THE OCCURRENCE OF PILI ASSOCIATED WITH A PLASMID OF THE W COMPATIBILITY GROUP

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SUMMARY

Using electron microscopy, it was found that the acquisition of the W group drug resistance plasmid S-a by normally pilus-less bacterial strains was associated with the appearance of pili. The loss of drug resistance markers in presumed R^- revertants was accompanied by a loss of pili. The numbers of pili present on transconjugant strains of the three bacterial species tested were 3.2 pili/cell for Salmonella typhimurium, and 0.19 pili/cell for both Escherichia coli and Pseudomonas aeruginosa. Negatively stained pili were about 12 nm thick and varied in length from 23 nm to 3,370 nm.

INTRODUCTION

Recently, a great deal of interest has been centred on drug resistance plasmids of the P, N and W compatibility groups (5, 6, 7) because they are able to transfer intergenerically to a wide range of bacterial hosts. The three groups are also related in that their DNA molecules show a relatively high degree of homology (8). The way in which these plasmids are transferred from donor to recipient is not known, but in the F and I compatibility groups it has been demonstrated that pili are required (9, 12). It is thus important to ascertain whether or not pili are determined by P, N and W plasmids. While RP1 (P group) pili have been clearly demonstrated (1), an exhaustive study of cells carrying N group plasmids using

electron microscopy has failed to reveal any filaments (4). So far, bacteria harbouring W plasmids have not been studied for pilus formation. In the present work, the W group R factor S-a (5), which determines resistance to the drugs chloramphenicol, streptomycin, ampicillin and sulphonamides, and which confers sensitivity to the cell wall bacteriophage PR4 (3, 15), is shown to have associated pili.

MATERIALS AND METHODS

Bacteria were very kindly supplied as follows. E. coli CR34 from R. H. Olsen, E. coli J53-1 S-a and P. aeruginosa PU21 S-a from G. Jacoby, and S. typhimurium LT2 strain SQ1139 from R. Bradley. V. Stanisich isolated phage PR4 (15), which plaques on strains carrying a plasmid of either of the P, N or W compatibility groups.

J53-1 carries "common" pili, which would obscure any S-a pili in the electron microscope, hence the plasmid was transferred to strains devoid of pili. Rifampicin resistant mutants of E. coli CR34 and S. typhimurium SQ1139 were selected and used as recipients in 2 hour broth matings with E. coli J53-1 S-a. Transconjugants were selected on nutrient agar plates containing 100 μg/ml rifampicin and 25 μg/ml chloramphenicol, and further checked for the presence of S-a by their sensitivity to phage PR4. For the intergeneric cross, the female was incubated for 30 minutes at 50°C immediately prior to mating to increase the frequency of transfer (11). A derivative of P. aeruginosa strain PU21 S-a lacking "common" pili (PU21/M6 S-a) was isolated by selecting for resistance to the pilus phage M6 (2). Presumed S-a revertants were selected by resistance to phage PR4.

Samples of E. coli and S. typhimurium strains were prepared for electron microscopy from overnight soft agar lawns. A few drops of 0.1M ammonium acetate solution were placed on the plate, and the soft agar was gently broken up with a loop. A carbon-coated support grid was touched onto the resulting suspension, then held under a lamp until the edges were just beginning to dry. The grid was finally washed in ammonium acetate and negatively stained with 0.2% sodium phosphotungstate. P. aeruginosa cells were mounted by floating a grid on the bacterial suspension for about 1 minute, followed by washing and negative staining. About 100 isolated random cells were scored for pili in the electron microscope.

RESULTS AND DISCUSSION

Table 1 shows the numbers of pili found on various

TABLE 1

PILUS COUNTS ON STRAINS WITH AND WITHOUT THE PLASMID S-a

Strain	Description	Pili/cell	% cells piliated
E. coli			
CR34 rif^r	Rifampicin resistant (pil^- fla^+)	0	0
CR34 S-a	R^+ transconjugant from J53-1 S-a X CR34 rif^r	0.19	13
P. aeruginosa			
PU21/M6 S-a	M6-resistant (pil^- fla^+)	0.19	18
PU21/M6 rev 1	R^- revertant from PU21/M6 S-a	0	0
S. typhimurium			
SQ1139 rif^r	Rifampicin resistant (pil^- fla^-)	0	0
SQ1139 S-a	R^+ transconjugant from J53-1 S-a X SQ1139 rif^r	3.16	80
SQ1139 rev A	R^- revertant from SQ1139 S-a	0	0

Note: pil refers to "common" pili and fla to flagella.

strains. It can be seen that only those carrying the plasmid are piliated. None were present on background strains (CR34 rif^r, SQ1139 rif^r) or R^- revertants (PU21/M6 rev 1, SQ1139 rev A). A point of obvious interest is that SQ1139

carried about fifteen times the number of pili found on CR34 S-a and PU21/M6 S-a, implying a more efficient expression of the plasmid by S. typhimurium. SQ1139 R46 (N group) was therefore examined in the electron microscope for the possible presence of N pili; none were found, confirming the results of Brodt et al (4). It was also noted that, with revertant strains, the loss of pili coincided with a loss of drug resistance. Thus the pili and the plasmid are clearly associated. However, it might be argued that a portion of the J53-1 chromosome determining "common" pili had been mobilized by the plasmid, but the pili associated with S-a are easily distinguished by their morphology.

The remote possibility that the pili are filamentous phage virions associated with S-a has been ruled out for the following reasons. Firstly, the longest pili (3,370 nm) were about twice as long as the longest filamentous phages. Secondly, no infectivity could be found associated with J53-1 S-a bacteria and SQ1139 S-a culture fluids. Thirdly, logarithmic phase shake cultures of all three R^+ strains revealed no pili on the cells, or detached from them, in the electron microscope; a filamentous phage would be produced in large numbers (about 10^{12} plaque forming units/ml). While the genes responsible for S-a pili are undoubtedly associated with the plasmid, it is impossible to state unequivocally that they are carried by it.

Cells from overnight soft agar lawns gave the largest numbers of pili so that the data in Table 1 was obtained from such cultures. Pili were found in lesser numbers on cells

from overnight static broth cultures, and bacterial lawns spread on nutrient plates, also incubated overnight. They were completely absent on logarithmic phase shake cultures.

The number of pili on individual cells was most frequently 1 in the cases of P. aeruginosa and E. coli, but was variable with S. typhimurium, the maximum observed on a single cell being 24. The lengths of the pili, measured from the cell edge, varied between about 23 nm and 3,370 nm, with an average of about 450 nm, which did not vary greatly from one species to another. The thickness of the pili was also similar for all three species at about 12 nm. They are thus twice as thick as the "common" pili of P. aeruginosa. The pili of the related P group plasmid RP1 are halfway between at 9 nm (1). While S-a pili are similar in appearance to F pili, they are thicker. The average for F pili is 9.5 nm (10). As expected, the pili were not apparently related to F pili since none of the strains plaqued F-specific RNA and filamentous bacteriophages.

The appearance of the pili on the three bacterial species is illustrated in Figs. 1-3. They are generally similar to F pili but without any form of terminal knob or other appendage (10). There is no indication of any helical structure or cross-striations. It was noted that the pili were evenly distributed around the cells of all three species. With P. aeruginosa, RP1 pili show a marked tendency to appear at the poles of the cell (1). This is not the case with S-a-associated pili.

It is interesting to note that pili have now been demonstrated for plasmids in two of the related compatibility

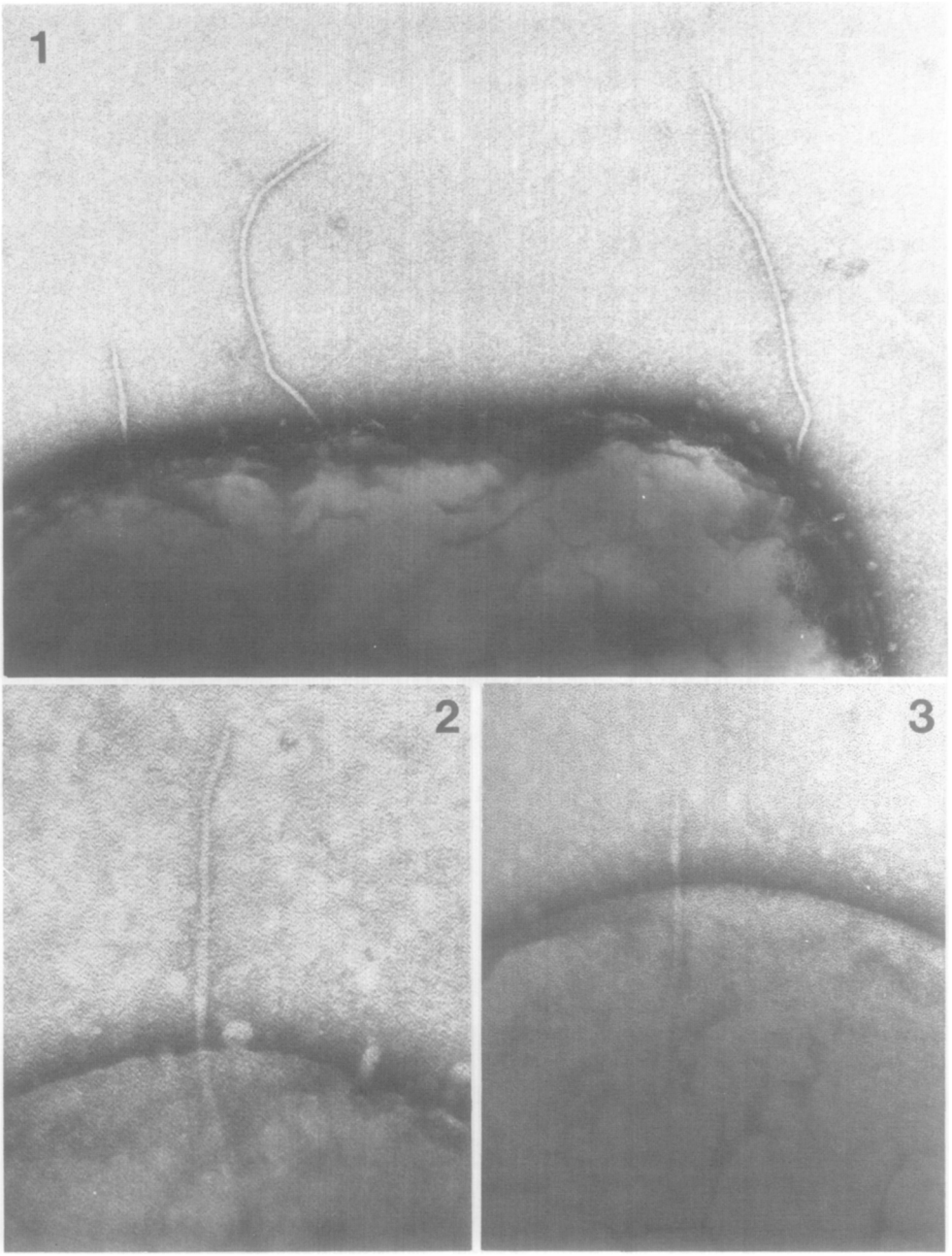

Fig. 1. Pili associated with the drug resistance plasmid S-a on S. typhimurium SQ1139 S-a, X 77,000. Fig. 2. A similar pilus on P. aeruginosa PU21/M6 S-a, X 123,000. Fig. 3. A short pilus on E. coli CR34 S-a, X 123,000.

groups P, N and W. Plasmids of group P have a specific RNA phage PRR1 (13), but there is no such phage for the N or W groups, and PRR1 does not adsorb to bacteria carrying N or W plasmids (3). This suggests that P and W pili are different. The only bacteriophages known to be associated with W plasmids are PR4, used here as a marker for transconjugants, and the similar phage PRD1 (14). Both adsorb to the bacterial cell wall and not to pili (1, 14). Attempts to isolate a W pilus phage are in progress since it would obviously be very valuable in determining whether or not W-associated pili are involved in conjugation. More detailed genetic and immunological studies on the pili described here are in progress.

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