JOURNAL OF BACTERIOLOGY, May 1989, p. 2287–2292
0021-9193/89/052287-06$02.00/0

Vol. 171, No. 5

Genetic Comparison of Bacteriophage PS17 and *Pseudomonas aeruginosa* R-Type Pyocin

TOMOYUKI SHINOMIYA* AND SAWAKO INA

Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan

Received 25 August 1988/Accepted 28 January 1989

PS17 is a bacteriophage of *Pseudomonas aeruginosa* that is serologically cross-reactive with phage tail-like bacteriocins called R-type pyocins. In addition to having immunological cross-reactivity, certain genes are functionally complementable between PS17 and R-type pyocins. To compare the genetic structures of PS17 and R-type pyocins, a physical map of PS17 genes was constructed by cloning phage DNA fragments on RSF1010-derived vector plasmids. The head and tail gene clusters were tandemly arrayed and together occupied about half of the 41-kilobase-pair PS17 chromosome. With use of these phage clones, the following results were obtained with respect to the genetic relationship between PS17 and R-type pyocins: (i) serological cross-reaction between PS17 and pyocin occurred for the major sheath protein and two components of the fiber, (ii) a certain pyocin mutation was complemented by cloned phage fragments, and (iii) the phage DNA fragment carrying sheath and core tube genes was shown to hybridize to the DNA fragment carrying the pyocin R2 genes.

Particulate bacteriocins with complex structures resembling those of bacteriophages have been found in various bacterial species (see reference 13 for review). Many strains of *Pseudomonas aeruginosa* are known to produce contractile phage tail-like bacteriocins, called R-type pyocins, which have been classified into five subgroups, R1 through R5, according to receptor specificity (7). The R-type pyocins are almost identical in morphology, antigenic property, and protein composition (12). Several temperate bacteriophages serologically cross-reactive with the R-type pyocins have been found (5, 6, 8). Certain components are interchangeable between pyocin R2 and bacteriophage PS17 (15). These results strongly suggest that the R-type pyocins and bacteriophages such as PS17 share a common ancestor. A detailed comparison of the genetic structures of the R-type pyocins and such bacteriophages will provide useful information on the evolutionary relationship between bacteriophages and particulate bacteriocins.

We previously analyzed the genetic structure of pyocin R2, an R-type pyocin produced by *P. aeruginosa* PAO (17, 18). A cluster of pyocin R2 genes 13 kilobase pairs (kbp) long was localized within a ca. 30-kbp chromosome region flanked by the *trpCD* and *trpE* genes. In this study, we constructed a genetic map of the head and tail genes of PS17 and examined the genetic relationship between R-type pyocins and the PS17 tail.

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* PML14 (8) and *Escherichia coli* C600 r^- m^- were used as hosts for cloning. Pyocin R2 (*prt*) mutants of *P. aeruginosa* PAO were those described previously (17).

Bacteriophages. PS17c1 (16) was used for cloning of PS17 genes. Temperature-sensitive mutants of PS17 were previously described (16).

Plasmids. Two plasmids, pKT230 and pMMB22, derived from a broad-host-range plasmid, RSF1010, were used as vectors to clone PS17 genes (1, 2) and were provided by M. Bagdasarian.

Microbiological methods. Nutrient broth (16) was used for cultivation of *P. aeruginosa* and *E. coli*. Transformation of *P. aeruginosa* with plasmid DNA was performed as described by Sano and Kageyama (14). For *E. coli*, a standard $CaCl_2$ method (11) was used. All pKT230 clones were obtained in *P. aeruginosa* PML14. Transformants were selected with 250 μg of streptomycin per ml, and those sensitive to 250 μg of kanamycin per ml were chosen. Clones on pMMB22 were obtained either in *P. aeruginosa* PML14 or in *E. coli* C600 r^- m^-. Transformants were selected with carbenicillin at 500 μg/ml for *P. aeruginosa* and at 100 μg/ml for *E. coli*. The recombinant DNAs isolated in *E. coli* were transferred to *P. aeruginosa* by DNA transformation.

Methods for titration of bacteriophage and pyocin were described previously (16). The ability of phage DNA clones to complement temperature-sensitive mutants of PS17 was assayed by titrating the mutant phages at 30 and 42°C, using cells carrying the plasmids. When the pMMB22-derived clones were tested, phage titers at 42°C were assayed in the presence and absence of 5 mM isopropyl-β-D-thiogalactopyranoside (IPTG).

Preparation and manipulation of plasmid DNA. Techniques used to prepare and handle plasmid DNA were those described by Maniatis et al. (11). Plasmid DNA was prepared by a boiling method and purified by centrifugation in a CsCl-ethidium bromide EtBr density gradient when necessary. Restriction enzymes were purchased from Takara Shuzo Co. Ltd. (Tokyo, Japan) and Toyobo Co. Ltd. (Osaka, Japan). The large fragment of *E. coli* DNA polymerase I, T4 DNA ligase, *E. coli* alkaline phosphatase, and BAL 31 nuclease were from Takara Shuzo.

Electrophoretic analysis of proteins. Culture samples (1 ml) were harvested by centrifugation, and the cell pellets were lysed in 200 μl of sample buffer as described by Laemmli (10). Samples were heated at 95°C for 5 min, and 10 μl per well was analyzed by electrophoresis on 12 or 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels (10). For analysis

* Corresponding author.

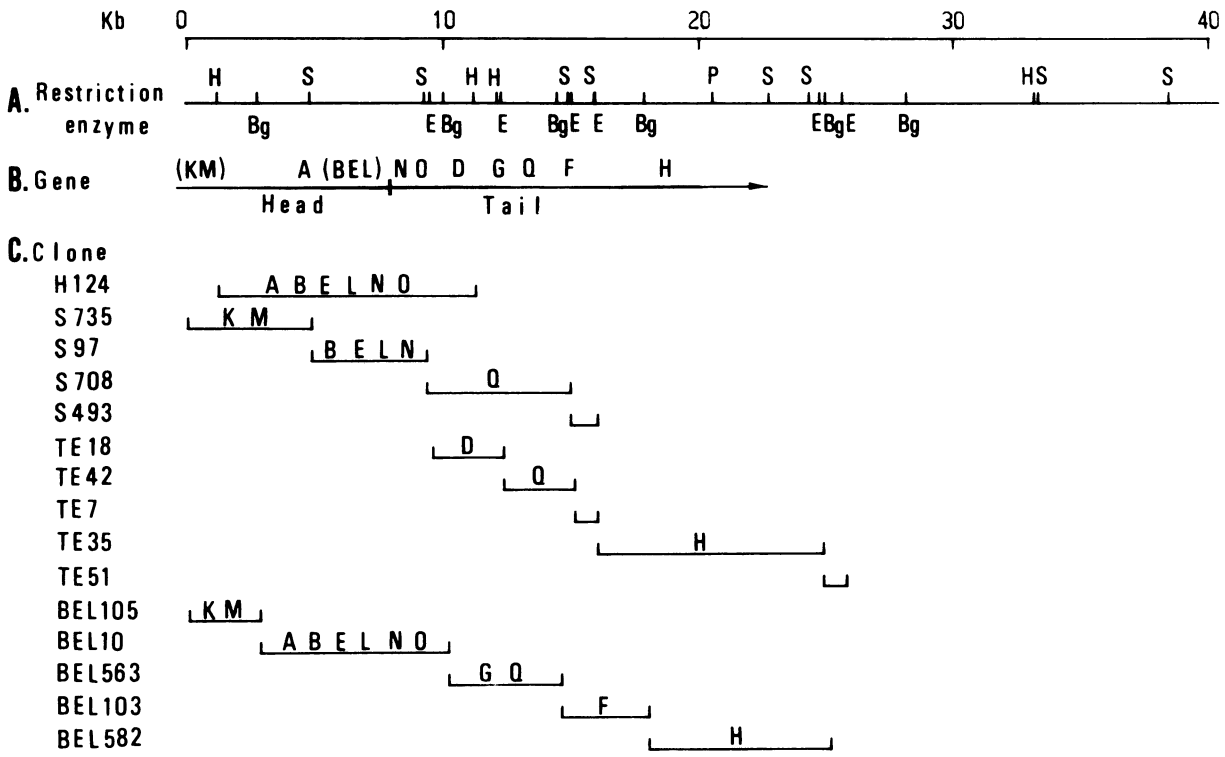

FIG. 1. Restriction and genetic maps of PS17. (A) Map of restriction enzyme cleavage sites. Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sma*I. (B) Genetic map compiled from the phage genes identified in the clones. Gene nomenclature is as previously described (16). (C) Phage DNA clones (direction of transcription from promoters on the vectors is from left to right). Phage genes on the cloned fragments were identified by complementation of temperature-sensitive mutations. H, *Hin*dIII clone on pKT230; S, *Sma*I clones on pKT230; TE, *Eco*RI clones on pMMB22; BEL, *Bgl*II clones on pMMB22.

of total proteins, the gels were stained with Coomasie brilliant blue R-250.

Immunoblotting techniques. For immunological detection of phage proteins after SDS-polyacrylamide gel electrophoresis, unstained gels were transferred to nitrocellulose filters (BA85; Schleicher & Schuell Co., Keene, N.H.) by electrophoresis as described by Towbin et al. (20). Enzyme-linked immunological detection of antigenic proteins, with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G antibody (Miles-Yeda Ltd., Rehovot, Israel) as a second antibody, was performed by the procedure of Blake et al. (3). As primary antibodies, rabbit antisera raised against PS17 (8), pyocin R1 (12), pyocin R1 sheath (21), pyocin R1 core tube (4), and pyocin R1 fiber (9) were used. The latter two antisera were kindly provided by S. Ishii.

Affinity purification of antibodies specific to the individual proteins of PS17 was done by eluting the bound antibodies from immunoblotted filters after excising the bands of interest by the method of Smith and Fisher (19). Dissociated PS17 proteins equivalent to 2×10^{10} PFU were electrophoresed in 10% polyacrylamide gels. The immunoblot using the antiserum against PS17 was stained for alkaline phosphatase activity for 8 min. The stained bands corresponding to the major phage proteins were excised from the filter. The filter fragments were eluted with 5 mM glycine hydrochloride (pH 2.3)–500 mM NaCl–0.5% Tween 20–100 μg of bovine serum albumin per ml, then with 130 mM NaCl–10 mM sodium phosphate buffer (pH 7.4)–0.5% Tween 20, 100 μg of bovine serum albumin per ml, and finally with 3 M ammonium thiocyanate. Eluates were mixed and used as affinity-purified antibodies.

Blot hybridization. Southern hybridization was carried out as described previously (18) at 65°C in 6× SSC (SSC is 0.15 NaCl plus 0.15 sodium citrate)–0.5% SDS–1× Denhardt solution.

RESULTS

Physical mapping of PS17 genes. A restriction enzyme map of PS17 is shown in Fig. 1A. PS17 DNA is a linear DNA of 41 kbp, and preliminary experiments, in which the *Hin*dIII fragments were cloned on R68.45, suggested that most of the structural genes were located in the left half. Two plasmid vectors, pKT230 and pMMB22, were used for further cloning and expression of the phage genes (Fig. 1C). Phage DNA digested with *Hin*dIII or *Sma*I was inserted into pKT230 at the *Hin*dIII or *Sma*I site, respectively (H or S series). In the latter case, the cohesive ends of PS17 were filled with DNA polymerase I before ligation to allow cloning of the terminal fragments. Another vector, pMMB22, carries the *lacI*^{q} gene and *tac* promoter, and expression of the gene inserted at the *Eco*RI site located downstream of the *tac* promoter can be induced by IPTG (2). The *Eco*RI and *Bgl*II fragments of PS17 were inserted into the *Eco*RI site of pMMB22 (TE and BEL series, respectively). The *Bgl*II-digested phage DNA and the *Eco*RI-digested vector DNA were ligated after blunting of the protruding ends with the large fragment of DNA polymerase I.

Phage genes located on the cloned fragments were identified by the ability to complement temperature-sensitive mutations of PS17 (Fig. 1C). Complementation activities were detected only in the clones in which the phage DNA fragments were inserted in an orientation such that the promoter for the kanamycin gene on pKT230 or the *tac*

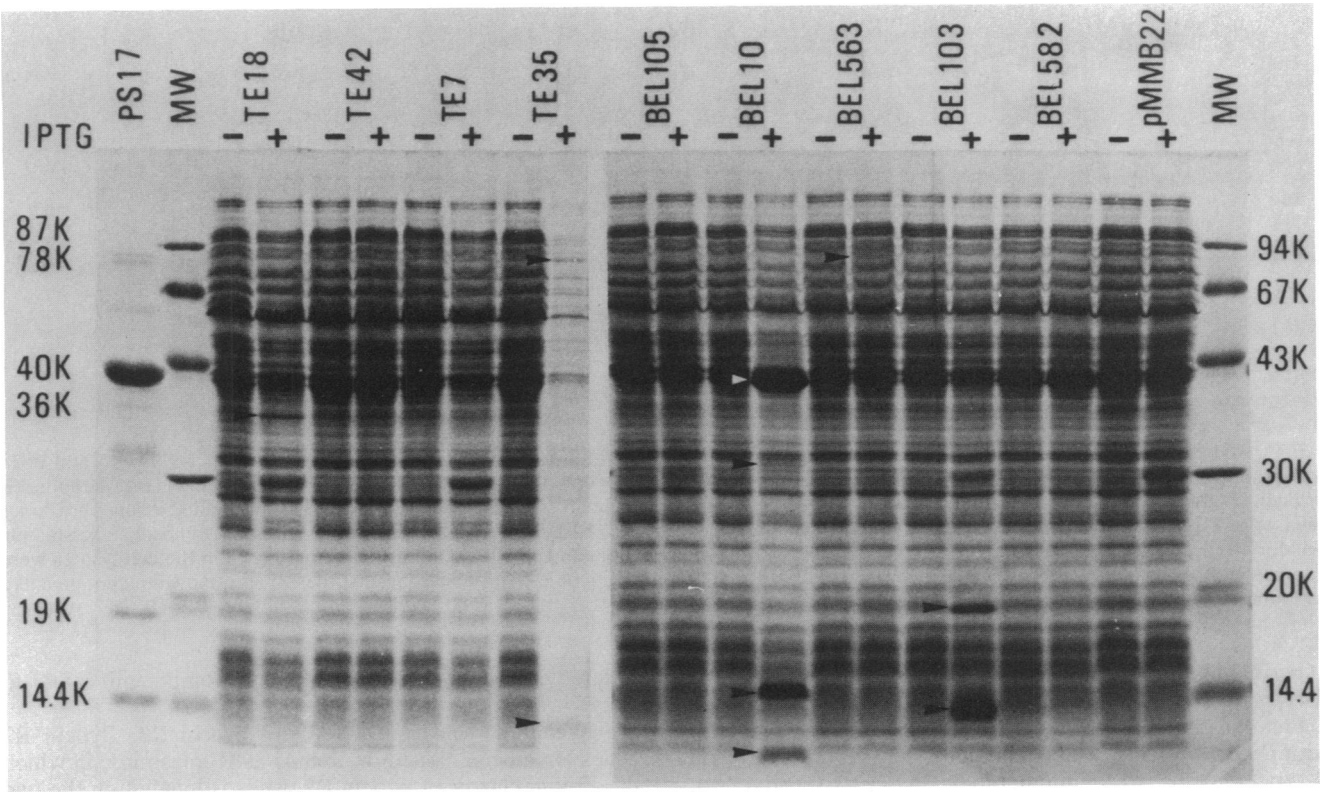

FIG. 2. Phage proteins produced by PS17 DNA clones. All lysates were prepared from IPTG-treated (+) and untreated (−) cultures as described in Materials and Methods. Samples (10 μl per well) were loaded on 12% polyacrylamide gels. After electrophoresis, proteins were stained with Coomasie brilliant blue. As standards, dissociated PS17 proteins (equivalent to 6×10^{10} PFU) and standard proteins were electrophoresed. Molecular weights of representative phage proteins are shown on the left; those of standard proteins are shown on the right. Arrowheads indicate phage proteins induced by IPTG.

promoter on pMMB22 was located to the left of the inserted fragments when PS17 DNA was oriented as shown in Fig. 1. Furthermore, complementation activities of pMMB22-derived clones were induced by IPTG. Therefore, the direction of transcription of the phage genes identified was assumed to be from left to right on the map shown in Fig. 1.

The order of the phage genes (Fig. 1B) was deduced from the results shown in Fig. 1C. The phage genes mapped included all of the head genes (A, B, E, K, L, and M) plus the tail genes (F, G, H, N, O, and Q) thus far identified. The head and tail genes appeared to form separate clusters. Gene D was previously considered necessary for both head and tail formation (16) but was localized within the cluster of tail genes.

Detection of phage proteins. Proteins encoded by the phage genes cloned under the *tac* promoter of pMMB22 were identified by SDS-polyacrylamide gel electrophoresis. Cell lysates were prepared from cultures grown in the presence or absence of IPTG (5 mM) and subjected to electrophoresis in SDS-polyacrylamide gels. Proteins in the gels were either stained with a protein stain or blotted to nitrocellulose filters by electrophoresis for immunological detection of phage proteins.

Protein patterns of the TE and BEL series are shown in Fig. 2. The moderately abundant protein at a molecular weight of 30,000 (30K protein) produced in cells carrying pMMB22, TE18, TE7, and BEL103 was probably the product of the streptomycin gene on pMMB22. The other proteins detected in the IPTG-treated samples but not in the untreated samples were assigned to products of the cloned phage genes (arrowheads in Fig. 2). The low levels of proteins in the IPTG-induced cells carrying TE35 resulted from a reduction in growth rate by IPTG, which occurred for unknown reasons.

Phage proteins were identified more clearly by immunoblotting, using the antiserum raised against purified phage particles. To relate these antigenic proteins to the phage proteins, antibodies against the individual phage proteins were affinity purified as described in Materials and Methods and used as primary antibodies for immunoblotting (Fig. 3).

TE18 (Fig. 3, lanes b) produced two antigenic proteins of about 36 kilodaltons that were reactive with antibodies to the 36K and 78K proteins, respectively, of PS17 (Fig. 3E and C). The 36K protein reactive with the anti-78K antibodies was thought to be the product of the truncated gene for the 78K protein. TE42 (lanes c) did not produce immunologically detectable proteins. TE35 (lanes d) produced several proteins reactive with the anti-PS17 antiserum. Two of them were assigned to the 87K and 30K proteins of PS17 (Fig. 3B and F). BEL10 (lanes e) produced the 14.4K phage protein (Fig. 3G). BEL563 (lanes f) produced the protein reactive with the anti-78K antibodies (Fig. 3C). BEL103 (lanes g) gave two bands at the 40K position that were cross-reactive with the 40K and 87K phage proteins, respectively (Fig. 3D and B). The protein reactive with the anti-78K antibodies was thought to be the product of the truncated 87K protein gene.

Phage proteins thus identified are summarized in Table 1. Localization of the phage proteins in the phage particles was deduced from previous results (16) and from immunoblotting

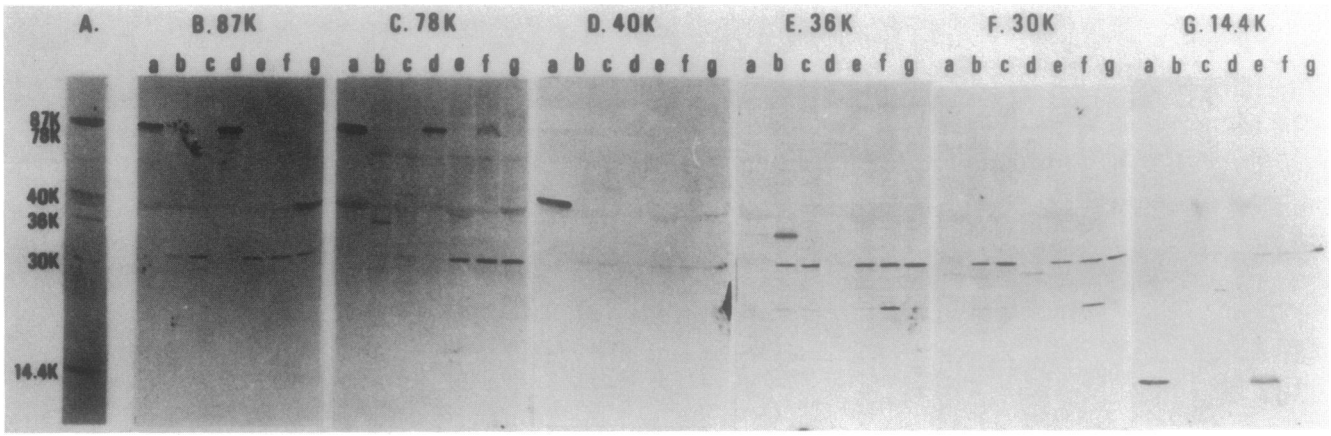

FIG. 3. Identification of phage proteins by immunoblotting with affinity-purified antibodies. (A) Immunoblots of PS17 with anti-PS17 antiserum from which the antibodies against the individual phage proteins were affinity purified as described in Materials and Methods; (B through G) immunoblots with the affinity-purified antibodies against the phage proteins indicated. Purified PS17 was loaded in lanes a. Clones used were TE18 (lanes b), TE42 (lanes c), TE35 (lanes d), BEL10 (lanes e), BEL563 (lanes f), and BEL103 (lanes g). Phosphatase staining was for 40 (B and D), 120 (C, E, and F), and 10 (G) min. Two bands of about 30 and 20 kilodaltons commonly seen in the cell lysates were non-phage proteins that could be detected even in the absence of primary antibodies. Antibodies against the 78K protein were contaminated with antibodies against the 87K protein.

experiments using the antisera against components of the R-type pyocin as described below.

Identification of phage proteins serologically cross-reactive with pyocin proteins. Previous results showed that PS17 and R-type pyocins were immunologically cross-reactive (8). Phage proteins cross-reactive with the pyocin components were identified by immunoblotting, using antisera against the isolated components of pyocin R1.

The antiserum against the pyocin R1 sheath bound to several proteins of pyocin R1, including the major sheath protein (data not shown). Affinity-purified antibodies against the major sheath protein of pyocin R1 (band 6) bound to the 40K protein of PS17 and BEL103 (Fig. 4A), a major constituent of the PS17 tail (16). We concluded that this 40K protein was the major sheath protein of PS17.

The fiber of pyocin R1 is composed of two proteins, 71K (band 2) and 31K (band 9) (9). The antiserum against the fiber of pyocin R1 gave strong bands at the positions of these proteins (Fig. 4B). When the same antiserum was reacted with purified PS17, two phage proteins, 78K and 36K, were identified. The 78K and 36K proteins were also detected in BEL563 and TE18, respectively. The antiserum against the isolated core tube of pyocin R1 did not bind significantly to phage proteins.

TABLE 1. Phage gene products of PS17 clones

Clone	Product	Method(s)[a]	Comment
TE18	36K	p, i	Fiber component
	36K	i	Truncated 78K protein
TE35	87K	p, i	Tail component
	30K	i	
	14K	p	
BEL10	40K	p	Major head protein
	30K	p	
	14.4K	p, i	Major head protein
	12K	p	
BEL563	78K	p, i	Fiber component
BEL103	40K	i	Major sheath protein
	40K	i	Truncated 87K protein
	19K	p	Core tube protein
	14K	p	

[a] Methods used for identification of products. p, Protein staining; i, immunoblotting.

Sequence homology of PS17 and pyocin R2. Pyocin R2 genes have been cloned on R68.45 as R′ plasmids, in which the 13-kbp cluster of pyocin R2 genes is located on the two contiguous *Hin*dIII fragments of 16 and 8 kbp (18). The

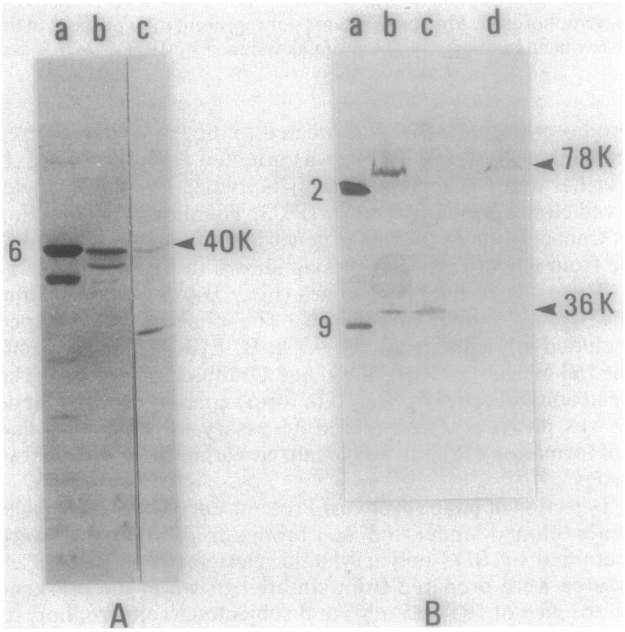

FIG. 4. Identification of phage proteins cross-reactive with antibodies against pyocin proteins. Immunoblotting experiments were carried out by using antibodies against the pyocin R1 components. (A) Antibodies against the major sheath protein of pyocin R1 (band 6). Lanes: a, pyocin R1; b, PS17; c, BEL103. (B) Antibodies against the fiber proteins of pyocin R1 (bands 2 and 9). Lanes: a, pyocin R1; b, PS17; C, BEL563; d, TE18. Cell lysates were prepared from IPTG-treated cultures. Numbers on the left of each panel are band numbers of pyocin R1 proteins. Cross-reactive phage proteins are marked with arrowheads. Amounts of pyocin R1 and PS17 were equivalent to about 6×10^{10} particles per well. The lower band in lane c of panel A is a nonspecific band (see legend to Fig. 3).

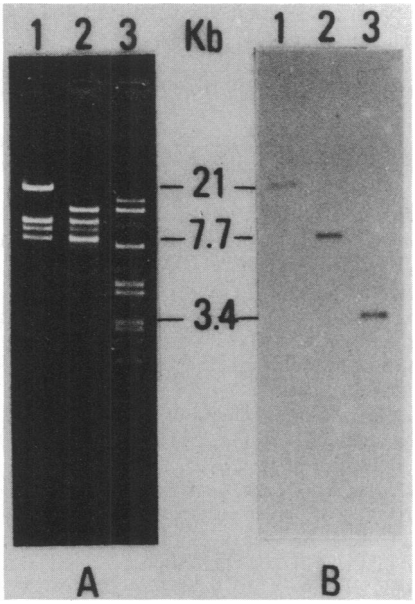

FIG. 5. Hybridization of PS17 DNA to pyocin R2 genes. PS17 DNA was digested with *Hin*dIII (lane 1), *Hin*dIII-*Pst*I (lane 2), and *Bgl*II-*Pst*I (lane 3), electrophoresed in agarose gel, and then subjected to Southern hybridization, using a 16-kbp ^{32}P-labeled *Hin*dIII fragment carrying the 11-kbp segment of the pyocin R gene cluster (18). (A) Ethidium bromide-stained agarose gel electropherogram; (B) autoradiograph of Southern hybridization.

major portion (11 kbp) of the pyocin R2 gene cluster is localized on the 16-kbp fragment. When these plasmids were digested with *Hin*dIII and subjected to Southern hybridization, using ^{32}P-labeled PS17 DNA as a probe, the 16-kbp fragment was labeled (data not shown). To define the homologous region in PS17 DNA, the 16-kbp fragment was isolated, labeled with ^{32}P, and hybridized to the PS17 fragments digested with various restriction enzymes. The 16-kbp fragment hybridized to the 21-kbp fragment of the *Hin*dIII digest, the 7.7-kbp fragment of the *Hin*dIII-*Pst*I digest, and the 3.4-kbp fragment of the *Bgl*II-*Pst*I digest of PS17 DNA (Fig. 5). The homologous region was thus assigned to the 3.4-kbp fragment restricted by *Bgl*II, which was cloned in BEL103 (Fig. 1).

Clone BEL103 carries gene F (Fig. 1) and the genes encoding at least four phage proteins, the 40K sheath protein, the product of the truncated gene for the 87K protein, the 19K putative core tube protein, and the 14K protein (Table 1). Since both gene F activity and production of the 40K sheath protein were lost by deleting approximately 0.1 kbp from the left end of the insert by BAL 31 nuclease (data not shown), gene F must encode the 40K sheath protein. Phage PS17 can complement sheath-deficient mutations of pyocin R2 (15), and the 40K sheath protein of PS17 was serologically cross-reactive with the sheath protein of pyocin R1, as shown above. Therefore, hybridization between the 3.4-kbp *Bgl*II fragments of PS17 and pyocin R2 is suggested to result primarily from sequence homology between the sheath genes.

Complementation of pyocin R2 mutations by PS17 clones. We reported previously that certain PS17 and pyocin R2 genes were exchangeable in function (15). We tested whether the cloned phage fragments could complement these pyocin R2 mutations. The pMMB22-derived clones were introduced into pyocin R2 mutants by DNA transformation. Pyocin production was induced by mitomycin C in the presence and absence of IPTG, and pyocin R2 activity in the lysates was titrated.

TABLE 2. Complementation of pyocin R2 mutation by PS17[a]

Prophage or plasmid	Complementation activity[b] with given marker				
	prt$^+$	*prtB186*	*prtF372*	*prtF377*	*prtK357*
PS17c2	10^3	10	10^2	10^2	10^3
pMMB22	10^4	0	0	0	0
TE18	10^3	0	0	0	10^3
TE42	10^4	0	0	NT	0
TE35	10^3	10	0	0	0
BEL10	10^4	0	0	NT	10
BEL563	10^3	0	0	0	0
BEL103	10^3	0	0	0	0
BEL582	10^4	0	0	0	0

[a] PS17c2 or plasmids were introduced into *P. aeruginosa* PAO3182 (*prt*$^+$) and its pyocin R2 mutants (17). Pyocin production in liquid culture was induced with mitomycin C in the absence or presence of 5 mM IPTG. Pyocin R2 activity in the lysates was titrated as described previously (17).

[b] Values except those for TE35 were obtained in the presence of IPTG. nt, Not tested.

Among the pyocin R2 mutations complemented by the PS17 prophage (*prtB*, *prtF*, and *prtK*), the *prtK* mutation was complemented efficiently by TE18 and weakly by BEL10 in the presence of IPTG (Table 2). Since these clones overlapped by about 0.5 kbp (Fig. 1), the phage gene responsible for complementation of the *prtK* mutation must be located in this overlapping region.

The *prtB* mutation was slightly complemented by TE35 in the absence of IPTG. The apparent lack of complementation activity in the presence of IPTG might have resulted from the toxic effect of this clone in the IPTG-induced cells.

Previous work showed that gene F of PS17 was responsible for complementation of the *prtF* mutation (15). Although BEL103 carried gene F and expressed its product, the sheath protein, it did not complement the *prtF* mutations. This clone may not have produced the phage sheath protein in an amount sufficient to bind to abundant core tube proteins of pyocin even under the strong *tac* promoter.

DISCUSSION

To clarify the genetic relationship between bacteriophage PS17 and R-type pyocins, a genetic map of head and tail genes of phage PS17 was constructed. The head and tail genes were localized in the left half of PS17 DNA (Fig. 1). The tail genes seemed to form a cluster similar in size to the pyocin R2 gene cluster (about 13 kbp [18]).

We compared the tail genes of PS17 and the R-type pyocin genes according to three criteria: (i) immunological cross-reaction of the products, (ii) DNA sequence homology detectable by hybridization, and (iii) functional complementation.

On the basis of these criteria, the sheath genes of PS17 and R-type pyocins must be homologous. Immunoblotting experiments revealed that the major sheath proteins were immunologically cross-reactive between PS17 and pyocin R1. Southern hybridization showed that the PS17 DNA segment of BEL103 carrying the sheath gene significantly hybridized to pyocin R2 genes. Moreover, the sheath-deficient mutants of pyocin R2 could be complemented by PS17 having intact sheath gene F (15).

Two components of the tail fiber were also immunologically cross-reactive between PS17 and pyocin R1. Although

we could not detect significant hybridization between the phage DNA fragments carrying the fiber genes and pyocin R2 genes under the conditions used in this work, the fiber components of PS17 and pyocin R2 are functionally exchangeable under certain conditions. Previous results (15) showed that phenotypically mixed phages with receptor and serological specificities of pyocin R2 were formed from PS17 lysogens of certain pyocin R2 mutants. We assumed that pyocin fibers accumulated in the pyocin R2 mutants deficient in base plate formation would associate with PS17. It is expected from this assumption that the phenotypically mixed phages will become the major products if mutations in the tail fiber genes are introduced into PS17. Actually, the lysogen of PS17 *tsG44* produced predominantly the phenotypically mixed phage (15). The study reported here shows that clone BEL563, carrying genes G and Q, produced the 78K fiber protein but that clone TE42, carrying only gene Q, did not. These results suggest that gene G encodes the 78K fiber protein and that its product can be substituted by the 71K fiber protein of pyocin R2, which is probably a distal component of the pyocin fiber (9, 12).

In addition to the three genes mentioned above, other three genes of PS17 and pyocin R2 are functionally related. Pyocin R2 mutation *prtB* and *prtK* can be complemented by PS17, and PS17 mutations of gene Q can be complemented by pyocin R2 (15). Complementation of the *prtK* mutations was convincingly confirmed by results obtained for the cloned PS17 fragments in TE18 and BEL10. Since these clones overlapped by only about 0.5 kbp, the protein that complemented the *prtK* mutation must be a small protein with a molecular weight of about 15,000 or less. Cells carrying BEL10 produced 14.4K and 12K proteins (Fig. 2), but cells carrying TE18 did not produce the corresponding proteins. Therefore, we could not identify the protein that complemented the *prtK* mutation. The *prtB* mutation seemed to be complemented by TE35.

In conclusion, at least six genes of PS17 tail and R-type pyocins are related with respect to immunological cross-reactions of products, functional exchangeability, or both. In particular, their sheath genes must be homologous.

ACKNOWLEDGMENTS

We thank M. Kageyama for useful discussions and critical reading of the manuscript. We also thank M. Bagdasarian for providing cloning vectors and S. Ishii for providing antisera for the pyocin components.

LITERATURE CITED

1. **Bagdasarian, M., R. Lurz, B. Rueckert, F. C. H. Franklin, M. M. Bagdasarian, J. Frey, and K. N. Timmis.** 1981. Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. Gene **16**:237–247.
2. **Bagdasarian, M. M., E. Amann, R. Lurz, B. Rueckert, and M. Bagdasarian.** 1983. Activity of the hybrid *trp-lac* (*tac*) promoter of *Escherichia coli* and *Pseudomonas putida*. Construction of broad-host-range, controlled expression vectors. Gene **26**:273–282.
3. **Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich.** 1984. A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. **136**:175–179.
4. **Hasegawa, T., and S. Ishii.** 1979. Isolation, homogeneity, and properties of core particles from pyocin R1. J. Biochem. **85**: 403–411.
5. **Honma, J. Y., and H. Shionoya.** 1967. Relationship between pyocin and temperate phage of *Pseudomonas aeruginosa*. III. Serological relationship between pyocines and temperate phages. Jpn. J. Exp. Med. **38**:213–224.
6. **Ito, S., and M. Kageyama.** 1970. Relationship between pyocins and a bacteriophage in *Pseudomonas aeruginosa*. J. Gen. Appl. Microbiol. **16**:231–240.
7. **Kageyama, M.** 1975. Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*, p. 291–305. *In* S. Mitsuhashi and H. Hashimoto (ed.), Microbial drug resistance. University of Tokyo Press, Tokyo.
8. **Kageyama, M., T. Shinomiya, Y. Aihara, and M. Kobayashi.** 1979. Characterization of a bacteriophage related to R-type pyocins. J. Virol. **32**:951–957.
9. **Kumazaki, T., Y. Ogura, and S. Ishii.** 1982. Isolation and characterization of pyocin R1 fibers. J. Biochem. **91**:825–835.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Ohsumi, M., T. Shinomiya, and M. Kageyama.** 1980. Comparative study on R-type pyocins in *Pseudomonas aeruginosa*. J. Biochem. **87**:1119–1126.
13. **Reanney, D. C., and H. W. Ackerman.** 1982. Comparative biology and evolution of bacteriophages. Adv. Virus Res. **27**:205–280.
14. **Sano, Y., and M. Kageyama.** 1977. Transformation of *Pseudomonas aeruginosa* by plasmid DNA. J. Gen. Appl. Microbiol. **23**:183–186.
15. **Shinomiya, T.** 1984. Phenotypic mixing of pyocin R2 and bacteriophage PS17 in *Pseudomonas aeruginosa* PAO. J. Virol. **49**:310–314.
16. **Shinomiya, T., and S. Shiga.** 1979. Bactericidal activity of the tail of *Pseudomonas aeruginosa* bacteriophage PS17. J. Virol. **32**:958–967.
17. **Shinomiya, T., S. Shiga, and M. Kageyama.** 1983. Genetic determinant of pyocin R2 in *Pseudomonas aeruginosa* PAO. I. Localization of the pyocin R2 genes cluster between *trpCD* and *trpE* genes. Mol. Gen. Genet. **189**:375–381.
18. **Shinomiya, T., S. Shiga, A. Kikuchi, and M. Kageyama.** 1983. Genetic determinant of pyocin R2 in *Pseudomonas aeruginosa* PAO. II. Physical characterization of pyocin R2 gene using R prime plasmids constructed from R68.45. Mol. Gen. Genet. **189**:382–389.
19. **Smith, D. E., and P. A. Fisher.** 1984. Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in *Drosophila* embryos: application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. J. Cell Biol. **99**:20–28.
20. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
21. **Yui, C.** 1971. Structure of pyocin R. I. Isolation of sheath from pyocin R by alkali treatment and its properties. J. Biochem. **69**:101–110.