STIMULATION OF PILUS FORMATION IN PSEUDOMONAS AERUGINOSA BY RNA BACTERIOPHAGE ADSORPTION

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SUMMARY

It is shown that the number of pili on P. aeruginosa increases when organisms are incubated with RNA phage or anti-pilus serum. To ascertain if this was due to the stimulation of pilus synthesis, pili were first coated with antibodies, then incubated with RNA phage. Additional pili without antibodies were produced, indicating that synthesis had been stimulated. Further experiments suggested a model for RNA phage penetration with the stimulated growth and retraction of pili.

INTRODUCTION

Electron microscopy has shown that there are many more pili on cells of P. aeruginosa to which RNA phages have been adsorbed than on untreated organisms (3). It was thought that the phages, attached to the sides of the pili, prevented retraction during negative staining, so producing an apparent increase in numbers. An alternative explanation, the stimulation of pilus growth by the adsorbed phages, was thought unlikely. However, when pilus retraction was demonstrated (1,2), an RNA phage penetration sequence involving both phenomena was not ruled out. This paper describes experiments which show that phage-stimulated pilus synthesis does occur.

MATERIALS AND METHODS

The RNA phage used was PP7 (4). P. aeruginosa strains PAO1 (PP7-sensitive) and PAO1264 (PP7-resistant) were kindly supplied by Prof. B. W. Holloway (5). Bacteria and phage were grown by

standard methods in Oxoid nutrient broth. Anti-pilus serum was made by injecting a cell-free pilus suspension into rabbits (2).

The experiments (using electron microscopy) were based on counting the pili, which are polar, on 100 poles and calculating the average pili/pole. Pili were labelled in suspension by shaking 5-6 hr shake-culture samples with antiserum, or PP7 suspension at a multiplicity of infection (m.o.i.) of 1000. Carbon-coated grids were floated on or immersed in the mixture to mount the cells after incubation. Negative staining in 0.75% sodium phosphotungstate followed. Pili were also labelled on grids (6) by mounting the bacteria, floating the grids on antiserum or PP7 suspension, followed by negative staining.

RESULTS AND DISCUSSION

The first experiment shows that increased piliation on PAO1, similar to that obtained with PP7-labelling (3) can be produced with antibodies (Table 1). After cells are incubated with antibodies or PP7, there are many more pili than on unlabelled cells.

TABLE 1. THE AVERAGE NUMBER OF PILI/POLE ON P. AERUGINOSA STRAIN PAO1 LABELLED WITH ANTIBODIES OR BACTERIOPHAGE PP7

Labelling method	Pili/pole		
	Unlabelled	Antibody-labelled (dilution 1:30)	PP7-labelled (m.o.i. 1000)
In suspension (10 min. at 37°)	0.1	4.1	5.6
On grid (15 min. at 37°)	1.0*	3.5	4.2

*Unlabelled cells were mounted on the grid which was floated for an equivalent time on plain broth.

This happens when organisms are labelled either in suspension or after mounting on grids, though the increase is not quite as marked with grid-labelling. This is important for subsequent experiments because the grid method only can be used for labelling over short periods (a few seconds). Lawn and Meynell obtained a greater increase using antibodies with Escherichia coli I-like sex pili in suspension (7). The increase with grid-labelling is not merely due to further incubation on broth since bacteria treated in this way (Table 1*) showed only a small increase in piliation relative to that produced with either phage or antibodies. It is possibly due to a few pili growing

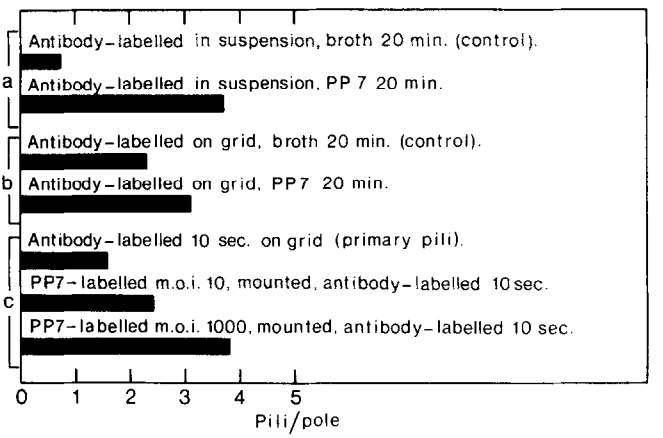

Fig. 1. Pili/pole on P. aeruginosa strain PAO1 after various labelling treatments. (a) Antibody-labelled in suspension with anti-pilus serum (1:50 dilution) at room temperature with agitation for 20 sec. Grids were immersed and moved about for 45 sec. to mount cells. They were then floated on broth (controls) or PP7 at 1 X 10^{12} p.f.u./ml for 20 min. at 37°C and negatively stained. (b) Bacteria were antibody-labelled on grids after mounting by flotation (see text), then they were floated on anti-pilus serum in broth (1:50) for 45 sec. followed by PP7 treatment as above. (c) Bacteria for primary pili were mounted by flotation then floated on 1:1 anti-pilus serum in broth for 10 sec. and negative stained. In addition, bacteria were mixed with PP7 at a nominal m.o.i. of 10 (actually gave 1-2 virions per pole adsorbed to pili) and also 1000. After 10 min. at 37°C, cells were mounted by flotation (10 min.) and antibody-labelled for 10 sec. as above.

out in contact with the surface of the carbon film which provides a different physical environment. The question is whether the increases with phage and antibodies are due to the prevention of pilus retraction during negative staining, the stimulation of new pili, or both.

To determine whether pilus growth was stimulated by PP7 adsorption, cells were antibody-labelled in suspension and on grids, both methods visibly coating all pili with antibodies. Grids were then floated on PP7 (1×10^{12} p.f.u./ml) and on broth as a control, for 20 min. at 37°C. The average pili/pole for each grid sample is shown in Fig. 1a,b. Additional unlabelled pili were obtained by floating on broth (Fig. 1b). Floating on PP7 produced additional pili labelled with phage, not antibodies (Fig. 1a,b PP7-treated). All samples showed approximately equal numbers of antibody-coated pili. Since there were no pili without antibodies before the grids were floated on broth or PP7, the additional ones must have been generated at this stage of the experiments. Taken together, the results so far (Table 1, Fig.1) indicate that PP7 and antibodies stimulate pilus growth.

The question of the very low piliation observed with unlabelled cells (Table 1) will now be considered. Since only about 10% have pili, it must be asked how phage or antibodies can stimulate further pili with none for initial adsorption. Cell penetration (7) is unlikely, particularly for a 25 nm PP7 virion. The presence of about 1 pilus/pole which retracts on drying is more plausible. To detect them, cells were grid-labelled with 1:1 anti-pilus serum in broth for 10 sec. at room temperature to prevent retraction but not to allow time for

further pili to grow. Two determinations gave 2·65 and 1·55 pili/pole. Antibody-labelling in suspension usually gave similar results (2). Thus there appear to be 1-2 primary pili/pole to which antibodies or phage adsorb to stimulate more.

To determine whether one or two phages could cause all the potential pili of a pole to grow, cells were PP7-labelled in suspension at low and high m.o.i. (Fig. 1c), mounted on grids, then labelled for 10 sec. with anti-pilus serum. The average count represents primary + induced pili. Fig. 1c shows that it takes more than one or two virions to produce maximum piliation.

Further information about the complex effects of phage adsorption was obtained as follows. Grids with cells were floated on PP7 suspension at 37°C for different times, then negative stained. Pili/pole was plotted against time (Fig. 2). To interpret the results, the time taken to label all the pili was determined using strain PAO1264, whose pili do not retract on PP7 adsorption (1,2). Grids with bacteria were floated on

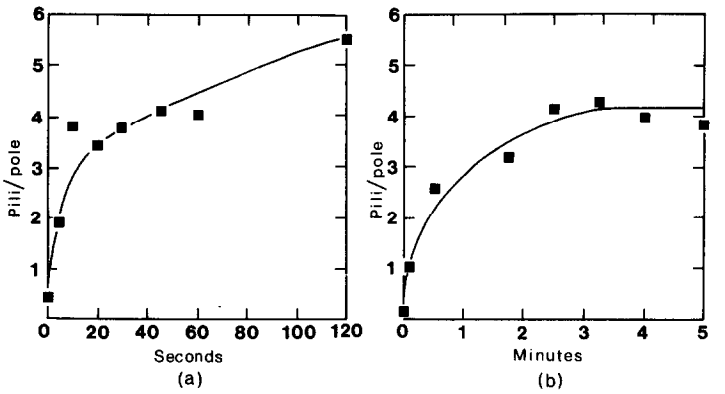

Fig. 2. Bacteria were mounted by floating grids on samples from two cultures. Grids were then floated for various times on 1 X 10^{12} p.f.u./ml PP7 suspension at 37°C, negative stained, and the pili/pole counted. (a) Change in piliation over 2 min. (first culture), (b) over 5 min. (second culture).

phage suspension for specific times, and pili with and without virions were counted (Table 2). The maximum visible labelling (80%) was reached after 10 sec., the remaining 20% of the pili probably having their attached virions obscured by the cell. In Fig. 2a, all primary pili would be labelled in 10 sec. The 2.8 pili/pole at this time is close to other values for primary pili given above. The average rate of pilus synthesis after

TABLE 2. PERCENTAGES OF PILI WITH VISIBLE ADSORBED PP7 VIRIONS AFTER LABELLING STRAIN PAO1264 ON GRIDS FOR VARIOUS TIMES

Time (sec.)	% Pili with phage
5	58
10	82
15	80
300	82

20 sec. is 48 sec./pilus on the grid. Maximum piliation is reached after 3 min. (Fig. 2b), much longer than is required to label all the pili. This again shows that PP7 increases piliation and does not merely prevent the retraction of existing pili, otherwise the full complement would be reached in 10 sec.

To test the effect of temperature on PP7-stimulated pilus growth, culture samples were cooled to different temperatures then mixed with PP7 to label the cells in suspension. Pili/pole was plotted against temperature (Fig. 3). Pilus synthesis started around 10°C and reached maximum efficiency at 25°C. The low piliation below 5°C suggests that retraction was set off by

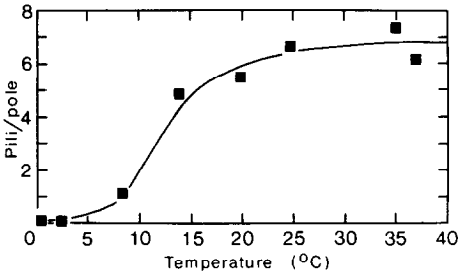

Fig. 3. Culture samples were cooled to given temperatures for ½-1¼ hr (according to temperature) to stabilize metabolic activity, then mixed with PP7 at 1 X 10^{12} p.f.u./ml (m.o.i. 1000) for about 10 min. at the same temperatures. Grids were then floated on the mixture and negative staining carried out at the same temperature. The graph shows changes in piliation with temperature. Lawn and Meynell found similar changes with E. coli I-like sex pili labelled with antibodies (7).

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the cooling prior to labelling; PP7-labelling at 0°C was efficient and should have maintained the primary pili (about 2 pili/pole).

CONCLUSION

A complex model for RNA phage penetration is proposed (Fig. 4). Experimental evidence supports all steps save (e) and

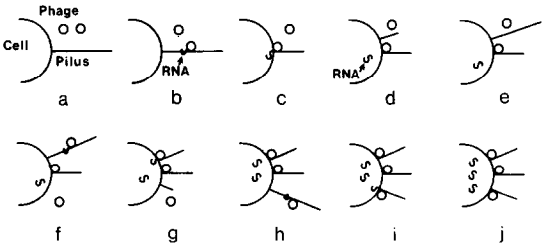

Fig. 4. Model for RNA phage adsorption and penetration. (a) Single pilus with unadsorbed phages, (b) one phage adsorbs and ejects short length of RNA onto the pilus surface, (c) the pilus retracts and the RNA penetrates the cell, (d) a second pilus starts to grow, (e) it reaches maximum length, (f) a virion adsorbs to it, (g) retraction occurs, (h), (i), (j), the cycle is repeated. Experimental evidence (1,2,4) supports all save steps (e) and (h) which are discussed.

(h), where pili grow to their full length before a virion can adsorb, but this seems probable. If a virion remained irreversibly attached before full length was attained, subsequent retraction would leave only a small fraction of the pilus protruding. This would average over a number of cells at much less than half the unretracted length already observed (1,2). It could well be the process of retraction triggered by phage adsorption, rather than adsorption alone, which stimulates further pilus growth. Antibodies could provide the same signal even though they prevent any actual shortening of the pili.

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