Characteristics and Purification of PRR1, an RNA Phage Specific for the Broad Host Range *Pseudomonas* R1822 Drug Resistance Plasmid

RONALD H. OLSEN AND DEANNA D. THOMAS

University of Michigan, Department of Microbiology, Ann Arbor, Michigan 48104

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A new drug resistance plasmid-dependent RNA containing phage resembling coliphage f2 in its particle size and density is described. The phage, PRR1, will only productively infect some R^+ hosts containing the *Pseudomonas* drug resistance plasmid R1822. The membrane filter-salt elution patterns, RNase sensitivity, inactivation in low ionic strength solutions, and host range serve to distinguish PRR1 from coliphage f2 and two other *Pseudomonas* RNA phages, 7s and PP7.

RNA containing bacteriophages specific for male strains of *Escherichia coli* or related *Enterobacteriaceae* containing the *E. coli* sex factor F have been studied extensively and have been shown to fall into three serologically distinct groups (16). Phages containing single-strand RNA have also been isolated on *Caulobacter* species (15) and *Pseudomonas aeruginosa* (5, 7). However, for the *Pseudomonas* and *Caulobacter* species, no requirement for phage growth associated with the presence of an extrachromosomal genetic element has been demonstrated. We will describe some of the characteristics of PRR1, whose growth is dependent on the presence of the broad-host-range drug resistance plasmid R1822. We also report some of the salient features of PRR1 which distinguish it from other RNA phages studied to date. We have recently described the isolation of the phage and some of the properties of the plasmid conjugation system (14). Earlier reports of Grinsted et al. (8) and Datta et al. (6) have also described some of the characteristics of the same or a related plasmid, respectively. A separate report by C. H. To and C. Brinton describes plasmid-dependent PRR1 phage receptor sites and their relationship to pili occurring in other systems.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains and growth media used for host range and phage growth studies have been previously described (14). Additional strains used for this study are described in the appropriate tables.

Bacteriophage growth and enumeration. Bacteriophage enumeration by the overlay technique was essentially described by Adams (1) and was used by us in previous studies on *Pseudomonas* phages (13). The procedures for PRR1 isolation from sewage have been reported (14). For the preparation of partially purified high-titer phage stock suspensions, tryptone glucose yeast extract (TGE) broth medium in flasks was inoculated with 100 ml of a logarithmically growing culture (approximately 1×10^8 cells/ml) of *P. aeruginosa* PAT904Rev1 (R1822) per liter. This suspension was infected at a multiplicity of 0.01 with PRR1 phage freshly grown on the above host in soft agar overlays from a dilution of a single-plaque isolation. After phage infection, CaCl_2 was added to 0.015%, and the cultures were incubated under static conditions for 6 h at 37 C. At the end of this incubation, an additional 200 ml (1×10^8 cells/ml) of host cell suspension was added per liter of lysate with mixing followed by the addition of MgCl_2 to 0.05 M. Incubation was static at 37 C for 16 h. Cell debris was sedimented from the lysate by centrifugation at $9,000 \times g$ for 30 min at 4 C. Phage was precipitated from the supernatant fluid of the foregoing by the addition of solid $(\text{NH}_4)_2\text{SO}_4$ to 40% at ambient temperature. The pH of the broth containing $(\text{NH}_4)_2\text{SO}_4$ was then adjusted to 8.0 by using NH_4OH . After 2 h at ambient temperature, the $(\text{NH}_4)_2\text{SO}_4$ -precipitated phage was pelleted by centrifugation at $9,000 \times g$ for 30 min at 4 C. The pellets formed per liter of lysate were suspended and pooled in 20 ml of buffer of the following composition: Tris, 0.01 M; NaCl, 1.17 M; tryptone, 0.5%; and CaCl_2 , 0.001 M; the pH was adjusted to 7.0 with HCl. After dispersion of the phage precipitate in buffer, the suspension was centrifuged at $10,000 \times g$ at 4 C and the supernatant fluid was exposed successively to 50 μg of RNase per ml for 60 min at 25 C and to 5 μg of DNase per ml with MgCl_2 (0.005 M) for 60 min at 25 C. This nuclease-treated suspension was then centrifuged at 28,000 rpm

in a Spinco type 30 rotor for 2.5 h at 5 C. The pellets resulting from this high-speed centrifugation were suspended in 0.5 ml Tris, 0.01 M, pH 7.0, and 0.5% tryptone (TTB).

The phage was then further purified in CsCl gradients. These were centrifuged at 35,000 rpm in a Spinco SW50.1 head at 4 C for 24 h. For this, 1.9062 g of CsCl, 0.82 ml of TTB-suspended phage, and 2.18 ml of TTB were mixed in the centrifuge tube and overlaid with mineral oil to fill the tube. After centrifugation, a phage band near the middle of the tube was clearly visible and subsequently was collected by pumping out the contents of the tube. The CsCl was removed from the suspension by dialysis against TTB.

By using the above procedure, crude lysates with approximately 10^{11} PFU/ml were obtained, and 60 to 70% of the initial titer was recovered in the CsCl gradient phage band. Partially purified phage suspensions obtained by these procedures were stored over chloroform at 2 C.

RESULTS

Host range of PRR1. Not all bacterial strains capable of maintaining the R1822 plasmid (confirmed by minimal inhibitory concentration testing of R⁺ and R⁻ isolates for their sensitivities to carbenicillin, neomycin, or tetracycline) were able to propagate PRR1. R⁺

strains studied previously (14) and capable of acting as conjugal donors of multiple antibiotic resistance may be grouped into several categories on the basis of PRR1 clear plaque morphology, titer increase testing, and the inhibition of surface lawn bacterial growth by spotting drops of purified concentrated phage suspension. These groupings and their associated properties are shown in Table 1. Of the strains listed in Table 1, group B, only PRR1 infection of *P. aeruginosa* FP⁺ broth cultures showed a positive titer increase result (10 ml of TGE broth with 10^7 bacteria and 10^4 PRR1 incubated overnight at 25 or 37 C). Thus, the remaining strains listed in Table 1, group B, although killed at high multiplicities of infection attendant to the spot test procedure, were unable to grow PRR1 in significant amounts to result in a detectable increase in titer after overnight incubation at 37 C. Isogenic R⁻ strains did not show a positive spot test result in any instance when spotted with the same phage suspensions. From these results it is clear that the maintenance of the R factor allowing donor activity shown previously (14) is not sufficient by itself to permit phage growth.

We have also determined the susceptibility of

TABLE 1. *Plaque-forming ability of PRR1*

R ⁺ indicator	R ⁺ strain designation ^a	Lytic response
A. <i>P. aeruginosa</i> <i>P. fluorescens</i> <i>P. putida</i> <i>E. coli</i> <i>S. typhimurium</i> <i>V. cholera</i>	PAT904(R1822) FP* PAO67(R1822) FP ⁻ PFO15.4SmR(R1822) PPO13.12(R1822) K12 W1177(R1822) F ⁻ Hfr H(R1822) ^c LW1895 Hfr C(R1822) B-WR3951(R1822) C(R1822) ^d LT2(R1822) VCF2(R1822) (596B, Inaba serotype)	Forms plaques of variable clarity ^b depending on R ⁺ clonal isolate tested (not genera or species)
B. <i>A. calco-aceticus</i> <i>N. pe:flava</i> <i>P. aeruginosa</i> <i>A. vinelandii</i> <i>P. mirabilis</i>	ACJ1(R1822) NPM(R1822) PAT2(R1822) FP ⁺ AVM(R1822) PM17(R1822)	Does not form clearly distinguishable plaques, but does elicit zones of inhibition when spotted on surface-inoculated lawns
C. <i>S. boydii</i>	SBM(R1822)	Indicator shows no evidence of phage susceptibility by above criteria, but does act as R1822 donor for conjugal mating

^a For source and other characteristics see Olsen and Shipley (14).

^b Plating by the overlay technique.

^c Stock culture, Dept. of Microbiology, Univ. of Mich. (PFO15.4SmR[R1822] × *E. coli* Hfr H).

^d Laboratory strain received from R. Greenberg, Dept. of Biochemistry, Univ. of Mich. (PFO15.4SmR[R1822] × *E. coli* C).

members of other plasmid compatibility groups to PRR1 infection including F, I, N, W, C, A, J, and T. These plasmid-containing strains showed no evidence of PRR1 plaque formation after spotting of surface lawns (unpublished data). These negative findings also support the observed specificity of PRR1 for R1822 plasmid-containing bacteria.

Efficiency of plating. PRR1 plaque-forming ability (number and clearness of plaques) was optimal among *Pseudomonas* species and some *Enterobacteriaceae*. Accordingly, we determined whether the plating efficiency was equivalent on these strains. The results are shown in Table 2. Of all the R⁺ strains and their clonal isolates tested, the plating efficiency was highest on *P. aeruginosa* PAT904(R1822) even though the phage was initially isolated by enrichment on *P. aeruginosa* PAL1822 with plaque isolation on *E. coli* W1177(R1822). Accordingly, *P. aeruginosa* PAT904(R1822) or its derivative, PAT904Rev1(R1822), has been used routinely as the plating indicator for quantitative estimates of PRR1 titer. The reduced plating efficiency on *Pseudomonas fluorescens* PF015.4SmR(R1822) or *Pseudomonas putida* PP013.12(R1822) probably reflects the effect of temperature on phage growth and not strain characteristics, for a similar diminution in plating efficiency obtains for *P. aeruginosa* PAL1822 at 30 C. The former strains are incapable of bacterial growth above 33 C, although they will propagate PRR1 in turbid broth culture at 37 C (unpublished data). The *P. fluorescens* strain used here was psychrophilic and capable of growth at 0 C. However, no PRR1 growth occurred at this temperature. Thus, in this respect, the growth temperature range is apparently phage dependent as observed for other *Pseudomonas* phages isolated in our laboratory (13).

TABLE 2. Efficiency of plating of PRR1 on various R⁺ hosts

Incubation temp	R ⁺ indicator	R ⁺ strain designation	Efficiency of plating ^a
37 C	<i>P. aeruginosa</i>	PAL1822	(1)
		PAT904(R1822)	5
		PA067(R1822)	0.8
	<i>E. coli</i>	K12 W1177(R1822)	0.2
		LT2(R1822)	0.3
		VCF2(R1822)	0.2
30 C	<i>P. aeruginosa</i>	PAL1822	0.1
	<i>P. fluorescens</i>	PFO15.4SmR(R1822)	0.1
	<i>P. putida</i>	PP013.12(R1822)	0.1

^aPlating was at 37 C on *P. aeruginosa* PAL1822 as unity.

Growth of PRR1. One-step growth kinetics of PRR1 are shown in Fig. 1. Infection of *P. aeruginosa* PAT904Rev1(R1822) resulted in a latent period of approximately 45 min, which is similar to that observed for PRR1 growth on either *P. aeruginosa* PA067(R1822), *E. coli* W1177(R1822), or *S. typhimurium* LT2(R1822) (not shown). However, the average burst size varied somewhat with the propagating host. For growth on R⁺ pseudomonads, a burst of 50 to 100 was commonly observed, whereas on *Enterobacteriaceae* a burst of 10 to 20 occurred. These values were calculated on the basis of singly infected cells and were consistent from experiment to experiment. The adsorption of phage was also greatest by using *Pseudomonas* species as hosts, whereby 70 to 90% of the phage was adsorbed in 10 min at 37 C with less than 20% adsorbed under similar conditions by using *Enterobacteriaceae*. Thus, of the susceptible hosts tested, it appears that PRR1 phage growth

FIG. 1. One-step growth of PRR1 on PAT904Rev1(R1822) at 37 C. Cells (1×10^8 /ml) in TGE broth medium containing 0.001 M CaCl₂ were infected at a multiplicity of 0.1 PFU/bacterium. Phage was adsorbed for 20 min under static conditions followed by serial dilution to approximately 100 PFU/ml with incubation continued under static conditions at 37 C. At the times indicated, samples were taken and plated as described previously (13). Unadsorbed phage was determined by filtering dilute, infected cell suspension through 0.22- μ m membrane filters prewashed with TGE broth medium. Burst size was calculated on the basis of adsorbed phage.

was optimal on pseudomonads both from the perspective of phage adsorption and synthesis. However, the burst size was somewhat less for PRR1 than that observed for coliphage f2 (12). All of our attempts to recover more phage progeny after single-cycle growth by sonication or chloroform-lysozyme treatment of infected cells were unsuccessful. In fact, artificial lysis of PRR1-infected pseudomonads or *E. coli* resulted in the release of phage progeny only slightly sooner than observed for unperturbed, infected cell suspensions. When PRR1 adsorption to various hosts was tested, it was found to be optimal when R⁺ cells were grown overnight on solid medium containing 500 μ g of carbenicillin per ml, followed by 2 to 3 h of growth in TGE broth medium to 1×10^8 /ml. Cells were used directly from these broth cultures. All attempts to identify R⁺ hosts or growth conditions showing lysis inhibition and corresponding large burst size as observed for coliphage f2 (12) thus far have been unsuccessful. Therefore, we must conclude that PRR1 differs significantly from f2 in its ability to direct phage progeny synthesis.

Nucleic acid content of PRR1. A phage suspension purified as described earlier was tested for its nucleic acid content by using the orcinol or diphenylamine procedures for the determination of RNA or DNA, respectively. Approximately 1.4×10^{14} phage in 1 ml were tested. The results of these determinations showed no detectable DNA present and an RNA concentration of 1.2×10^{-11} μ g of RNA/PFU in the original crude lysate. If one assumes that RNA content for PRR1 is similar to the f2 group of RNA phages and if complete recovery of PRR1 particles through the simple purification procedure is used, approximately one plaque-forming phage is produced per seven progeny phage particles. This ratio of viable to total phage progeny was similar to that observed for f2 phage (10).

Sedimentation constant of PRR1. A mixture of f2 and PRR1 was cosedimented on a 5 to 20% sucrose gradient to further characterize PRR1 particle size in relation to the well-studied f2 phage. The result of this determination is shown in Fig. 2. It is apparent from the results of this experiment that the sedimentation properties of PRR1 are identical or closely related to those observed for f2. The maximum number of PFU for both phages appear in the same fraction. Therefore, on the basis of the sedimentation coefficient reported for f2 (10), PRR1 sediments at approximately 80S and hence has a particle size similar to f2.

Buoyant density. A dilute mixture (1×10^6 PFU) of partially purified f2 and PRR1 phages

was run in a CsCl density gradient to estimate the buoyant density of PRR1 in relation to f2. The results of this determination are shown in Fig. 3 and indicate similar, but not identical, buoyant densities for the two phages. The pitch of the gradient run under these conditions, as determined by weighing samples of fractions collected in the region of the phage peaks, was 0.004 g/fraction. In Fig. 3, the dashed line represents the intercept obtained by extrapolation of the PRR1 profile major axis which occurred at a density of 1.453. Thus the buoyant density of PRR1 is similar to those values reported for f2 and related phages (10, 17). This observation taken with the sedimentation coefficient determination shown in Fig. 2, buoyant density in Fig. 3, and morphology shown in Fig. 5, indicates a similar structure for PRR1 and the f2 group of phages.

Comparison of f2 and Pseudomonas RNA phages by the membrane filtration method. Previous work by Watanabe et al. (16) has shown correspondence between the serological grouping of RNA coliphages and their elution from membrane (Millipore Corp.) filters ex-

FIG. 2. Cosedimentation of PRR1 with RNA coliphage f2 in a 5 to 20% sucrose gradient. Gradients were produced by using a Beckman gradient forming device and contained in addition to sucrose, 0.01 M Tris, pH 7.0, and 0.5% tryptone. A mixture of PRR1 and f2 (0.2 ml) was added to the top, and gradients were centrifuged at 39,000 rpm at 20 C for 70 min in a Spinco SW50.1 rotor. Samples were collected dropwise from the bottom, diluted, and plated on appropriate indicator bacteria.

FIG. 3. Coseimentation of PRR1 and coliphage f2 in CsCl. CsCl was added to a mixture of PRR1 and f2 in 0.05 M Tris, pH 7.0, and 0.5% tryptone to result in a mean density of 1.4 g/ml. A sample of this mixture was centrifuged at 35,000 rpm at 4 C for 43 h in a Spinco SW50.1 rotor. Fractions were collected dropwise from the bottom of the tube and subsequently diluted and plated on appropriate hosts.

posed to a salt gradient. In view of the similarity of f2 and PRR1 with respect to particle size and density, we accordingly used this technique for the further comparison of f2 and PRR1. Two other *Pseudomonas* RNA phages, 7s and PP7, which are serologically related (4, 5), were included as a control for the grouping criteria and for comparison with PRR1. The results of these determinations are shown in Fig. 4. Phages 7s and PP7 were each filtered and eluted simultaneously with f2, allowing for an estimate of the reproducibility of f2 determinations and hence a subsequent estimate of the significance of differences occurring between phages. It is

apparent from these data that *Pseudomonas* RNA phages 7s and PP7 are similar although not identical based on the salt elution profiles. These phages are also significantly different from f2. PRR1 phage grown on either of two different *Pseudomonas* strains is markedly different from the other *Pseudomonas* RNA phages or coliphage f2. No phage was passed by the filter at a 1 M NaCl concentration, and PRR1 was resistant to elution at lower NaCl concentrations. Thus, PRR1 also differs from the other phages tested by Watanabe et al. (16), resulting in the three groupings of RNA coliphages. However, there is some indication that the host used for growth of PRR1 has some influence on its salt elution properties. When PRR1 was grown on *E. coli* Hfr(R1822), the partially purified phage suspension exhibited a tendency to be eluted at higher salt concentrations than was observed for R⁺ *Pseudomonas*-grown PRR1. However, this is the only instance of an effect of growth host on PRR1 properties observed for phage preparations grown on various hosts. This particular phage preparation was used here to indicate, however,

FIG. 4. Filtration and elution patterns of *Pseudomonas*, PRR1, and f2 RNA phages. High-titer phage stock suspensions were diluted into 0.01 M Tris, pH 7.0, and 1.0 M NaCl. A 10-ml amount was slowly filtered through a membrane-type filter which had been prewashed with Tris-salt (1.0 M) buffer. Adsorbed phage was determined by titrating filtrates after successive filtration of 10 ml of Tris-salt buffer solutions of decreasing salt concentration. No decrease in phage titer occurred by mere exposure to these solutions. Elution profiles reflect cumulative elution of phage with decreasing salt concentration.

the possibility of a host effect and to assure that f2 properties distinguishing it from PRR1 were not primarily a reflection of the presence of *E. coli* sex factor required for f2 growth.

Effect of various treatments on the viability of RNA phages. In view of the distinctly different membrane filtration elution patterns observed for f2, PRR1, and the *Pseudomonas* RNA phages, it was decided to compare them on the basis of their susceptibility to several treatments. The choice of the treatments reflects preliminary observations with regard to difficulties occurring during the initial preparation of purified PRR1 phage stocks by using procedures commonly employed in the purification of other phages, specifically, the addition of EDTA and RNase either in buffer or in broth. The treatments used and their effects on RNA phage survival are shown in Table 3. PRR1 grown on two R⁺ *Pseudomonas* strains and on an R⁺ *E. coli* Hfr strain were compared with each other as well as with 7s and PP7 phages grown on an R⁻ strain of one of the *Pseudomonas* hosts and with f2 grown on an R⁻ *E. coli* Hfr host. These data indicate that the survival of PRR1 is independent of the growth host, unlike the variance in salt elution seen in Fig. 4 when *E. coli* and *Pseudomonas* grown PRR1 are compared. When PRR1 is compared with 7s, PP7, and f2, its unusual sensitivity to EDTA is apparent. In Tris buffer-EDTA the inactivation of PRR1 is particularly evident. PRR1 is also sensitive to high concentrations of RNase (50 µg/ml), and this sensitivity is enhanced in Tris buffer suspension. Thus, from

these and similar experiments suspension of PRR1 in Tris buffer, while not deleterious to phage survival by itself, serves to potentiate phage inactivation by EDTA and RNase. These effects occur independently of the growth host.

Morphology of PRR1. A typical electron micrograph of a negatively stained, purified preparation of PRR1 is shown in Fig. 5. From this it is apparent that PRR1 is an isometric RNA phage whose morphology is similar to that described for other RNA phages (5, 7, 10, 15). Its average diameter, determined by measurement of electron micrographs and comparison with appropriate standards for size calibration, was 25 nm. Thus, the particle size of PRR1 is similar to that reported for the f2 group of male-specific *E. coli* RNA phages (f2, fr, MS2, R17, M12) proposed by Hohn and Hohn (10). However, this similarity in morphology is apparently trivial in relation to the distinctly different properties shown for PRR1 elsewhere in this report when compared with other RNA phages.

DISCUSSION

There is precedent for the inability of some of our R⁺ strains to serve as plaque-forming hosts for PRR1, although their behavior with respect to their ability to serve as plasmid donors was normal. For example, *Proteus mirabilis* is capable of harboring F-genotes which, when reintroduced into *E. coli*, are observed to have retained all of their original properties (11). The F-genote-harboring *Proteus*, however, is unable to promote plaque formation by male-specific

TABLE 3. Comparison of RNA phage survival after various treatments

Phage/growth host	Expt no.	Percent survival ^a			
		TGE EDTA ^c	Tris ^b EDTA ^c	TGE RNase (50 µg/ml)	Tris ^b RNase (50 µg/ml)
PRR1.PAT904(R1822)	I	66	0 ^d	62	
	II		0	65	32
	III	35	0	53	30
PRR1.PAO67(R1822)	III	36	0	51	23
PRR1.Hfr H(R1822)	III	41	0	52	12
7s.PAO67 ^e	III	83	100	64	72
PP7.PAO67	III	84	70	51	62
f2.LW1895 Hfr C	I	93	109	122	
	II		125	124	117
	III	85	100	97	95

^a Incubated for 60 min at 37 C under the conditions specified. TGE broth medium was treated similarly and used as 100% survival. No decrease in titer occurs during this exposure time of these phages in broth at 37 C. Surviving PRR1 plated on PAT904(R1822); 7s and PP7 plated on PAO67; f2 plated on LW1895 Hfr C.

^b 0.05 M, pH 7.0.

^c 0.05 M.

^d Zero equals less than 0.001% survival.

^e *Pseudomonas* phage 7s was received from B. W. Holloway, Monash University, Clayton, Victoria, Australia.

FIG. 5. PRR1 phage morphology. PRR1 purified as described in Materials and Methods was negatively stained with 2% phosphotungstic acid at pH 7.0 as described by Bradley (5).

MS2 phage. When phage growth was determined by a sensitive titer increase test in broth, it was shown that *Proteus* could propagate MS2. A similar situation occurred with some of the R⁺ strains we examined (see section B of Table 1). Although not all of the strains included in this group showed a significant increase in PRR1 titer when tested in broth, these may represent an extreme case of poor PRR1 phage adsorption under those conditions. The important aspect of this, in any case, is that no R⁻ strains have ever been observed to propagate or to be inhibited by PRR1. Among those R⁺ strains which showed plaque-forming ability, we also observed some variation in PRR1 susceptibility, as indicated in Table 2. In view of these results, *P. aeruginosa* PAT904Rev1(R1822) has been selected as the indicator strain for quantitative estimation of phage titer.

Our preliminary observations on some of the properties of PRR1 reveal characteristics serving to clearly distinguish it from other RNA phages studied to date. However, some indications of diversity allowable within simple structure and limited genetic content characteristic of these phages have been shown previously. For example, the RNA coliphages described by Bishop and Bradley (2, 3) had properties unlike other male-specific *E. coli* RNA phages. Thus, within the coliphages some indication of diversity was evident. Also, the somewhat larger size of Q β (17) and the specificity of its replicase (9) served to distinguish it from other male-specific coliphages.

Interestingly, with regard to the latter physiological parameter, it is apparent in the case of

PRR1 that the host bacteria contribution to PRR1 RNA replication has been genetically conserved throughout the evolution of the disparate R⁺ hosts plating PRR1 or, alternatively, replicase function(s) is either part of the R1822 plasmid or entirely specified by the PRR1 genome.

The physiology of infection of *Pseudomonas* RNA phages has not been studied to date. Although their host range is apparently not plasmid dependent, one of them, PP7, has been shown to adsorb to pili in a manner analogous to the adsorption of male-specific coliphages (4). Similar adsorption of *Caulobacter* phages to pili has also been demonstrated by Schmidt and Stanier (15). Therefore, besides their small size, RNA phages isolated to date share a common requirement for pili for their adsorption. In this regard, PRR1 is not an exception (C. H. To and C. Brinton, manuscript in preparation). The results of our preliminary studies with PRR1, however, infer properties distinctly different from other phages studied, in particular, its unusual sensitivity to RNase in broth or buffer suspension prior to adsorption in the early stages of infection, membrane filter elution, EDTA sensitivity, and ability to grow on diverse bacterial strains. These properties distinguish it from the other *Pseudomonas* RNA phages and from the coliphages. PRR1 RNase sensitivity resembles that observed for coliphages with defective A-protein or other structural anomalies, as recently summarized by Hohn and Hohn (10) and, as shown in Table 3, these properties occur independently of the host used to produce phage crops.

To our knowledge, PRR1 is the first transfera-

ble plasmid dependent RNA phage isolated which is unrelated to male-specific coliphages. The potential for promiscuity shown for the R1822 plasmid, then, may make PRR1 phage one of the most ubiquitous viruses in the microbial world.

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