Functional Modification of the Plasmid RP1-specified Pilus by *Caulobacter vibrioides*

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Caulobacter strains which contain plasmid RP1 are resistant to carbenicillin, kanamycin and tetracycline and can serve as donors of the plasmid, but such R⁺ strains are not susceptible to the RP1-specific phage PRR1. Since both conjugation and phage PRR1 infection are mediated by the plasmid-specified pilus, the lack of phage binding suggests some functional alteration of the pilus. In this report we demonstrate that RP1 pili are produced by *Caulobacter* R⁺ cells, but unlike the RP1 pili produced by PRR1-susceptible strains, the *Caulobacter* R⁺ pili do not inactivate phage PRR1. Antibody produced against *Caulobacter* R⁺ pili prevents phage PRR1 binding to RP1 pili from PRR1-susceptible strains of *Pseudomonas aeruginosa* and *Escherichia coli*, and also inhibits plasmid transfer by those strains. Antibody produced against RP1 pili from *P. aeruginosa* and *E. coli* inhibits plasmid transfer both by those strains and by *Caulobacter* R⁺ donors. Phage PRR1 rendered non-infectious by RNAase treatment prevents plasmid transfer by *P. aeruginosa* and *E. coli* but not by *Caulobacter*.

INTRODUCTION

Although plasmids have not been reported to occur naturally in the differentiating bacterium *Caulobacter*, several plasmids have been introduced into organisms of this genus. Alexander & Jollick (1977) demonstrated the transfer of plasmid RP1 to *Caulobacter crescentus* WS12 and *Caulobacter vibrioides* CV6. Ely (1979) subsequently transferred plasmids RP4, RK2, R702, R68.45 and R46 to several auxotrophic derivatives of *C. crescentus* CB15.

In their study of RP1 expression in *Caulobacter*, Alexander & Jollick (1977) found that although the *Caulobacter* R⁺ strains expressed the three RP1-specified antibiotic resistance markers (kanamycin, tetracycline and carbenicillin), these strains did not become susceptible to the plasmid-dependent phages PRD1 or PRR1 (as determined by a spot test using high titre phage lysates or by plaque formation using lower titre phage suspensions). In contrast, the RP4-containing *Caulobacter* strains of Ely (1979) were susceptible to high titres of those phages by the spot test but neither PRD1 nor PRR1 formed plaques on his strains.

Plasmid RP1 is apparently intact in *Caulobacter* R⁺ strains, since the strains can serve as donors of the plasmid, which when transferred to *Escherichia coli* or *Pseudomonas aeruginosa* is fully expressed including susceptibility to PRR1 and PRD1. Since plasmid RP1 specifies a new pilus which has been shown to be the phage PRR1 receptor organelle (Olsen & Thomas, 1973) and is considered also to be the organelle of conjugation, the fact that *Caulobacter* R⁺ strains can serve as effective donors of the plasmid yet remain insensitive to the plasmid pilus-specific phage PRR1 suggested the following possibilities: (1) *Caulobacter* R⁺ strains do not produce RP1-specified pili and plasmid transfer by *Caulobacter* involves a pre-existing structure, e.g. common pili; (2) RP1 codes for two kinds of pili and *Caulobacter*

R⁺ strains produce the conjugal pilus but not the phage-binding pilus; or (3) RP1 codes for a single pilus type which serves both functions but in *Caulobacter* R⁺ strains the pilus is modified so that it serves only the conjugal function, and cannot bind phage PRR1. In this report we present evidence which supports the third possibility.

METHODS

Organisms. The bacterial strains used are listed in Table 1. RNA phage PRR1 and DNA phage PRD1 were obtained from Professor R. J. Olsen, Department of Microbiology, University of Michigan, Ann Arbor, U.S.A.

Chemicals and media. Chemicals and antibiotics were obtained from Sigma. Bacterial media were from either Difco or BBL. RNAase A was purchased from Worthington Biochemical Corp.

The complete medium for the growth of *Caulobacter vibrioides* WS48 was the peptone yeast extract (PYE) medium of Poindexter (1964). The complete medium for the growth of *Pseudomonas aeruginosa* PAO67, *Escherichia coli* CSH29 and *Yersinia enterocolitica* W277 was either nutrient medium, trypticase soy medium or TGE medium (Olsen & Shipley, 1973). Phage PRR1 or PRD1 was propagated on TGE agar by the agar overlay method of Adams (1959).

The chemically defined (minimal) medium for the growth of prototrophic *Caulobacter* strains was described by Jollick & Tran (1975). The minimal medium for the growth of *Y. enterocolitica* W277 was the same as that for the *Caulobacter* strains, but with the addition of 5 µg thiamin ml⁻¹ and 0.5 mg sodium citrate ml⁻¹. For the selective growth of auxotrophic *E. coli* CSH29, the minimal medium was supplemented with 10 µg tryptophan ml⁻¹ and 5 µg thiamin ml⁻¹. The selective medium for R⁺ strains was the appropriate minimal medium with the minimal inhibitory concentration of carbenicillin, tetracycline or kanamycin.

Mating procedures. Donor and recipient cell titres were adjusted to approximately 3 × 10⁸ colony-forming units (c.f.u.) ml⁻¹, mixed at a ratio of 1:1 or 1:10 and then incubated at the optimum growth temperature of the recipient (30 °C for *C. vibrioides* WS48 and *Y. enterocolitica* W277 and 37 °C for *E. coli* CSH29) for 3 h. The mating mixture was then diluted and plated on the appropriate selective medium. Transconjugant colonies were counted after 48 h when *E. coli* CSH29 or *Y. enterocolitica* W277 was the recipient, and after 1 week when *C. vibrioides* WS48 was the recipient.

Phage susceptibility test. Bacterial strains were tested for phage susceptibility by the agar layer method of Adams (1959) and by the routine test dilution (RTD) procedure of Blair & Williams (1961). Nutrient agar was used as the base layer for phage susceptibility tests. In all cases 0.2 ml of a 24 h broth culture of the bacterial strain to be tested was added to 4.0 ml of molten soft (0.6%, w/v) agar kept at 46 °C. The molten agar was poured on to the base layer and allowed to harden, and then single drops (0.01 ml) of serial 10-fold dilutions of phage suspension containing about 10⁹ plaque-forming units (p.f.u.) ml⁻¹ were placed on the surface. The plates were incubated at the appropriate temperature and examined after 24 and 96 h.

Assay of unadsorbed phage PRR1. The absorbance of an 18 h broth culture of bacteria was adjusted to A₄₂₀ = 0.045, which corresponds to a cell concentration of about 10⁸ cells ml⁻¹. Phage PRR1 at a multiplicity of infection of 0.1 was added to the culture. The sample was mixed thoroughly and incubated for 10 min at 37 °C for strain CSH29, or at 30 °C for strain WS48. At the end of the incubation a 0.1 ml sample was removed, diluted 1:100 in cold PYE medium, mixed gently, and filtered through a 0.45 µm Millipore filter. The filtrate was diluted and 0.1 ml of each dilution was added to 4 ml of soft agar with 0.2 ml of an 18 h broth culture of the indicator strain PAO67, and then plated. Unadsorbed phage was expressed as the percentage of input phage that remained infectious.

Table 1. *Bacterial strains*

Strain	Characteristics	Source
<i>Caulobacter vibrioides</i> WS48	Prototroph, PRR1 ^r , PRD1 ^r	Authors
<i>Escherichia coli</i> CSH29	Trp ⁻ , B ₁ ⁻ , PRR1 ^r , PRD1 ^r	Cold Spring Harbor
<i>Yersinia enterocolitica</i> W277	Prototroph, PRR1 ^r , PRD1 ^r	R. Hodinka
<i>Pseudomonas aeruginosa</i> PAO67	His ⁻ , FP ⁻ , PRR1 ^s , PRD1 ^s , Car ^r , Tet ^r , Kan ^r (RP1)	R. Olsen

Derivatives of WS48, CSH29 and W277 containing plasmid RP1 (indicated in the text by the suffix R⁺) are all resistant to carbenicillin (Car), tetracycline (Tet) and kanamycin (Kan). In addition, CSH29R⁺ and W277R⁺ are susceptible to the plasmid-dependent phages PRR1 and PRD1, whereas WS48R⁺ is not. Plasmid RP1 is maintained in PAO67, and all references to PAO67 in this report mean PAO67R⁺.

Phage PRR1 titre increase experiment. PYE broth cultures of strains WS48 and WS48R⁺ were adjusted to about 10^8 c.f.u. ml⁻¹. A 7 ml portion of the cell suspension was mixed thoroughly with 1.0 ml of a suspension of phage PRR1 (5×10^2 p.f.u. ml⁻¹) and 0.11 ml of the mixture was added to each of 72 tubes containing 0.9 ml of PYE medium. The tubes were incubated overnight at 30 °C. Then 3 ml of soft agar and 0.2 ml of PAO67 culture were added to each tube and the mixtures were plated on nutrient agar plates. Plaques were counted after 15 h incubation at 37 °C.

Phage inactivation by sheared surface appendages (shearates). *Caulobacter* strains were grown on PYE agar plates for 48 h and *E. coli* strains and PAO67 were grown on TGE agar plates for 24 h. Plates for R⁺ cells were supplemented with antibiotics inhibitory to R⁻ cells. Cells were harvested by scraping the surface growth into 100 ml of appropriate broth. The cells were then collected by centrifugation, using a GSA rotor, for 15 min at 8000 rev. min⁻¹. Each cell pellet (about 2 g wet wt) was resuspended in 15 ml of sterile distilled water and the cell surface appendages (pili and flagella) were removed by blending in a Waring blender for 60 s at low speed. The cells were pelleted again in an SS34 rotor for 15 min at 12000 rev. min⁻¹. The supernatant (referred to as the shearate) was then exposed to u.v. light for 10 min, with agitation. At the end of irradiation, 0.9 ml of shearate was added to each of 14 tubes containing 0.1 ml of a suspension of phage PRR1 (about 1.6×10^2 p.f.u. ml⁻¹) and 10 µg chloramphenicol. The shearate/phage suspension was incubated for 30 min at 37 °C. Then 3 ml of soft agar and 0.2 ml of PAO67 culture were added to each tube and the mixtures were plated on nutrient agar plates. Plaques were counted after 15 h incubation at 37 °C.

Effect of *Caulobacter* R⁺ supernatant on phage adsorption of other R⁺ shearates. Broth cultures of strains WS48R⁺, CSH29R⁺ and PAO67 were centrifuged, using a GSA rotor, at 8000 rev. min⁻¹ for 10 min and the supernatants were filtered. Strains CSH29R⁺ and PAO67 were grown on TGE agar plates and the cells were scraped off and resuspended in the filtered supernatants. After 1 h incubation, the cells were centrifuged, resuspended in 15 ml of sterile distilled water and sheared in a Waring blender. The mixture was centrifuged, using an SS34 rotor, for 15 min at 12 000 rev. min⁻¹ at 4 °C. The shearate was dialysed against 12 l of distilled water for 48 h. A 0.9 ml portion of the shearate and 0.1 ml of a suspension of phage PRR1 (5×10^3 p.f.u. ml⁻¹) were then mixed gently and incubated at 37 °C for 1 h. Then 4 ml of soft agar and 0.2 ml of PAO67 culture were added and the mixture was plated on nutrient agar plates. Plaques were counted after 15 h incubation at 37 °C.

Anti-shearate antiserum preparation. Lyophilized shearates were dissolved in sterile saline to a concentration of 1 mg ml⁻¹. Equal volumes of the shearate/saline solution and Freund's complete adjuvant were homogenized. Rabbits (each weighing about 2.3 kg) were bled for pre-immune serum, and then inoculated weekly by the following schedule: 1st week, 2 ml antigen intraperitoneally; 2nd week, a total of 2 ml subcutaneously (four locations); 3rd week, resting; 4th week, a total of 2 ml subcutaneously (four locations). On the 5th week, serum was collected and stored at -18 °C. The presence of antibodies against shearate components was demonstrated by Ouchterlony's double diffusion method (Ouchterlony, 1968).

Effect of anti-shearate antiserum on phage adsorption to whole cells. Bacteria were grown to approximately 10^8 c.f.u. ml⁻¹ in broth and 1 ml of the culture was mixed with 1 ml of antiserum and chilled for 10 min. A 0.01 ml portion of phage PRR1 suspension (about 9×10^7 p.f.u. ml⁻¹) was added to the mixture which was then placed in the cold for another 10 min. A 1 ml portion of this mixture was added to 99 ml of chilled PYE broth and mixed by gentle inversion. The suspension was filtered through a 0.45 µm Millipore filter. Then 0.1 ml of the filtrate and 0.2 ml of PAO67 culture were added to 4 ml of molten soft agar and the mixture was plated on nutrient agar plates. Plaques were counted after 15 h incubation at 37 °C.

Phage inactivation by shearates in the presence of antiserum. Portions of 0.1 ml each of shearate and antiserum were mixed and kept at room temperature for 10 min; 0.1 ml of a suspension of phage PRR1 (about 3×10^3 p.f.u. ml⁻¹) was then added. The tubes were kept at room temperature for an additional 10 min before 4 ml of soft agar and 0.2 ml of PAO67 culture were added to each tube and the mixtures were plated on nutrient agar plates. Plaques were counted after 15 h incubation at 37 °C.

Bacterial conjugation in the presence of antiserum. The plasmid RP1-containing strains PAO67, CSH29R⁺ and WS48R⁺ were used as donors in matings with *Y. enterocolitica* W277 as the recipient. Prior to mating, the donor cell titres were adjusted to approximately 10^7 c.f.u. ml⁻¹ and the recipient cell titres to approximately 10^8 c.f.u. ml⁻¹. A 0.5 ml portion of the suspension of donor cells was first mixed with an equal volume of antiserum and incubated for 10 min at the appropriate temperature, and then 1 ml of the recipient culture was added. The mating mixture was incubated for 3 h at 30 °C and then plated on selective agar plates.

Bacterial conjugation in the presence of phage PRR1. Phage PRR1 (3.8×10^{10} p.f.u. ml⁻¹) was mixed with RNAase A to give a concentration of 1 mg RNAase ml⁻¹. RNAase renders PRR1 non-infectious only after adsorption and release of phage RNA. Strains PAO67 and WS48R⁺ were chosen as donors and W277 as the recipient. Equal volumes (0.5 ml) of the donor culture and the PRR1/RNAase preparation were first mixed and kept at room temperature for 15 min, then 0.5 ml of the recipient culture was added. The mating mixture was directly plated on selective agar plates.

Electron microscopy. Bacterial shearates and bacteriophage were examined using a Siemens Elmiskop I electron microscope. Specimens were negatively stained with 2.0% (w/v) uranyl acetate.

RESULTS

Adsorption of phage PRR1. Adsorption of phage PRR1 to all R⁺ transcipts was determined. *Pseudomonas aeruginosa* PAO67, the donor strain that was used for the propagation of phage PRR1, was also used as a positive control. *Caulobacter vibrioides* WS48 and *E. coli* CSH29 were used as negative controls. The results, expressed as the percentage of input phage adsorbed, showed that *P. aeruginosa* PAO67 cells adsorbed 22% of input phage and *E. coli* CSH29R⁺ cells adsorbed 28%, whereas *C. vibrioides* WS48R⁺ cells and the two negative controls adsorbed less than 5% of input phage.

Phage PRR1 titre increase. The titre increase experiment is more sensitive than the standard phage susceptibility spot test in detecting minimal phage replication in apparently non-productive hosts. Plasmid RP1-containing *Caulobacter* strains showed no apparent susceptibility to phage PRR1 as determined by the standard phage susceptibility tests. To determine whether low levels of phage PRR1 were being produced by strain WS48R⁺, phage titre increase experiments were done. The total phage input in these experiments was about 7 p.f.u. per tube. The average number of plaques from 72 tubes of WS48/PRR1 was 4.11 p.f.u. per plate, with a range of 0 to 18. The average number of plaques from WS48R⁺/PRR1 was 4.07 p.f.u. per plate, with a range of 0 to 13. Statistical analysis of variance showed no significant difference ($P < 0.05$) in phage productivity between WS48 and WS48R⁺.

Phage inactivation by various shearates. Shearates generated from R⁺ and R⁻ strains were examined by electron microscopy. Strain WS48 shearates contained two different cellular appendages: flagella about 20 nm in diameter and common pili about 5 nm in diameter (Fig. 1a). Strain WS48R⁺ shearates, in addition to flagella and common pili, contained an appendage about 10 nm in diameter (Fig. 1b, c). An identical 10 nm diameter appendage was also seen in CSH29R⁺ shearates. When CSH29R⁺ shearate was incubated at room temperature with phage PRR1, binding of the phage to the 10 nm appendage confirmed that it was the R pilus (Fig. 2). Specific binding of phage PRR1 to R pili in WS48R⁺ shearates could not be seen by electron microscopy.

Shearates generated from PAO67 and CSH29R⁺ showed significant phage adsorption, PAO67 shearate adsorbing 30% of input phage PRR1 and CSH29R⁺ shearate adsorbing 50%. Shearates from CSH29, WS48 or WS48R⁺ showed little or no phage adsorption (Table 2). Thus, although the RP1-specific pili were present in WS48R⁺ shearates, they did not adsorb phage PRR1.

Effect of Caulobacter WS48R⁺ supernatant on phage adsorption of other R⁺ shearates. This experiment was designed to examine the possibility that WS48R⁺ produced an extracellular substance which might modify the RP1 pilus phage-attachment site so that the pilus could not function in phage adsorption, as seen in *Caulobacter* R⁺. With a phage PRR1 input of 520 p.f.u. per plate, PAO67 shearate showed about 28% phage inactivation regardless of whether the cells were pre-incubated in *Caulobacter* WS48R⁺ supernatant or the original PAO67 culture supernatant. CSH29R⁺ shearate showed about 46% phage inactivation regardless of whether the cells were pre-incubated in WS48R⁺ supernatant or the original CSH29R⁺ supernatant. Thus, extracellular alteration of the phage adsorption pilus by a product of *Caulobacter* R⁺ could not be detected.

Anti-shearate antiserum preparation. The presence of antibodies against shearate antigens was demonstrated by Ouchterlony's immunodiffusion method. Lines of identity of precipitation were detected between PAO67 shearate and anti-PAO67 and anti-CSH29R⁺ shearate antiserum. Lines of identity were also seen between CSH29R⁺ shearate and anti-CSH29R⁺ and anti-PAO67 shearate antiserum as well as between WS48R⁺ shearate and anti-PAO67 and anti-CSH29R⁺ shearate antiserum. The immunodiffusion tests were used only to confirm that common plasmid-specified antigens were present in the shearates of the various R⁺ strains. We did not identify the specific antigens. Cross-reactivity among the R⁻ strains was not seen.

Fig. 1. Electron micrographs of shearates from *C. vibrioides* WS48 (a) and the isogenic RP1-containing transconjugant WS48R⁺ (b). Note the presence of flagella (F) and common pili (P) in both preparations while a third filament of approximately 10 nm diameter, the RP1-specified pilus (RP), is seen only in the shearate of the RP1-containing strain. The inset (c) shows, at higher magnification, a *C. vibrioides* flagellum (right) and the RP1-specified pilus. The bar markers represent 100 nm.

Fig. 2. Electron micrograph of phage PRR1 bound to a bundle of RP1-specified pili from *E. coli* CSH29R⁺. Note flagella (F) and RP1-specified pilus (RP). The bar marker represents 100 nm.

Table 2. *Phage PRR1 inactivation by shearates of several R⁺ and R⁻ strains*

Source of shearate	No. of plaques per plate*	Phage inactivation (%)
PAO67	112.3	30
CSH29R ⁺	80.2	50
WS48R ⁺	162.1	0
CSH29	157.2	1.6
WS48	155.1	2.9
Blank control	159.7	0

* Mean of 14 determinations.

Effect of anti-shearate antiserum on phage PRR1 adsorption to whole cells. As described above, PAO67 and CSH29R⁺ cells adsorbed phage PRR1 while WS48R⁺ did not. If RP1 pili are produced in WS48R⁺ with modified phage attachment sites yet are unaltered antigenically, antibody produced against WS48R⁺ shearate should also react with RP1 pili produced in other R⁺ strains. The effect of various anti-shearate antisera on phage adsorption by PAO67 and CSH29R⁺ cells was therefore determined. Antiserum against WS48 shearate served as the negative control. In the presence of WS48 antiserum, PAO67 cells inactivated 22.8% of input phage and CSH29R⁺ cells inactivated 27.1%. Antiserum against WS48R⁺ shearate inhibited phage adsorption by these two susceptible strains, to about 2% and 4% of input phage, respectively, suggesting the presence in the WS48R⁺ shearate of material that is antigenically similar to plasmid RP1-specified phage-adsorbing pili.

Phage inactivation by shearates in the presence of antiserum. The effects of the various anti-shearate antisera on phage inactivation by shearates was also determined. In the presence of blank or pre-immune serum, PAO67 shearate adsorbed about 17% of input phage PRR1

and CSH29R⁺ shearate adsorbed about 45 %. (The difference in phage inactivation capacity between PAO67 shearate and CSH29R⁺ shearate is probably the result of CSH29R⁺ shearate containing more R pili than PAO67 shearate, due either to the method of culturing or to greater production of R pili by CSH29R⁺.) Antiserum against CSH29 shearate had little or no effect on phage inactivation by either PAO67 shearate or CSH29R⁺ shearate. However, when antisera against shearates from RP1-containing strains were used to treat the shearates, the phage inactivation capacity of the shearates was reduced. When PAO67 shearate was treated with anti-shearate antiserum against CSH29R⁺, WS48R⁺ or PAO67, its phage inactivation capacity dropped to less than 5 %, and when CSH29R⁺ shearate was treated with anti-shearate antiserum against CSH29R⁺, WS48R⁺ or PAO67, its phage inactivation capacity dropped to less than 30 %.

Effect of anti-shearate antisera on plasmid RP1 transfer. The effect of various antisera on plasmid transfer was determined using the RP1-containing strains PAO67, CSH29R⁺ and WS48R⁺ as donors with *Y. enterocolitica* W277 as the recipient (Fig. 3). Antiserum against the shearate of one strain agglutinates the intact cells of that same strain as well as those of the isogenic R⁻ strain. For example, in the WS48R⁺ × W277 cross, WS48R⁺ whole cells were agglutinated by either anti-WS48 or anti-WS48R⁺ shearate antiserum, thus the mating frequency was reduced because there were fewer WS48R⁺ cells to serve as donors. Strain

Fig. 3. Effect of anti-shearate antisera on plasmid RP1 transfer in three crosses. The numbers of transconjugants in the blank controls (100% transconjugants) were: PAO67 × W277, 2.60×10^2 c.f.u. per 10^{-1} dilution plate; CSH29R⁺ × W277, 2.83×10^2 c.f.u. per 10^{-1} dilution plate; WS48R⁺ × W277, 1.55×10^2 c.f.u. per 10^0 plate.

Table 3. *Effect of intact and RNAase-treated phage PRR1 on the viability of R⁺ and R⁻ bacterial strains*

The viable counts of the untreated controls (100% survival) were (c.f.u. ml⁻¹): PAO67, 4.7×10^7 ; W277, 3.4×10^7 ; W277R⁺, 2.3×10^7 ; WS48R⁺, 2.8×10^8 .

Strain	Viability (% of control)		
	RNAase only	PRR1 only	PRR1 + RNAase
PAO67	95.4	31	90.4
W277	97.8	100	99.2
W277R ⁺	94.0	6.3	81.0
WS48R ⁺	99.6	100	100

W277 was chosen as the recipient because it was not agglutinated by any of the antisera. Anti-CSH29 shearate antiserum and anti-WS48 shearate antiserum were used as negative controls. When PAO67 or CSH29R⁺ was the donor, the mating frequency was reduced by at least 50% in the presence of R⁺ shearate antisera. The effect of anti-shearate antisera on transconjugant formation was especially marked in the WS48R⁺ × W277 cross: when donor WS48R⁺ was treated with anti-CSH29R⁺ shearate antiserum or anti-PAO67 shearate antiserum, transfer of the plasmid could not be detected.

Plasmid RP1 transfer in the presence of phage PRR1. The experiments described above showed that antiserum against WS48R⁺ shearate inhibits conjugation and phage PRR1 inactivation by other R⁺ strains, suggesting that RP1-specific conjugal pili as well as material that is antigenically similar to phage-binding pili is present in WS48R⁺ shearates. Since WS48R⁺ can transfer RP1 but not adsorb phage PRR1, it was still unclear whether (a) two different RP1-encoded pili were present on all R⁺ strains, with the phage-binding pili being functionally but not antigenically modified in WS48R⁺, or (b) a single R pilus serving both functions was present in all R⁺ strains, but was modified in WS48R⁺ so that it functioned only as the organelle of conjugation. If the first possibility were true, that is, if there are two kinds of R pili, one for conjugation and one for phage binding, then PAO67, harbouring plasmid RP1, should conjugate and transfer RP1 with relatively normal frequency even if the phage-binding pili were coated by inactivated phage.

RNA phage attachment to cell-associated F pili is followed almost immediately by a loss of phage infectivity and the concomitant sensitization of the phage RNA to RNAase (Valentine & Wedel, 1965; Danziger & Paranchych, 1970). Thus, in the presence of RNAase, a phage-susceptible host should be protected from infection by some RNA phages. The results in Table 3 show that such is the case with phage PRR1 for the R⁺ strains used in this study. Strains PAO67 and W277R⁺, both susceptible to phage PRR1, were protected by RNAase A as compared with the controls. Strains WS48R⁺ and W277, on the other hand, were not affected by any of the treatments.

The effect on transconjugant yields of phage PRR1 treated with RNAase was also determined. In the PAO67 × W277 cross, when donor cells were incubated with phage PRR1 plus RNAase, although phage infection was prevented, the mating frequency was nonetheless reduced to 25% of the control value (from 293 transconjugants to 73 transconjugants on the 10⁻¹ dilution plates). In the WS48R⁺ × W277 cross, phage PRR1 plus RNAase had no effect on the mating frequency.

Crosses of the same strains (PAO67 × W277 and WS48R⁺ × W277) which were exposed to phage PRR1 without RNAase were also performed. In both cases, transconjugant formation was reduced, as would be expected, since W277R⁺ is susceptible to the phage. These data show that when non-infectious phage PRR1 binds to susceptible R⁺ strains they no longer serve as donors.

DISCUSSION

An alteration of plasmid RP1 pilus function in *Caulobacter vibrioides* WS48R⁺ has been demonstrated in this study. *Caulobacter* R⁺ strains express RP1-encoded transferability and antibiotic resistance markers, but they are insensitive to the RP1-dependent phage PRR1. The physical integrity of RP1 in *Caulobacter* R⁺ is implicit because when RP1 is transferred from *Caulobacter* R⁺ to *E. coli* CHS29 or *Y. enterocolitica* W277, all the RP1-encoded markers are expressed in the transconjugants. Since both RP1 transfer and phage PRR1 susceptibility involve pili, the RP1 pilus in *Caulobacter* R⁺ must be different from that in other R⁺ strains.

Gene transfer among *Caulobacter* species readily occurs (Jollick & Schervish, 1972); however, pili of the F or P type are not seen. *Caulobacter* R⁻ species produce only common pili. Evidence from this study indicates that *C. vibrioides* WS48R⁺ does not use pre-existing pili for RP1 transfer. The evidence is as follows: (1) WS48R⁺ produces RP1-specified pili as seen by electron microscopy; (2) antibody produced against WS48 shearates (containing common pili) does not inhibit RP1 transfer by other R⁺ strains, while antibody against WS48R⁺ shearates (containing both common pili and RP1 pili) does, suggesting that an RP1-specific conjugal pilus is present in WS48R⁺ shearates; (3) RP1 transfer in the WS48R⁺ × W277 cross was blocked by antibody produced against shearates of PAO67 or CSH29R⁺. If WS48R⁺ did use common pili for RP1 transfer, the inhibition of RP1 transfer effected by PAO67 or CSH29R⁺ shearate antibody would not have occurred, since there is no cellular appendage in the PAO67 shearate or the CSH29R⁺ shearate which is antigenically similar to the *Caulobacter* common pilus.

Assay of phage PRR1 adsorption by whole cells and shearates indicated that PRR1 infection in WS48R⁺ was defective in the initial step, i.e. phage adsorption. These facts suggested the possibility that two RP1-specified pili, one for phage binding, the other for conjugation, may exist and that the phage-binding pilus is absent in WS48R⁺. However, antiserum produced against WS48R⁺ shearates inhibits the phage PRR1 inactivation capacity of either whole cells or shearates of PAO67 or CSH29R⁺. This suggests that the phage-binding pilus is not absent from WS48R⁺ shearate. We may therefore conclude either (a) two different RP1-encoded pili are present on both WS48R⁺ and the other R⁺ strains, and the conjugal pilus is functional in both but the phage-binding pilus is modified in WS48R⁺, or (b) a single pilus which serves both functions is present in WS48R⁺ and the other R⁺ strains, but is modified in the *Caulobacter* R⁺ strains so that it functions only as the organelle of conjugation. Another possibility cannot be excluded at this point, that is, the possible cross-reactivity of the antibodies produced against the *Caulobacter* R⁺ conjugal pilus to phage-binding pili in other R⁺ strains. However, the inhibition of plasmid transfer by inactivated phage PRR1 shows that there is only one kind of RP1 pilus produced and that in WS48R⁺ the RP1-specified pilus serves only the conjugal function.

The basis of the functional modification of the RP1 pilus in *Caulobacter* has not been established. Several possible mechanisms can be invoked; however, our data do indicate that *Caulobacter* does not modify the pilus by any extracellular mechanism. We speculate that an intracellular function that modifies the pilin protein to produce the final extracellular structure is missing in *Caulobacter*. Compositional analysis of RP1-specified pili produced by *Caulobacter* R⁺ and other R⁺ strains is in progress.

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