CURRENT MICROBIOLOGY Vol. 38 (1999), pp. 239–243

**Current Microbiology**
An International Journal


# The Clinically Isolated FIZ15 Bacteriophage Causes Lysogenic Conversion in *Pseudomonas aeruginosa* PAO1

Sergio Vaca-Pacheco,[1] Gloria L. Paniagua-Contreras,[1] Octavio García-González,[1] Mireya de la Garza[2]

[1]Laboratorio de Genética, Escuela Nacional de Estudios Profesionales Iztacala, Universidad Nacional Autónoma de México, Apartado postal 314, Tlalnepantla, Edo. de Méx., 54090, México
[2]Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados del IPN, Apdo. postal 14-740, México D.F. 07000, Mexico

Received: 31 August 1998 / Accepted: 20 November 1998

**Abstract.** FIZ15 bacteriophage, from a human clinical isolate of *Pseudomonas aeruginosa*, causes lysogenic conversion in the *P. aeruginosa* strain PAO1. The prophage-conferred phenotypes are: (1) increased resistance to phagocytosis by mouse peritoneal macrophages; (2) increased resistance to killing by normal human serum, and (3) increased adhesion to human buccal epithelial cells. These phenotypes are related to the prophage-induced change at the level of its own bacterial receptor, which appears to be the O-antigen.

*Pseudomonas aeruginosa* is an opportunistic pathogen associated with infections in patients suffering from cystic fibrosis and in immunocompromised hosts [5]. Adhesion is an important virulence property of this invasive and toxigenic Gram-negative bacillus, since adhesion allows host colonization and subsequent invasion to cause a localized or systemic infection. It has been shown that adhesion of *P. aeruginosa* to epithelia can be mediated by pili [13, 16, 19, 24], alginate [10], and other bacterial components [8, 18, 20] including exoenzyme S [1].

It is well known that some bacteriophages carry genes coding for toxins or products that modify the external surface of their lysogenic bacteria, conferring on them some specific properties such as resistance to killing by complement or antigenic differences [2, 3, 6]. However, increased bacterial adhesion to epithelial cells owing to prophage has been reported only for coliphage λ [23].

As nearly all clinical *P. aeruginosa* strains are lysogens [12], it is tempting to suppose that bacteriophages may play a role in the virulence of this bacterium. Therefore, in a search for native bacteriophages that might improve characteristics of virulence to *P. aeruginosa*, we have isolated a group of temperate bacteriophages from human clinical *P. aeruginosa* isolates. One of the phages was chosen (FIZ15), and in a preliminary study the increase in adhesion of *P. aeruginosa* PAO1 lysogen to human buccal epithelial cells (HBC) was reported [22]. In this work we report a further characterization of this lysogenic conversion. This phage increased the levels of at least three virulence properties of PAO1: resistance to killing by human complement, resistance to phagocytosis by mouse peritoneal macrophages, and adhesion to human buccal epithelial cells.

*Correspondence to:* M. de la Garza

## Materials and Methods

**Media.** Media for bacterial cell growth were tryptic soy broth (TSB) and agar (TSA), and nutrient broth (NB) and agar (NA). All from Bioxon, México.

**Bacteria and bacteriophages.** *Pseudomonas aeruginosa* PAO1 and the temperate bacteriophage D3 were kindly provided by B.W. Holloway (Monash University, Australia). FIZ15 phage was isolated from a *P. aeruginosa* clinical isolate obtained from an infected human wound. PAO1(FIZ15) is a PAO1 strain lysogenic for FIZ15. Stability of the lysogen was assessed by subculturing three times ten colonies of the original strain and testing them for spontaneous phage release onto a lawn of PAO1, and for failure of FIZ15 phage to form plaques onto lawns of each lysogen. Upon lysogenization, FIZ15 conferred streptomycin resistance to PAO1. PIZ3 is a streptomycin-sensitive revertant obtained by mutagenizing PAO1(FIZ15) with ethyl-methane-sulfonate as described by Miller [15]. PIZ31 is a PAO1 lysogen for the FIZ15 phage obtained from PIZ3. The lysogenic state of PIZ3 and PIZ31 was tested by the failure of FIZ15*c* to form plaques onto lawns of PIZ3 or PIZ31 and by spontaneous phage release onto lawns of PAO1. For the phage adsorption experiments, nitrous acid-induced, clear-plaque mutants of FIZ15 and D3 were used (FIZ15*c* and D3*c*). *P. aeruginosa* PAO1/FIZ15 is a spontaneous PAO1 mutant resistant to FIZ15*c*, which

was obtained by spreading about $10^9$ CFU over a lawn of about $10^{10}$ FIZ15*c* in NA, followed by three single-colony isolations. PIZ1 is a spontaneous PAO1 mutant resistant to serum, isolated by three successive treatments in 75% normal human serum. Sensitivity of strains to streptomycin was tested in NA plus 40 μg/ml of the antibiotic.

**Adhesion assay.** Bacterial adhesion to HBC was performed by a modification of the methods described by Woods [24] and Reid [17] as described elsewhere [23].

**Antisera preparation.** PAO1 and PAO1(FIZ15), grown on NA for 18 h at 37°C, were harvested in 0.85% NaCl, and the suspension was heated in a boiling water bath for 2 h. The bacterial suspension was twice washed and diluted in 0.85% NaCl to an $A_{590}$ of 0.3. Mouse antiserum against PAO1 or PAO1(FIZ15) was obtained by immunizing CD1 mice by two intraperitoneal injections with $1 \times 10^8$ CFU given at intervals of 4 days. The immune sera were collected by cardiac puncture 7 days after the last injection, pooled, and heated for 1 h at 56°C to inactivate the complement, and stored at −20°C.

New Zealand adult rabbits were immunized by four intravenous injections of 0.5, 1.0, 1.5, and 2.0 ml of PAO1 bacterial suspension, mixed with complete Freund's adjuvant, given at 7-day intervals. Immune serum was collected 7 days after the last injection, heated for 1 h at 56°C, and stored at −20°C.

**Agglutination assay.** Serial twofold dilutions of the immune antiserum, made in PBS (0.145 M NaCl; 0.05 M sodium phosphate, pH 7.0), were mixed with 0.5 ml of a bacterial suspension (grown on TSA, boiled, and washed with PBS) at a concentration of $5 \times 10^9$ CFU/ml. Mixtures were incubated at 37°C for 18 h together with appropriate controls, centrifuged at 1000 *g* for 5 min, and the pellet was gently agitated to observe the agglutination. Agglutination titers were reported as the reciprocal of the highest dilution of serum showing macroscopic agglutination.

**Resistance to human serum bactericidal activity.** Resistance of the bacterial strains to human serum bactericidal activity was tested as described by Timmis et al. [21].

**Adsorption of phages to *P. aeruginosa* strains.** About $1 \times 10^8$ exponentially growing bacterial cells of each strain were mixed with FIZ15*c* or D3*c* phages at a multiplicity of infection (m.o.i.) of 0.05 in a final volume of 0.6 ml. Mixtures were incubated without shaking at 37°C, and 6 min later aliquots of 0.05 ml were removed and added to 0.45 ml of NB containing 5 drops of chloroform. After a 30-min incubation period at 37°C, samples were diluted in NB and assayed for the amount of free phage by plating on PAO1.

**Phagocytosis.** Phagocytosis was assayed by a modification of the method described by Holloway and Cooper [11]. Peritoneal macrophages were collected from normal CD1 mice, centrifuged and adjusted to $2 \times 10^6$ cells/ml in Dulbecco's modified Eagle medium (Gibco), pH 7, by counting in a Neubauer hemocytometer. Bacteria grown at saturation in NB were washed and resuspended in Hanks' Balanced Salts Solution (HBSS) plus 0.014% $CaCl_2$, 0.01% $MgSO_4$, 0.035% $NaHCO_3$, and 0.002% phenol red at a concentration of $2 \times 10^4$ CFU/ml. One ml of bacterial suspension was added to 1 ml of macrophage suspension, and the mixture was incubated in a gyratory water bath shaker at 20 rpm for 1 h. After this time, the mixture was centrifuged at 250 *g* and the supernatant fluid was conveniently diluted in HBSS to quantify the number of surviving bacteria by viable count in NA. In each experiment, a control was included to which boiled macrophages (100°C for 1 h) were added. The percentage of phagocytosed bacterial cells was calculated as follows: [Bacteria in control tube − bacteria in experimental tube/bacteria in control tube] × 100.

Table 1. Agglutination titers of wild-type and lysogenic *Pseudomonas aeruginosa* with hyperimmune sera[a]

| Strain | Rabbit antiserum vs. PAO1 | Mouse antiserum vs. | |
|---|---|---|---|
| | | PAO1 | PAO1(FIZ15) |
| PAO1 | 1:20480 | 1:32 | 1:128 |
| PAO1(FIZ15) | 1:81920 | 1:512 | 1:2048 |

[a] Serial twofold dilutions of antiserum (made in PBS) were mixed with $5 \times 10^9$ heat-killed bacterial cells. After incubation at 37°C for 18 h, mixtures were centrifuged, and the pellet was gently agitated to observe the agglutination. Agglutination titers are reported as the reciprocal of the highest dilution of serum showing macroscopic agglutination.

## Results and Discussion

**FIZ15-induced surface alteration on PAO1.** In searching for temperate bacteriophages able to modify some virulence-related properties of *P. aeruginosa*, 210 phages were isolated from the same number of clinical strains by spotting aliquots of the culture supernatants onto lawns of PAO1, the genetically and biochemically most studied *P. aeruginosa* strain. After three single plaque isolations, a stock was obtained for each phage and spotted as mentioned above, for obtaining lysogens from the center of the turbid lysis zone. Only 80 phages formed stable lysogens, and they belonged to 19 distinct phage immunity groups. From these lysogens, PAO1(FIZ15) showed a fourfold increased agglutination with rabbit antiserum raised against PAO1 (Table 1), suggesting that FIZ15 prophage caused a change on the surface of the lysogenic bacteria. This result was confirmed when cross agglutination of both strains with mouse antisera against PAO1 and PAO1(FIZ15) was measured. Agglutination titers for each strain with the heterologous antiserum differed 16-fold (Table 1).

It has been reported that the O-antigen-specific D3 bacteriophage causes lysogenic conversion in *P. aeruginosa*, and the corresponding lysogen loses the ability to adsorb this phage [11], owing to a modification of the side chains of LPS consisting in the acetylation of position 4 of fucosamines, and the change in the bonding between the trisaccharide repeats from $\alpha 1 \rightarrow 4$ to $\beta 1 \rightarrow 4$ [14]. As neither FIZ15 nor D3 phages were able to grow on the lysogen for the other phage (data not shown), adsorption of both phages to the corresponding lysogens was measured. As expected, FIZ15 and D3 adsorbed well to PAO1, but none of them was able to adsorb to either lysogen (Table 2). This result suggests that both phages could be using and modifying the same receptor on the PAO1 surface, a conclusion further supported by the finding that a spontaneous FIZ15-resistant PAO1 mutant did not permit the adsorption of FIZ15 or D3 (PAO1/

Table 2. Adsorption of phages FIZ15*c* and D3*c* to wild type, lysogenized, and mutants of *Pseudomonas aeruginosa*

| Strain | % Adsorption of[a] | |
|---|---|---|
| | FIZ15*c* | D3*c* |
| PAO1 | 92.4 ± 0.8 | 93.0 ± 2.6 |
| PAO1(FIZ15) | 3.0 ± 0.3 | 1.7 ± 0.4 |
| PAO1(D3) | 2.8 ± 0.7 | 3.5 ± 0.9 |
| PAO1/FIZ15 | 2.5 ± 1.1 | 4.3 ± 0.3 |
| PIZ1 | 2.6 ± 1.6 | 4.8 ± 0.5 |
| PIZ3 | 81.5 ± 2.3 | ND[b] |
| PIZ31 | 93.0 ± 1.8 | 57.3 ± 2.2 |

[a] Exponential phase-grown bacteria were mixed with phage particles at a multiplicity of infection of 0.05. After standing at 37°C for 6 min without agitation, aliquots were removed and assayed for the amount of free phage by plating on PAO1. Results are mean ± standard deviation of three independent assays.
[b] ND, not done.

FIZ15 in Table 2). PAO1/FIZ15 did not liberate phages when picked onto a lawn of PAO1, showing that it does not possess the prophage.

**Increased resistance to serum and phagocytosis conferred by FIZ15 phage.** To determine whether the surface change on the bacteria conferred by the FIZ15 prophage was related to serum and phagocytosis susceptibility, these virulence properties were measured to PAO1 and its lysogen-carrying FIZ15. As can be seen in Table 3, PAO1(FIZ15) showed a 6- and 20-fold higher survival in 75% and 80% normal human serum, respectively, than PAO1. Susceptibility of PAO1(FIZ15) to phagocytosis by mouse peritoneal macrophages was two times lower than that of PAO1.

Phage receptor alteration and phagocytosis and serum resistance are related phenotypes, since PAO1/FIZ15 was as resistant to phagocytosis as PAO1(FIZ15) and showed a very high resistance to serum (Table 3). On the other hand, a spontaneous, serum-resistant PAO1 mutant (PIZ1) was unable to adsorb both FIZ15 and D3 phages (Table 2).

For reasons that are not readily apparent, another phenotype conferred by FIZ15 to PAO1 is resistance to streptomycin. Since streptomycin resistance also could be due to the FIZ15-related outer membrane modifications and is the most easily screened phenotype of those conferred by FIZ15 to PAO1, in order to obtain a FIZ15 mutant phage unable to cause lysogenic conversion, four independent streptomycin-sensitive mutants of PAO1(FIZ15) were obtained by EMS-mutagenesis. As all mutants exhibited a similar phenotype, the data of only one, designated PIZ3, are presented here. The lysogenic state of PIZ3 was tested by the failure of FIZ15*c* to form plaques onto lawns of PIZ3 and by spontaneous phage release onto lawns of PAO1. PIZ3 apparently reverted to the wild-type phenotype since it did not show increased survival in 75% serum (Table 3) and was able to adsorb FIZ15 phage (Table 2). The mutation(s) responsible(s) for these changes are located on the prophage genome, since the strain PIZ31, obtained by lysogenizing PAO1 with the phage obtained from PIZ3, behaved like PAO1 or PIZ3 (Tables 2–4). PIZ31 was immune to superinfection by FIZ15*c* and liberated phage when picked onto a lawn of PAO1.

Table 3. Killing by serum and phagocytosis of *Pseudomonas aeruginosa* strains

| Strain | % Survival in[a] | | % Phagocytosis[b] |
|---|---|---|---|
| | 75% NHS (R) | 80% NHS (R) | |
| PAO1 | 9.3 ± 1.8 (1.0) | 1.0 ± 0.1 (1.0) | 56.7 ± 6.0 |
| PAO1(FIZ15) | 57.9 ± 0.1 (6.2) | 20.7 ± 1.3 (20.7) | 29.4 ± 4.0 |
| PAO1/FIZ15 | 100 ± 0.0 (10.7) | 36.0 ± 1.0 (36.0) | 28.0 ± 0.2 |
| PIZ1 | 38.0 ± 9.0 (4.1) | 15.0 ± 3.0 (15.0) | ND[c] |
| PIZ3 | 8.5 ± 0.7 (0.9) | ND | ND |
| PIZ31 | 8.8 ± 0.7 (0.9) | ND | ND |

[a] $2 \times 10^6$ exponential phase-grown bacteria were mixed with human serum and PBS in a final volume of 0.5 ml to achieve 0%, 75%, and 80% serum. After incubation at 37°C for 3 h with agitation, the number of surviving bacteria was assayed by viable count. Mean ± standard deviation of three independent experiments, (R, mean % survival of each strain divided by that of PAO1).
[b] $2 \times 10^6$ mouse peritoneal macrophages were mixed with $2 \times 10^4$ bacterial cells in a final volume of 2 ml. After incubation for 1 h at 37°C with agitation, mixtures were centrifuged, and the number of surviving bacteria was assayed by viable count. Mean ± standard deviation of three independent experiments.
[c] ND, not done.

Table 4. Adhesion of *Pseudomonas aeruginosa* strains to human buccal epithelial cells (HBC)

| Strain | Bacteria per HBC[a] | % Adhesion[b] |
|---|---|---|
| None added | 1.8 ± 0.9 (19) | — |
| PAO1 | 21.5 ± 1.7 (11) | 100 |
| PAO1(FIZ15) | 35.6 ± 1.3 (11) | 165 |
| PAO1/FIZ15 | 71.0 ± 4.6 (5) | 330 |
| PAO1(D3) | 28.9 ± 0.2 (3) | 134 |
| PIZ1 | 47.0 ± 0.2 (3) | 218 |
| PIZ3 | 22.5 ± 1.9 (3) | 104 |
| PIZ31 | 21.1 ± 1.9 (3) | 98 |

[a] $5 \times 10^4$ HBC were mixed with $5 \times 10^6$ bacteria and incubated in agitation at 37°C for 2 h. The mixture was washed three times to discard unattached bacteria, stained with erythrosine B, and an aliquot was air-dried onto a glass slide. Preparation was counterstained with methylene blue, examined by light microscopy, and the number of bacteria adhered on 40 HBC was counted and averaged. Mean ± standard deviation (number of independent assays).
[b] Mean percentage adhesion of each strain divided by that of PAO1.

**Adhesion to human buccal epithelial cells.** Adhesion of *P. aeruginosa* to eukaryotic cells appears to be a complex phenomenon, since several bacterial components have been implicated in experimental adherence of this bacterium to distinct target cells [1, 8, 10, 13, 16, 18–20, 24]. Woods et al. suggested that the adhesion of *P. aeruginosa* to HBC is mediated solely by fimbriae [24]. However, preincubation of HBC with purified pili of *P. aeruginosa* did not inhibit completely the bacterial adherence [8].

The possibility that FIZ15 phage increases adhesion of PAO1 to human epithelial cells was tested. Indeed, PAO1(FIZ15) showed an adhesion value 1.6 times higher than that of PAO1 (Table 4). The FIZ15-resistant mutant, the serum-resistant mutant, and the PAO1(D3) lysogen also showed increased adhesion (3.3, 2.1, and 1.3 times, respectively, as compared with PAO1, Table 4). The lysogens for the non-receptor-converting mutant phage (PIZ3 and PIZ31) adhered to HBC in similar proportion as PAO1 (Table 4).

We have shown evidence that PAO1 strains lysogenic for FIZ15 or D3 are unable to adsorb both phages, suggesting that these phages use and modify the same receptor on the bacterial outer membrane. Probably this common receptor is the O-antigen. Phages D3 [11] and FIZ15 confer to their lysogens increased resistance to phagocytosis and increased adhesion to HBC; thus, they are closely related phages, perhaps belonging to the same immunity group, since D3*c* is unable to form plaques on PIZ31 (data not shown), although it adsorbs to this train, though to a lesser extent than FIZ15*c* (Table 2). This quantitative difference in adsorption of D3*c* and FIZ15*c* to PIZ31 suggests that each phage uses different regions of the O-antigen as its receptor. It has been reported that adhesion of PAO1 to HBC occurs by an energy-independent process, since heat-killed bacteria show adhesion values quite similar to those of live ones [24]. In agreement with this result, adhesion of PAO1 to HBC was not affected by the addition of an uncoupler of oxidative phosphorylation (2,4-dinitrophenol at 250 μg/ml) to the mixture bacteria–HBC during the adherence test, showing that adhesion of this strain does not require ATP synthesis (data not shown). Adhesion of PAO1(D3) was also not affected by the uncoupler (data not shown). On the contrary, PAO1(FIZ15) adhesion decreased with the uncoupler (from 35.6 to 12.7 bacteria/HBC). In addition, phage D3 did not confer streptomycin resistance. These data suggest that, although FIZ15 and D3 are closely related phages, they are not identical. These phages probably differ in their genetic contents, or integrate in distinct sites, or they cause different O-antigen modifications. Although the D3 phage has already been extensively characterized, this is the first report showing that this phage confers increased adhesion to HBC.

Apparently the phenotypes conferred by FIZ15 to PAO1 are due to the prophage-induced change at the level of its own receptor, as a FIZ15-resistant PAO1 mutant showed a high degree of adhesion to HBC, in addition to resistance to serum and phagocytosis; and, conversely, a spontaneous serum-resistant PAO1 mutant was unable to adsorb FIZ15 and adhere to HBC in greater numbers than its parental PAO1 strain. Furthermore, an EMS-induced streptomycin-sensitive mutant of PAO1(FIZ15), the PIZ3 strain, was able to adsorb FIZ15 phage and had lost increased serum resistance and adhesion to HBC, with the mutation(s) located in the phage chromosome, as can be inferred by the fact that, when this phage lysogenized PAO1 (strain PIZ31), it did not cause the conversion. Taken together, these results strongly suggest that surface alteration (probably LPS) is involved in resistance to serum and phagocytosis, and in adhesion to HBC. It is not known how many genes are involved in the O-antigen conversion mediated by D3, but it has been proposed that they may be three [14], encoding a β-fucosamine transferase, an inhibitor of α-fucosamine transferase and a fucosamine acetylase.

It has been shown that two accessory genes [7] of λ phage are involved in the conversion of *E. coli*: *bor* in resistance to serum [4] and *lom* in adhesion to HBC [23]. Both *lom* and *bor* are expressed in the lysogen and encode outer membrane proteins, which are present in low numbers per cell (about 100 copies of Lom and 2000 of Bor), a feature used to theorize that Bor may protect against serum killing through an enzymatic rather than structural role in the outer membrane [4]. It is noticeable that both λ and D3 phages show remarkable similarities in the genomic organization of their respective immunity regions, and a strong repressor-insensitive promoter has been located in the rightmost end of the D3 genome, in a position comparable to that reported for the lambda *bor* gene [4, 9].

All the bacterial surface changes conferred by λ or other phages may play an important role in the natural selection of phage and bacteria in animal hosts. Because of this selective advantage, *bor* and *lom* genes may be widespread among temperate phages. It will be necessary to look for *bor*- and *lom*-like genes in FIZ15 and D3 to ascertain whether the genetic bases of conversion in *E. coli* and *P. aeruginosa* are similar.

**ACKNOWLEDGMENTS**

We thank G. Hernández and S. Pérez for their technical assistance, and L. Kameyama for critical reading of the manuscript. This investigation was supported by DGAPA, UNAM (IN-206597).

## Literature Cited


1. Baker NR, Minor V, Deal C, Shahrabadi MS, Simpson DA, Woods DE (1991) *Pseudomonas aeruginosa* exoenzyme S is an adhesin. Infect Immun 59:2859–2863
2. Barksdale L, Arden SB (1984) Persisting bacteriophage infections, lysogeny, and phage conversions. Annu Rev Microbiol 28:265–299
3. Barondess JJ, Beckwith J (1990) A bacterial virulence determinant encoded by lysogenic coliphage λ. Nature 346:871–874
4. Barondess JJ, Beckwith J (1995) *bor* gene of phage λ, involved in serum resistance, encodes a widely conserved outer membrane lipoprotein. J Bacteriol 177:1247–1253
5. Bodey GP, Bolivar R, Fainstein V, Jadeja L (1983) Infections caused by *Pseudomonas aeruginosa*. Rev Infect Dis 5:279–313
6. Cheetman BF, Katz ME (1995) A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. Mol Microbiol 18:201–208
7. Court D, Oppenheim AB (1983) Phage lambda's accessory genes. In: Hendrix RW, Roberts JW, Stahl FW, Weisberg RA (eds) Lambda II. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory, pp 251–277
8. Doig P, Sastry PA, Hodges RS, Lee KK, Paranchych W, Irvin RT (1990) Inhibition of pilus-mediated adhesion of *Pseudomonas aeruginosa* to human buccal epithelial cells by monoclonal antibodies directed against pili. Infect Immun 58:124–130
9. Farinha MA, Allan BJ, Gertman EM, Ronald SL, Kropinski AM (1994) Cloning of the early promoters of *Pseudomonas aeruginosa* bacteriophage D3: sequence of the immunity region of D3. J Bacteriol 176:4809–4815
10. Hata JS, Fick RB (1991) Airway adherence of *Pseudomonas aeruginosa*: mucopolysaccharide binding to human and bovine airway proteins. J Lab Clin Med 117:410–422
11. Holloway BW, Cooper GN (1962) Lysogenic conversion in *Pseudomonas aeruginosa*. J Bacteriol 84:1321–1324
12. Holloway BW, Krishnapillai V (1975) Bacteriophages and bacteriocins. In: Clarke PH, Richmond MH (eds) Genetics and biochemistry of *Pseudomonas*. London: Wiley, pp 99–132
13. Irvin RT, Doig P, Lee KK (1989) Characterization of the *Pseudomonas aeruginosa* pilus adhesins: confirmation that the pilin structural protein subunit contains a human epithelial cell-binding domain. Infect Immun 57:3720–3726
14. Kuzio J, Kropinski AM (1983) O-antigen conversion in *Pseudomonas aeruginosa* PAO1 by bacteriophage D3. J Bacteriol 155:203–212
15. Miller JH (1980) Experiments in molecular genetics. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory, p 138
16. Ramphal R, Sadoff C, Pyle M, Silipigni JD (1984) The role of pili in the adherence of *Pseudomonas aeruginosa* to injured tracheal epithelium. Infect Immun 44:38–40
17. Reid G, Brooks HGL, Bacon DF (1983) In vitro attachment of *Escherichia coli* to human uroepithelial cells: variation in receptivity during the menstrual cycle and pregnancy. J Infect Dis 148:412–421
18. Saiman LK, Ishimoto K, Lory S, Prince A (1990) The effect of piliation and exoproduct expression on the adherence of *Pseudomonas aeruginosa* to respiratory epithelial monolayers. J Infect Dis 161:541–548
19. Sato H, Okinago K (1987) Role of pili in the adherence of *Pseudomonas aeruginosa* to mouse epidermal cells. Infect Immun 55:1774–1778
20. Sexton M, Reen DJ (1992) Characterization of antibody-mediated inhibition of *Pseudomonas aeruginosa* adhesion to epithelial cells. Infect Immun 60:3332–3338
21. Timmis KN, Manning PA, Eckarti C, Timmis JK, Moll A (1981) Serum resistance in *E. coli*. In: Levy SE, Clowes RC, Koenig EL (eds) Molecular biology, pathogenicity and ecology of bacterial plasmids. New York, London: Plenum Press, pp 133–144
22. Vaca PS, Arce J, Oliver G, Arenas D, Argüello F (1989) FIZ15 bacteriophage increases the adhesion of *Pseudomonas aeruginosa* to human buccal epithelial cells. Rev Lat Amer Microbiol 31:1–5
23. Vaca PS, García O, Paniagua GL (1997) The *lom* gene of bacteriophage λ is involved in *Escherichia coli* K12 adhesion to human buccal epithelial cells. FEMS Microbiol Lett 156:129–132
24. Woods DE, Straus DC, Johanson WG, Berry VK, Bass JA (1980) Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect Immun 29:1146–1151