), ×80,000; (C) PRD1 adsorbed to the cell wall of P. aeruginosa PAO67(RP1), ×100,000; (D) PRD1 adsorbed to the cell wall of E. coli J53(R388), ×80,000. The bars are 100 nm.*

cation and/or restriction observed for RP1 DNA (unpublished data) when transferred between hosts of known DNA modification and restriction specificity.

**Sedimentation constant of PRD1.** A mixture of lambda, P22, $\phi$X174, and PRD1 phages was cosedimented on a 5 to 20% sucrose gradient to characterize PRD1 in relation to some other well-studied phages (Fig. 4). By using as a reference the sedimentation coefficients reported for lambda (30) and $\phi$X174 (10), a sedimentation coefficient for PRD1 of 357 was

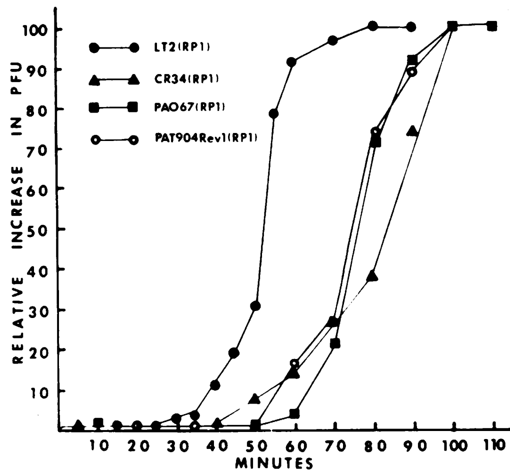


FIG. 2. *One-step growth of PRD1 on Pseudomonas, Salmonella, and Escherichia hosts. Approximately $10^8$ cells/ml logarithmically growing in TGE broth medium at 37 C were infected at a multiplicity of 0.01 phage/cell and incubated under static conditions for 20 min. Following phage adsorption, the culture was diluted into prewarmed broth to approximately 100 infective centers/ml and incubation continued with aeration at 37 C. All samplings were plated on S. typhimurium LT2(RP1) indicator bacteria with appropriate dilution of samples at the times indicated. Unadsorbed phage at 20 min was determined by filtration and subtracted from subsequent phage titers.*

calculated. The sucrose gradient was linear, as evidenced by comparing the positions in the gradient of the other phages, and the sedimentation behavior for PRD1 was consistent with its morphology and size.

**Buoyant density of PRD1.** Our initial attempts to determine the buoyant density of PRD1 yielded unusual results. Using a CsCl solution having a mean density of 1.6 g/cm$^3$, which is usually used for the purification of DNA phages, resulted in greater than 90% inactivation of PRD1, and the surviving PFUs were recovered from the top (light) portion of the gradient. We were able to prevent the CsCl inactivation of PRD1 by including 0.5% tryptone (Difco) in the CsCl solution. However, the unexpected buoyancy of PRD1 and the electron micrographs of phage resembling those for the lipid-containing PM2 phage prompted us to consider that PRD1 may contain lipid. We therefore ran CsCl equilibrium density gradients starting with a mean density of approximately 1.38, and included in the same tube for calibration checks an RNA phage, f2, having a buoyant density of 1.43 (11). The results of this determination are shown in Fig. 5. The pitch of the gradient was a density change of 0.004 g/cm$^3$ per fraction as determined by weighing samples intermediate to the two phage peaks. The buoyant density calculated for PRD1 run under these conditions was 1.348. This density and the membranous morphology of PRD1 are reminiscent of similar observations on phages PM2 (6) and $\phi 6$, which are known to contain lipid.

**Nucleic acid composition of PRD1.** Nucleic acid extracted from PRD1 lysates as described previously (26) was subjected to chromatogra-

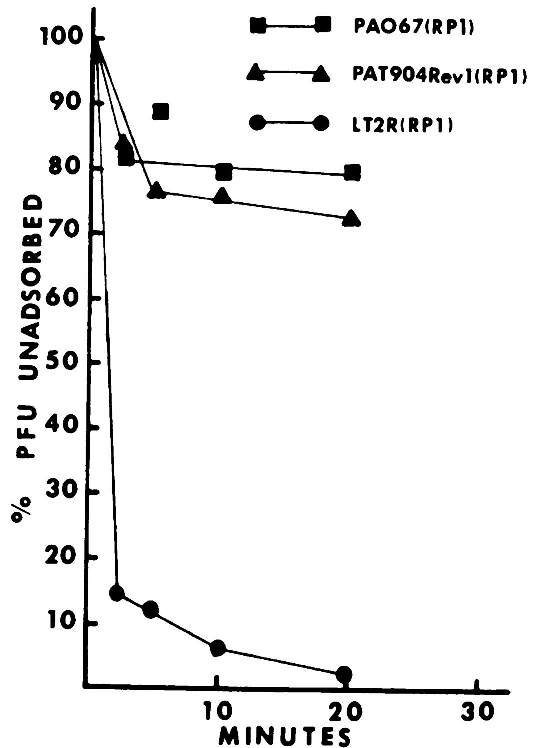


FIG. 3. *Adsorption kinetics of PRD1. Unadsorbed phage was determined as described in Table 4 except that a multiplicity of one phage per bacterium was used.*

TABLE 4. *PRD1 phage adsorption and cell kill*[a]

| Host cells | % PRD1 adsorbed | % cells killed |
|---|---|---|
| *S. typhimurium* LT2(RP1) | 60 | 95 |
| *P. aeruginosa* PAT904(RP1) | 40 | 47 |
| *P. aeruginosa* PA067(RP1) | 17 | 56 |

[a] For this, a culture growing logarithmically at 37 C and containing $10^8$ bacteria/ml was mixed with 5 × $10^8$ PFU/ml phage suspension in TGE broth and mixed. $CaCl_2$ was then added to 0.015%, and phage was adsorbed at 37 C under static conditions for 20 min followed by dilution in prewarmed TGE broth and plating for surviving bacteria or unadsorbed phage on *S. typhimurium* LT2(RP1).

TABLE 5. *Effect of growth host on efficiency of plating (EOP)*

| Phage growth host | EOP[a] on RP1-containing indicator strains[b] | | | | |
|---|---|---|---|---|---|
| | LT2 | CR34 | B | PAT904 | PA067 |
| *S. typhimurium* LT2(RP1) | 1 | 0.3 | 0.03 | 0.4 | 0.8 |
| *E. coli* K-12 CR34(RP1) | 1 | 0.6 | 0.08 | 0.6 | 1.2 |
| *E. coli* B(RP1) | 1 | 0.1 | 0.02 | 0.6 | 0.9 |
| *P. aeruginosa* PAT904(RP1) | 1 | 0.2 | 0.03 | 0.4 | 0.8 |
| *P. aeruginosa* PA067(RP1) | 1 | 0.3 | 0.07 | 0.6 | 0.8 |

[a] For the various phage suspensions, the EOP on LT2(RP1) was taken as unity regardless of the growth host.

[b] See text for genus-species designations corresponding to abbreviated nomenclature used here.

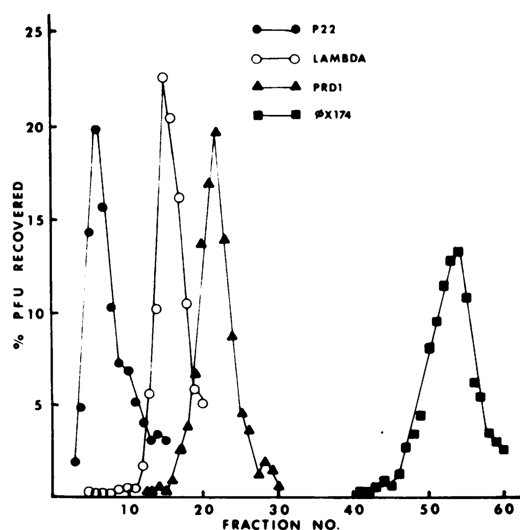


FIG. 4. *Sedimentation of PRD1 in a 5 to 20% sucrose gradient. Gradients were produced using a Beckman gradient-forming device and contained, in addition to sucrose, 0.01 M Tris (pH 7.0) and 0.5% tryptone (Difco). A mixture of 0.2 ml of PRD1, lambda, P22, and φX174 phages was added at the top and the gradient was centrifuged at 22,000 rpm at 25 C for 45 min in the Spinco SW50.1 rotor. Samples were collected dropwise from the bottom, diluted, and plated on appropriate indicator bacteria.*

phy using the procedures of Bendich (1). The $R_f$ values for PRD1 nucleic acid bases corresponded to those expected for adenine, guanine, cytosine, and thymine. PRD1 DNA was 49.2% G+C calculated from several experiments. For this work we used DNA from a psychrophilic *Pseudomonas* phage, PX4, as a reference standard. PX4 has been shown previously to contain DNA with 44.4% G+C (22). When PRD1 DNA was subjected to thermal denaturation (Fig. 6), the percent G+C, calculated from inspection of the $T_m$ profile, was 50.3. Thus the percent G+C determined by chromatography and $T_m$ determinations are in close agreement. The $T_m$ profile is also typical of that observed for double-stranded DNA since these DNA preparations were resistant to alkali hydrolysis and sensitive to thermal denaturation.

**Sedimentation of PRD1 DNA.** The sedimentation properties of PRD1 DNA are shown in Fig. 7A. The molecular weight of PRD1 DNA is estimated to be $24 \times 10^6$ as determined from the position of PRD1 DNA (Fig. 7A) relative to that for PR1 DNA ($39 \times 10^6$ daltons, M. Richmond, personal communication), or linear lambda DNA ($34 \times 10^6$ daltons [27]). Further analysis of PRD1 DNA in alkaline sucrose gradients (Fig. 7B) indicates that PRD1 DNA is linear based on the expected change in $S$ value (25). This estimate of the size of PRD1 DNA is also compatible with the dimensions of the intact phage particle when this relationship is compared with that for the *Salmonella* phage P22.

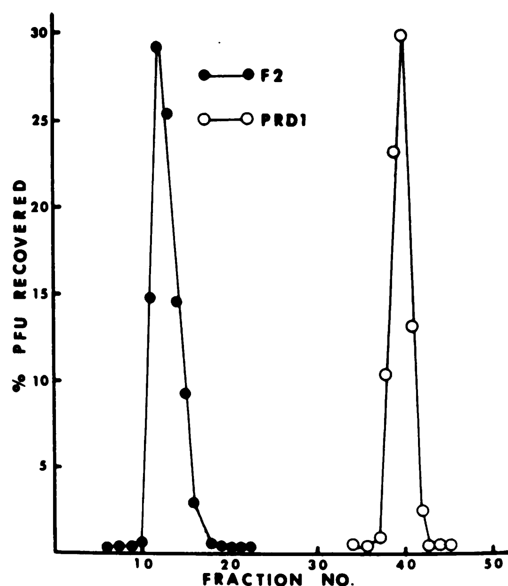


FIG. 5. *Cosedimentation to equilibrium of PRD1 and f2 phages in CsCl. CsCl was added to a mixture of PRD1 and f2 in 0.05 M Tris (pH 7.0)- 0.5% tryptone (Difco), to result in a mean density of 1.38 g/cm³. A portion of this mixture was centrifuged at 35,000 rpm for 48 h at 4 C in the Spinco SW50.1 rotor. Fractions were collected dropwise from the bottom of the tube, diluted, and plated on appropriate hosts.*

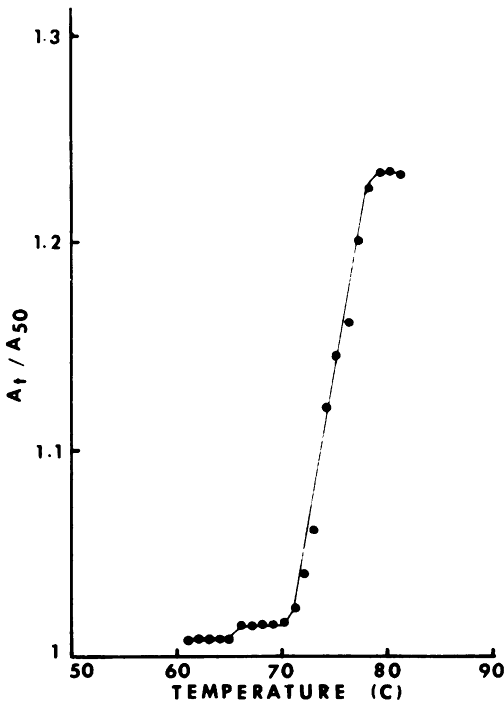


FIG. 6. *Melting profile of PRD1 DNA. Phage DNA was prepared from a purified lysate as reported previously for Pseudomonas phage CB3 (26). The $T_m$ determination was done as reported by Mandel and Marmur (16) in 0.1 SSC. Calculation of percent G+C was according to Marmur and Doty (17).*

**Solvent sensitivity of PRD1.** Our initial difficulties with the preparation of high-titer lysates and subsequent observations on phage morphology indicated PRD1 may contain lipid. Accordingly, we tested the sensitivity of PRD1 to agents known to inactivate lipid-containing phage (6, 29) and these results are shown in Table 6. *Salmonella* phage P22 was generally resistant to agents known to disrupt lipid-containing phage. PRD1 was sensitive to chloroform when exposed in broth, and this chloroform sensitivity was significantly enhanced when PRD1 was exposed to Tris-chloroform. Ethyl ether had no effect on PRD1. This result differs from observations on the lipid-containing phage $\phi$6 (29), but duplicates observations on the lipid-containing coliphage $S_d$ summarized by Tikhonenko (28). Phage $S_d$ also is disrupted by chloroform treatment but not by ether. Accordingly, our observations lead us to the tentative view that PRD1 phage may contain lipid. However, an alternative view is possible. PRD1 may have a structural protein required for infectivity that is uniquely chloroform sensitive. We consider this possibility less likely since, on the basis of morphology, buoyant density, and chloroform sensitivity, PRD1 clearly resembles three other phages known to contain lipid. Furthermore, one of these phages, $S_d$, is inactivated by chloroform but not other solvents as we also observe for PRD1.

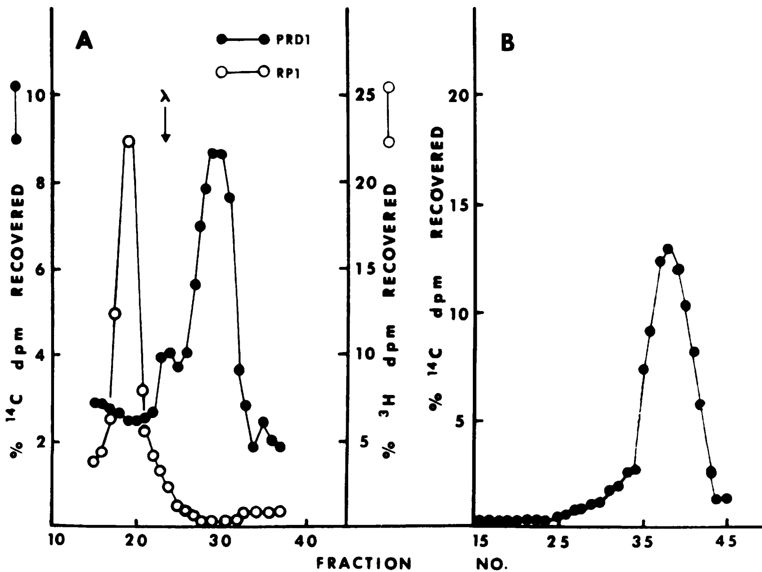


FIG. 7. *Sucrose gradient centrifugation of lambda, PRD1, and RP1 plasmid DNA. Experimental techniques are as previously described (21, 26). Samples were centrifuged at 39,000 rpm for 100 min at 20 C in the Spinco SW50.1 rotor. Sedimentation is from right to left. (A) Neutral sucrose gradient of lambda, PRD1 and RP1 DNA. (B) Alkaline gradient of PRD1 DNA.*

TABLE 6. *Inactivation of PRD1 by solvents and sodium deoxycholate*

| Treatment[a] | % Survival[b]/Phage | |
|---|---|---|
| | P22 | PRD1 |
| T-chloroform | 93 | 43 |
| T-ethyl ether | 101 | 109 |
| T-toluene | 98 | 79 |
| T-sodium deoxycholate | 92 | 78 |
| Tris | 109 | 80 |
| Tris-chloroform | 88 | 0.4 |
| Tris-ethyl ether | 77 | 92 |
| Tris-toluene | 76 | 68 |
| Tris-sodium deoxycholate | 92 | 64 |

[a] 0.5 ml of each phage stock suspension was added to a flask containing TGE broth (T) or Tris, 0.05 M (pH 7.0). To a flask containing both phages the indicated additions were made as follows: chloroform (10 ml); ethyl ether (10 ml); toluene (10 ml); and sodium deoxycholate to 0.5%. The resulting 50-ml mixtures were swirled in a water bath shaker at 80 rpm at 7 C for 24 h.

[b] Calculated as the percent surviving phage in relation to a T broth control phage suspension. Phase separation for solvent-containing mixtures occurred prior to removal of samples for dilution and titering using PA067(RP1) in this instance for PRD1 titer and LT2 for P22 titer.

## DISCUSSION

PRD1 is clearly unlike other phages described to date when its plasmid-dependent cell-wall adsorption, possible lipid content, DNA content, and broad host range are considered together. The ubiquity of RP1 and related plasmid-dependent phages is also apparent in view of the facility with which these phages have been isolated from sewage samples obtained from geographically distant areas (unpublished data). Previously described plasmid-dependent phages have had their host range confined to a particular plasmid group. For example, bacteria containing the *E. coli* sex factor, F, or related plasmids plate the male-specific *E. coli* phages (20); the I compatibility group phage, If1, attacks bacteria containing I-like plasmids (14); the N compatibility group specific phage, Ike, attacks bacteria containing N group plasmids (13). In addition, we have described an RNA phage, PRR1, whose host range is confined to the P-group plasmids (21, 23). These phages, in most instances, have been either pilus-specific RNA phages or filamentous single-stranded DNA phages. The icosahedral morphology of PRD1 represents an example of a new type of plasmid-dependent phage. It differs significantly from the small RNA phages or single-stranded DNA containing filamentous phages both requiring the expression of plasmid genes for their adsorption.

Of particular interest to us is the observation that plasmids of three distinctly different compatibility groups have in common the genetic information for PRD1 receptors, although the P group (RP1 and RP4), W group ($R_{s\text{-}a}$ and R388), and N group (R15, R46, and N3) plasmids do not share many other properties. For example, the host range of the P group is broad (4, 7, 23), whereas the N group plasmids are confined to some genera of the *Enterobacteriaceae* (3). The plasmids we used here of the P, W, and N groups also vary widely with regard to their molecular weight and DNA composition.

Interestingly, a further degree of complexity with regard to criteria for relatedness of plasmids is introduced when taking into consideration the exception that a W group plasmid, R7K, will not allow plating of the phage. From this observation we draw the inference that the specification of the PRD1 receptor has not necessarily been uniformly conserved (with R7K) throughout the evolution of the P, W, and N compatibility group plasmids. It is possible, however, that plasmids specifying the PRD1 receptor may have evolved from a common ancestral plasmid or, alternatively, that the plasmids allowing plating of PRD1 evolved independently through a common host from which was obtained the genetic information requisite for PRD1 adsorption. In addition, new phage type-compatibility-type plasmids may have been generated by recombination between unrelated plasmids in a given host (9).

The observations of others (P. Guerry, R. W. Hedges, N. Datta, and S. Falkow, manuscript in preparation) also suggest homology between plasmids of differing compatibility groups considered here. For example, $R_{s\text{-}a}$ and N3, group W and N, respectively, show 8 to 10% homology, RP1 and $R_{s\text{-}a}$, group P and W, respectively, show 5% homology, and RP1 and N3, group P and N, respectively, show 2 to 4% homology. However, it should be pointed out that the homologous regions of these plasmids need not necessarily represent receptor site information. An alternate hypothesis is possible, namely, that the receptor site configuration requirement of PRD1 is not as stringent as presumed for other cell wall adsorbing phages.

In summary, it is clear that PRD1 represents a new and unique class of phages whose growth is plasmid-dependent. In this case, PRD1 susceptibility is contingent on a plasmid modification of the cell surface. Additionally, the critical host contributions to phage growth and host bacterium adaptability to a plasmid-specified

cell wall modification have been genetically conserved. This has occurred throughout the evolution of hosts having disparate DNA composition and physiological properties.

**ACKNOWLEDGMENTS**

We are grateful to Deanna Thomas for her technical assistance.

This investigation was supported by Public Health Service Research grant AI07533 to Ronald H. Olsen from the National Institute of Allergy and Infectious Diseases and the American Cancer Society Institutional Research grant IN-40N to The University of Michigan.

**LITERATURE CITED**

1. Bendich, A. 1957. Methods for characterization of nucleic acids by base composition. p. 715-723. *In* S. P. Colowick, and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.
2. Datta, N., and R. W. Hedges. 1971. Compatibility groups among *fi*⁻ R factors. Nature (London) **234**:222-223.
3. Datta, N., and R. W. Hedges. 1972. Host ranges of R factors. J. Gen. Microbiol. **70**:453-460.
4. Datta, N., R. W. Hedges, J. Shaw, R. B. Sykes, and M. H. Richmond. 1971. Properties of an R factor from *Pseudomonas aeruginosa*. J. Bacteriol. **108**:1244-1249.
5. De Czekala, A., D. Luk, and P. Bartl. 1972. Large-scale production of lambda bacteriophage and purified lambda deoxyribonucleic acid. Appl. Microbiol. **23**:791-795.
6. Espejo, R. T., and E. S. Canelo. 1968. Properties of bacteriophage PM2: a lipid-containing bacterial virus. Virology **34**:738-747.
7. Grinsted, J. J., R. Saunders, L. C. Ingram, R. B. Sykes, and M. H. Richmond. 1972. Properties of an R factor which originated in *Pseudomonas aeruginosa* 1822. J. Bacteriol. **110**:529-537.
8. Guerry, P., D. J. LeBlanc, and S. Falkow. 1973. General method for the isolation of plasmid deoxyribonucleic acid. J. Bacteriol. **116**:1064-1066.
9. Hedges, R. W., and N. Datta. 1973. Plasmids determining I pili constitute a compatibility complex. J. Gen. Microbiol. **77**:19-25.
10. Hoffman-Berling, H., H. C. Kaerner, and R. Knippers. 1966. Small bacteriophages. Advan. Virus Res. **12**:329-370.
11. Hohn, T., and B. Hohn. 1970. Structure and assembly of simple RNA bacteriophages. Advan. Virus Res. **16**:43-98.
12. Holloway, B. W., and B. Rolfe. 1964. Host genome control in host induced modification of *Pseudomonas aeruginosa* phages. Virology **23**:595-602.
13. Khatoon, H., and R. V. Iyer. 1971. Stable coexistence of R*fi*⁻ factors in *Escherichia coli*. Can. J. Microbiol. **17**:669-675.
14. Lawn, A. M., G. G. Meynell, E. Meynell, and N. Datta. 1967. Sex pili and the classification of sex factors in the *Enterobacteriaceae*. Nature (London) **216**:343-346.
15. Lowbury, E. J., A. Kidsom, H. A. Lilly, G. A. J. Ayliffe, and R. J. Jones. 1969. Sensitivity of *Pseudomonas aeruginosa* to antibiotics: emergence of strains highly resistant to carbenicillin. Lancet **II**:448-452.
16. Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. p. 195-206. *In* L. Grossman, and K. Moldave (ed.), Methods in enzymology, vol. 12. Academic Press Inc., New York.
17. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109-118.
18. Meselson, R., R. Yuan, and J. Heywood. 1972. Restriction and modification of DNA. Annu. Rev. Biochem. **41**:447-466.
19. Meynell, E., G. G. Meynell, and N. Datta. 1968. Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. Bacteriol. Rev. **32**:55-83.
20. Meynell, E., and N. Datta. 1965. Functional homology of the sex-factor and resistance transfer factors. Nature (London) **207**:884-885.
21. Olsen, R. H., and D. D. Thomas. 1973. Characteristics and purification of PRR1, an RNA phage specific for the broad host range *Pseudomonas* R1822 drug resistance plasmid. J. Virol. **12**:884-885.
22. Olsen, R. H., E. S. Metcalf, and J. K. Todd. 1968. Characteristics of bacteriophages attacking psychrophilic and mesophilic pseudomonads. J. Virol. **2**:357-364.
23. Olsen, R. H., and P. Shipley. 1973. Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. J. Bacteriol. **113**:772-780.
24. Radke, K. L., and E. C. Siegel. 1971. Mutation preventing capsular polysaccharide synthesis in *Escherichia coli* K-12 and its effect on bacteriophage resistance. J. Bacteriol. **106**:432-437.
25. Rhoades, M., L. A. MacHottie, and C. A. Thomas, Jr. 1968. The P22 bacteriophage DNA molecule. I. The mature form. J. Mol. Biol. **37**:21-40.
26. Sobieski, R., and R. H. Olsen. 1973. Cold-sensitive *Pseudomonas* RNA polymerase. I. Characterization of the host dependent cold-sensitive restriction of phage CB3. J. Virol. **12**:1375-1383.
27. Studier, F. W. 1965. Sedimentation studies of the size and shape of DNA. J. Mol. Biol. **11**:373-390.
28. Tikhonenko, A. S. 1970. Ultrastructure of bacterial viruses. Plenum Press, Inc., New York.
29. Vidaver, A. K., R. K. Koski, and J. L. Van Etten. 1973. Bacteriophage $\phi$6: a lipid-containing virus of *Pseudomonas phaseolicola*. J. Virol. **11**:799-805.
30. Weigle, J. 1966. Assembly of phage lambda *in vitro*. Proc. Nat. Acad. Sci. U.S.A. **55**:1462-1466.