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Isolation and properties of mutants of the FP2 sex factor of *Pseudomonas aeruginosa*

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SUMMARY

Two new types of mutants of the FP2 sex factor have been isolated in males of *P. aeruginosa* strain PAT. Males harbouring FPd mutants are unable to mediate either sex factor or host chromosome transfer, although they retain the exclusion and precipitation characteristics of wild-type males. Males harbouring the FPs mutant apparently have an altered cell surface as indicated by their loss of precipitability, and although their donor properties are similar to those of wild-type males they show a slightly reduced conjugal fertility.

A previously described sex factor mutant FP* (Stanisich & Holloway, 1972) can be transferred to males carrying either the FPs or FPd factors, and the heterozygous strains produced show restoration of wild-type properties, i.e. wild-type precipitation characteristics of the FPs mutants, and transferability of FPd mutants respectively. This suggests that the mutations of the FPs and FPd factors are recessive to the alleles carried by the FP* factor. The ability to produce such heterozygous strains supports the view that at least two copies of the FP2 factor occur in strain PAT males.

1. INTRODUCTION

The FP2 sex factor of *Pseudomonas aeruginosa* shares with the sex factors of the Enterobacteria the ability to promote its own transfer, as well as that of host chromosome, to recipient bacteria (Holloway, 1969; Holloway, Krishnapillai & Stanisich, 1971). Although recombinant formation between strains harbouring FP2 occurs at low frequency (10^{-7} to 10^{-9}/donor cell), a mutant male of strain PAT has been isolated which forms recombinants with both donor and recipient bacteria at similar frequencies (10^{-3} to 10^{-5}/donor cell) (Stanisich & Holloway, 1972). This property of the mutant donor, termed 'extended fertility', is due to a mutation of the sex factor (FP2-2) and strains harbouring it are given the phenotypic designation FP*. The mutant FP2-2 can be transferred to both donor and recipient bacteria, and in the former instance results in the formation of males heterozygous for FP2-2 and the wild-type factor FP2. Such heterozygotes display the extended fertility phenotype indicating that the mutation of FP2-2 is dominant to the wild-type allele of FP2.

This paper describes the isolation of new mutants of the FP2 sex factor in

Table 1. *Bacterial strains*

Strain	Genotype	Mating phenotype due to sex factors carried	Parent(s)	Reference
PAT900	*his-404*, *str-1100*	FP⁻	PAT404	Stanisich & Holloway, 1972
PAT404	*his-404*, *str-1100*, FP2	FP+	PAT3	Stanisich & Holloway, 1969*b*
PAT906	*his-404*, *str-1100*, FP2-2	FP*	PAT904	Stanisich & Holloway, 1972
PAT967	*met-1105*	FP⁻	PAT964	Stanisich & Holloway, 1972
PAT973	*met-1105*, FP2	FP+	PAT967, PAT404	Stanisich & Holloway, 1972
PAT974	*met-1105*, FP-2	FP*	PAT967, PAT906	This paper
PAT993	*his-404*, *str-1100*, FP2-3	FPd	PAT900, PAT973	This paper
PAT996	*his-404*, *str-1100*, FP2-6	FPd	PAT900, PAT973	This paper
PAT998	*his-404*, *str-1100*, FP2-8	FPd	PAT900, PAT973	This paper
PAT999	*his-404*, *str-1100*, FP2-9	FPs	PAT900, PAT973	This paper
PAT1201	*his-404*, *str-1100*, FP2	FP+	PAT900, PAT458	This paper
PAT1220	*his-404*, *str-1100*, FP2-11	FPd	PAT900, PAT973	This paper
PAT1221	*his-404*, *str-1100*, FP2-11, FP2-2	FP*	PAT1220, PAT974	This paper
PAT1222	*his-404*, *str-1100*, FP2, FP2-2	FP*	PAT404, PAT974	This paper
PAT1223	*his-404*, *str-1100*, FP2-9, FP2-2	FP*	PAT999, PAT974	This paper
PTO13	*trp-6*, *chl-4*, FP2	FP+	PAT420, PAO286	Stanisich & Holloway, 1969*b*
PTO30	*trp-6*, *chl-4*	FP⁻	PTO13	Stanisich & Holloway, 1969*b*
PAO381	*leu-38*, *str-7*, FP2	FP+	PAO98, PTO13	Stanisich & Holloway, 1969*a*
PAO38	*leu-38*	FP⁻	Strain PAO	Holloway, 1955

PAO strains were formerly designated strain 1, PAT as strain 2.

PTO strains are recombinant lines derived from PAT (FP+) × PAO (FP⁻) matings.

Antibiotic resistance gene: *str*, streptomycin; *chl*, chloramphenicol.

Amino acid requirement genes: *his*, histidine; *met*, methionine; *trp*, tryptophan; *leu*, leucine.

FP2 = strain PAT sex factor.

FP2-2, FP2-3, etc., are sex factor mutants derived from FP2.

Mating phenotypes: FP⁻ = recipient; FP+ = wild-type male; FP* = male with 'extended fertility' phenotype; FPd = male with reduced transfer ability; FPs = male lacking the precipitation phenotype. These FP designations are used to represent either the mating properties of the strain, i.e. its recombinant formation with indicator male and female bacteria, or the particular type of sex factor carried by the strain, i.e. wild-type or mutant.

P. aeruginosa strain PAT. These have been recognized by the inability of males harbouring such mutants to mobilize either sex factor or host chromosome transfer, a criterion which has been employed for the isolation of F and R factor mutants in the Enterobacteria (Ohtsubo, Nishimura & Hirota, 1970; Achtman, Willetts & Clark, 1971) in addition to the selection available using insensitivity to sex specific bacteriophages (Hirota, Fujii & Nishimura, 1966; Nishimura, Ishibashi, Meynell & Hirota, 1967; Ohtsubo *et al.* 1970). These mutants of FP2 have been characterized by a variety of genetic tests including a 'dominance test' using the FP* mutant FP2-2, as substitute for the wild-type sex factor. This test is similar to that described for the analysis for *Flac* mutants in *Escherichia coli* (Finnegan & Willetts, 1971; Achtman, Willetts & Clark, 1972).

2. MATERIALS AND METHODS

Bacterial strains. The bacterial strains used were as shown in Table 1.

Media. Nutrient Broth (NB), Heart Infusion Broth (HIB), Nutrient Agar (NA) and Minimal Medium (MM) were as previously described (Stanisich & Holloway, 1972).

Mating on the plate, determination of mating type by replica plating, and *infectious transfer of sex factor* were as previously described (Stanisich & Holloway, 1972).

Mutagenesis of donor bacteria. Late exponential phase cultures of donor bacteria in 0·1 M citrate buffer (pH 5) were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (250 μg/ml) for 15 min at 37 °C. The culture was then centrifuged, washed once and the cell pellet resuspended in NB. Five ml of this suspension was used as inoculum to 30 ml NB, and this incubated overnight at 37 °C with aeration.

Infectious transfer of sex factor from the mutated culture to a recipient population used overnight cultures diluted in fresh broth to a density of *c.* 10^8 cells/ml and with an input ratio of *c.* 50:1 in favour of the donor strain. After 4 h incubation at 37 °C without shaking, the mixture was diluted and plated on to selective MM to isolate derivatives of the recipient strain, and these were further tested for inheritance of FP2.

Determination of precipitability. Late exponential phase cultures in NB (*c.* 10^9 cells/ml) were centrifuged and the cell pellets resuspended in one half the volume of citrate buffer at various pH values. The suspensions were maintained at 37 °C for 30 min before the precipitation of the bacteria was determined.

3. RESULTS

(i) *Isolation of strains harbouring mutant sex factors*

Sex specific phages for *P. aeruginosa* strains carrying the FP2 sex factor have not yet been reported, consequently selection for male strains harbouring mutant sex factors involves the screening of donor populations for bacteria with an altered ability to transfer either sex factor or host chromosome to recipient lines.

There is evidence to suggest that there are at least two copies of FP2 per cell in strain PAT (Stanisich & Holloway, 1972). Therefore the isolation of mutant males from mutagenized populations would not be expected unless the mutations involved were dominant to the wild-type alleles carried by unmutated sex factors in the same cell. This difficulty can be overcome by transferring the mutagenized sex factors to an FP$^-$ recipient strain. Although it is possible that in any particular mating event more than one FP2 factor may be transferred, a proportion of the recipients would be expected to receive only a single sex factor, and it is amongst

Table 2. *Mating phenotypes among recipient populations infected with sex factors from a mutated donor strain*

Recombinant formation in matings with			
An FP$^+$ donor strain	An FP$^-$ recipient strain	*c.* frequency/ 100 clones tested	Mating phenotype
−	+	95·0	FP$^+$
+	−	3·0	FP$^-$
+	+	1·8	FP*
−	−	0·2	FPd

PAT900 (*his-404*, *str-1100*) was used as a recipient of sex factor from an NG treated population of PAT973. Derivatives of the recipient were isolated on histidine supplemented MM and 1500–2000 colonies tested by replica plate mating for recombinant formation with indicator male (PAT973) and female (PAT967) bacteria. Plates were incubated at 37 °C for 48 h and recombinant formation scored as + or −.

these that recessive as well as dominant mutations of FP2 would be isolated. This transfer procedure also reduces the possibility of isolating chromosomal rather than sex factor mutations affecting donor functions. Mutants of the former type have been reported in *P. aeruginosa* strain PAO (Loutit, 1969; Pemberton & Holloway, 1972), and show either an increased or decreased conjugal fertility.

To isolate sex factor mutants in strain PAT, the male strain PAT973 (*met-1105*, FP2) was treated with nitrosoguanidine and the mutagenized population used as donor of sex factor in a mating with the recipient strain PAT900 (*his-404*, *str-1100*) (see Materials and Methods). After mating, the mixture was plated on to histidine supplemented MM to isolate clones of the recipient population, and these were then tested for recombinant formation with indicator male (FP$^+$) and female (FP$^-$) bacteria using the replica plating technique. In this test clones derived from recipients inheriting a wild-type sex factor can be distinguished from uninfected bacteria by their ability to form recombinants with the indicator FP$^-$ strain, but not with the indicator FP$^+$ strain (since matings between two wild-type male strains are of low fertility). In addition, bacteria inheriting a mutant sex factor can be distinguished from those with the wild-type factor by their altered mating pattern when their responses with both indicator strains are compared.

Table 2 shows the frequency of the four mating-type classes detected amongst the PAT900 derivatives tested. The two most frequent classes represent the mating patterns expected of wild-type male and female strains respectively, while the remaining classes show the patterns predicted for strains harbouring mutant sex factors. Those clones (1·8 %) which showed recombinant formation with both the indicator strains and which were therefore suspected to be mutants of the 'extended fertility' type (i.e. FP*), were found on purification to be mixtures of wild-type donor and recipient bacteria. From four independent experiments, a total of 15 clones were isolated which did not yield recombinants with either of the indicator strains. Of these only eight (0·2 %) were found to have a recombination frequency at least 100-fold lower than that obtained with the wild-type PAT404. These males (FPd phenotypes) apparently harbour mutant sex factors which cannot mediate recombinant formation in conjugation. Four such non-sibling FPd strains were given the sex factor numbers FP2-3, FP2-6, FP2-8 and FP2-11, and their properties were studied in detail. One of the remaining strains carrying the mutant factor FP2-9 showed only a slight reduction in recombinant formation, but differed from the other males in its precipitation characteristics (FPs phenotype; see below).

(ii) *Characterization of strains harbouring mutant sex factors*

(*a*) *Recombinant formation*

The ability of the PAT900 FPd and FPs derivatives to act as donors in matings with the FP$^-$ strain, and as recipients in matings with a wild-type (FP$^+$) and an 'extended fertility' (FP*) male, was quantitated in plate mating experiments

Table 3. *Mating properties of male and female lines of* P. aeruginosa *strain PAT*

Test strain	Sex factor	Mating phenotype	Recombination frequency/10^7 indicator bacteria in matings with PAT967 (FP$^-$)*	PAT973 (FP$^+$)	PAT974 (FP*)
PAT900	.	FP$^-$	0	486·00	3366
PAT404	FP2	FP$^+$	78	0·82	1190
PAT906	FP2-2	FP*	1176	6127·00	3560
PAT993	FP2-3	FPd	0	0·09	872
PAT996	FP2-6	FPd	0	0·09	899
PAT998	FP2-8	FPd	0	0·04	942
PAT1220	FP2-11	FPd	0	0·04	744
PAT999	FP2-9	FPs	32	0·20	842

The male strains PAT404 and PAT906 were compared to the FPd and FPs derivatives of PAT900 in plate matings with the indicator PAT967 (FP$^-$) and its FP$^+$ and FP* derivatives. One tenth ml aliquots of saline suspensions (*c.* 2×10^9 cells/ml) of each parent were plated to MM. Aliquots of a diluted male suspension were used if a high recombination frequency was expected. Plates were incubated at 37 °C for 48 h.

* The recombination frequency given here is per 10^7 cells of the test strain.

using PAT967 (*met-1105*) and its male derivatives. Included for comparison were matings using PAT900 (*his-404*, *str-1100*) and analogous FP+ (PAT404) and FP* (PAT906) strains, and in all instances selection for recombinants was on minimal medium.

The results given in Table 3 show that the FPd and FPs derivatives of PAT900, in contrast to PAT900 itself, produce recombinants at high frequency with the FP* male but at low frequency with the FP+ male. This mating response, seen also with PAT404, is characteristic of strains harbouring the FP2 sex factor and confirms that these derivatives have arisen as a result of sex factor transfer from the mutagenized PAT973 donor population to the PAT900 recipient.

However, it is apparent that these strains have not inherited the wild-type FP2 factor as they show a reduced donor ability (as compared to PAT404) in matings with the recipient strain PAT967. This reduction is most marked with the four FPd strains which yield recombinants at frequencies of *c.* 10^{-8}/male cell, some 500-fold lower than similar matings with PAT404. It is concluded that these strains have inherited mutants of FP2 which are unable to mediate chromosome transfer, although still retaining the exclusion property characteristic of the wild-type factor. In contrast, the FPs derivative behaves as a typical FP+ strain except for a slight reduction in recombinant formation. However, it will be seen below that this strain also harbours a mutant of FP2.

(*b*) *Precipitation response*

In strain PAT, donor and recipient lines can be distinguished by their mating properties with indicator FP+ and FP− strains, and by their differential precipitation properties in citrate buffer. This is shown in Table 4, where the characteristic

Table 4. *Precipitation of FP− and sex factor carrying strains of* P. aeruginosa *in citrate buffer*

Strain	Sex factor carried	Mating phenotype	pH 3·0	3·6	4·0	4·6	6·2
PAT404	FP2	FP+	+	(+)	−	+	+
PAT900	.	FP−	+	(+)	−	−	−
PAT1201	FP2	FP+	+	(+)	−	+	+
PAT974	FP2-2	FP*	+	(+)	−	+	+
PTO13	FP2	FP+	+	−	−	−	−
PAO381	FP2	FP+	+	−	−	−	−
PTO30	.	FP−	+	−	−	−	−
PAO38	.	FP−	+	−	−	−	−
PAT1220*	FP2-11	FPd	+	(+)	−	+	+
PAT999	FP2-9	FPs	+	(+)	−	−	−

The precipitation of FP+ and FP− bacteria was determined by the procedure in Materials and Methods.

+ = precipitation; (+) = partial precipitation; − = no precipitation.

* Strain PAT1220 carrying FP2–11, gave the same precipitation response as isolates carrying FP2-3, FP2-6 and FP2-8 which also show the FPd mating phenotype.

responses of donor and recipient lines of strains PAT, PAO and PTO (a PAT × PAO recombinant line) are given, together with the responses of strains carrying the suspected FPd and FPs mutants.

It is seen that all strains are precipitated at pH 3·0, and then pass through a stable phase around pH 3·6–4·0. However, at pH values greater than 4·6, donor and recipient lines of PAT but not of PAO or PTO show marked differences in precipitation response. The precipitation of PAT donors is apparently associated with the presence of the FP2 factor, since recipient lines acquiring either a wild-type FP^+ or the mutant FP* factor simultaneously acquire the precipitation phenotype (strains PAT1201 and PAT974 respectively). This result suggests that the FP2 factor controls a surface difference between donor and recipient bacteria which results in their differential precipitation, in particular ionic environments. However the inability to detect a similar difference between male and female lines of strains PAO and PTO, suggests that the precipitation phenotype conferred by FP2 is not invariably expressed but is dependent also on the host genome. For instance, it is likely that precipitability would be influenced by the presence or absence of specific host-determined components of the cell surface. In this respect strain PAT differs from PAO as indicated by its distinct colonial morphology and its less efficient adsorption, but not plating, of the phages E79, F116 and B3 (J. Watson, personal communication; P. Chandler, personal communication; Holloway & Rolfe, 1964).

The four FPd derivatives show the same precipitation characteristics as the donor PAT404, suggesting that these strains harbour sex factors which confer the wild-type precipitation phenotype although defective in some aspect of conjugation leading to recombinant formation (Table 3). In contrast, the FPs derivative (PAT999) fails to show the characteristic precipitation of FP^+ strains but behaves identically to the FP^- strain PAT900. It is concluded that PAT999 harbours a mutant FP2 factor defective in some function controlling the precipitation phenotype.

(c) *Sex factor transfer from the mutant male strains*

The wild-type male PAT404 and the FPd and FPs derivatives of PAT900 were tested for their ability to transfer sex factor to the recipient PAT967 (*met-1105*). After mating, derivatives of PAT967 were isolated on methionine supplemented MM and single clones tested for the inheritance of sex factor by replica plating to an indicator male strain. In this test, bacteria inheriting FP2 can be distinguished from uninfected recipients by their inability to form recombinants in matings with the donor strain PAT404.

It is seen (Table 5) that transfer of sex factor from the wild-type male PAT404 occurs at a frequency of *c.* 10%, while that from the FPs derivative PAT999 is only half as efficient. When the resulting FP2-9 derivatives of PAT967 were examined for donor ability and precipitation response, they were found to have the same properties as their parent PAT999; that is, a precipitation phenotype like that of recipient (FP^-) strains although maintaining a donor ability similar to that of

wild-type (FP+) donors. However, the recombination frequency obtained in matings with recipients was again some fivefold less than that with a corresponding wild-type male, suggesting that the mutation resulting in non-precipitability is associated with a slightly reduced conjugal fertility.

Table 5. *Sex factor transfer from males of* P. aeruginosa *strain PAT*

	Derivatives of PAT967 isolated				
Male strain	Sex factor carried	Mating phenotype	No. tested	No. forming recombinants with FP + donor	FP transfer/ 100 clones of PAT967
PAT404	FP2	FP+	335	303	9·5
PAT999	FP2-9	FPs	318	302	5·0
PAT1220	FP2-11*	FPd	345	345	0·0

Exponential phase cultures of PAT404 (FP+) and the FPd and FPs derivatives of PAT900 were mixed with the recipient PAT967 to a cell density of *c.* 5×10^8 cells/ml using a 20:1 ratio. After incubation at 37 °C for 6 h with gentle aeration, derivatives of PAT967 were isolated on methionine supplemented MM and single clones tested for their ability to form Met+[His+] recombinants in a replica plate mating with the donor strain PAT404.

* Similar results were obtained for strains carrying FP2-3, FP2-6 and FP2-8.

In contrast, sex factor transfer was not detected from the FPd derivatives of PAT900 ($< 0{\cdot}3\,\%$), suggesting that these strains cannot mediate either sex factor (Table 5) or host chromosome transfer (Table 3) to recipient bacteria. In this respect they are similar to the transfer defective (Tra−) mutants of *E. coli* (Achtman, Willetts & Clark, 1971, 1972). The non-transferability of these sex factors seems at variance with the observation of their inheritance from a mutated donor population, and it may be that in this instance transfer was due to a 'helper effect' provided by unmutated sex factors coexisting in the same cell. This possibility is further examined using the mutant factor FP* (see below).

(iii) *Properties of male strains heterozygous for FP2*

It has been shown that FP* males can transfer their sex factor (FP2-2) to FP+ as well as FP− bacteria, the former event resulting in the formation of males heterozygous for the FP* and FP+ factors (Stanisich & Holloway, 1972). Since these strains exhibit the 'extended fertility' phenotype characteristic of FP* males, it can be concluded that the FP* mutation is dominant to the wild-type allele carried by FP2. Strains which were presumed to be heterozygous for the FP* and FPd factors, and the FP* and FPs factors respectively, were constructed by mating PAT974 (*met-1105*, FP2-2) with each of the FPd and FPs derivatives of PAT900, and screening the latter for the inheritance of the 'extended fertility' phenotype. These strains and a comparable FP* FP+ derivative of PAT404, were used as donors of sex factor to the recipient strain PAT967. The purpose of the experiment was to determine whether transfer of the FPd factors could be initiated

from strains harbouring FP*, thereby indicating that the FPd mutations were recessive to the corresponding alleles carried by FP*.

After mating, clones derived from the recipient PAT967 population were isolated on methionine supplemented MM and were found to be of two sizes – large and small. The isolation of such colonial variants from matings involving FP* males have previously been reported, together with the observation that the inheritance of FP* is correlated with the small colonial type (Stanisich & Holloway, 1972). By determining the number of such colonies and then including the percentage

Table 6. *Transfer of sex factor from heterozygous males of* P. aeruginosa *strain PAT*

Donor strain	Sex factor carried	PAT967 derivatives able to form recombinants in matings with: An FP+ donor	PAT967 derivatives able to form recombinants in matings with: An FP− recipient	Mating phenotype	No. obtained	Frequency/100 large colonies of PAT967 isolated
PAT1222	FP2-2, FP2	+	−	FP−	210	72
		−	+	FP+	58	20
		+	+	FP*	23	8
PAT1220	FP2-2, FP2-11	+	−	FP−	372	94
		−	−	FPd	14	4
		+	+	FP*	8	2
PAT999	FP2-2, FP2-9	+	−	FP−	322	88
		−	+	FPs	21	6
		+	+	FP*	20	6

PAT404 (FP+) and the FPd and FPs derivatives of PAT900 were infected with FP* from PAT974. The heterozygous males obtained were used as donors of sex factor to the recipient PAT967. Exponential phase cultures of donor and recipient bacteria were mixed to a cell density of *c.* 5×10^8/ml in a 50:1 ratio and after 4 h incubation at 37 °C without shaking, derivatives of PAT967 were isolated on methionine supplemented MM. Two colony types, large and small, were found after 36 h incubation at 37 °C. The tabulated results show the mating phenotypes found among the large colonies determined by replica plate mating.

* The results shown were obtained from the heterozygote of PAT1220. Similar results were obtained from strains with FP2-3, FP2-6 and FP2-8.

of FP* colonies obtained among the large colony types (Table 6) the frequency of transfer of FP* from each of the donors FP* FP+, FP* FPd and FP* FPs was estimated as being 63%, 33% and 40% respectively. Transfer of FP+, FPd and FPs to PAT967 was determined by screening the large colony types for their ability to act as recipients or donors in matings with male and female indicator strains respectively. This was found to occur at frequencies of 20%, 4% and 6% respectively (Table 6).

It is likely that these figures are underestimates of the actual transfer frequencies of these sex factors, since cells inheriting these together with FP* cannot readily be distinguished from those which inherit FP* alone. Nevertheless, the recovery

of cells displaying the FPd mating phenotype suggests that FP* can provide the transfer function defective in the FPd mutants, and hence that these mutations are recessive to the alleles carried by FP* and probably also to those carried by the wild-type factor. A similar restoration of transfer function probably accounts for the transfer of these FPd factors from the mutated donor population from which they were initially derived. The precipitation characteristics of the FP* FPs heterozygote used in the transfer experiment (Table 6) was found to be identical to those obtained with FP* and FP+ bacteria (see Table 4), indicating that the wild-type function had been restored and that the mutation of FPs is also recessive to the corresponding allele carried by FP*.

The heterozygous strains constructed for these experiments were generally stable for the extended fertility phenotype, and segregants displaying the FP+, FPs or FPd mating phenotypes did not occur at frequencies greater than 0·5%, although two of the FP* FPd strains showed up to 20% segregation of FPd bacteria. The isolation and maintenance of these heterozygous strains supports the previous conclusion that at least two copies of FP2 occur in males of *P. aeruginosa* strain PAT (Stanisich & Holloway, 1972).

4. DISCUSSION

Three classes of FP2 mutants have been isolated in *P. aeruginosa* strain PAT. Of these, the mutant designated FP* has been particularly useful in providing evidence that at least two copies of FP2 occur in strain PAT, and in demonstrating the presence of transfer defective sex factors. It has been shown that the mutation determining the extended fertility (FP*) phenotype is dominant to the wild-type allele, since this phenotype is expressed in strains heterozygous for FP* and either the wild-type FP+ or the mutant FPd or FPs factors. In contrast, the mutations conferring the FPd and FPs phenotypes are recessive to the alleles carried by the FP* factor and hence probably also to those of the wild-type sex factor.

To determine whether the latter conclusion was valid, an attempt was made to transfer the FP+ factor into the FPd males using a heterozygous FP* FP+ donor to effect pair formation (Stanisich, 1972). Strains inheriting the FP+ mating phenotype were not isolated at frequencies > 0·5%, indicating that the FPd mutations were either dominant to the wild-type alleles, hence masking transfer of FP+, or, more likely, that the resident FPd factor exhibited entry exclusion towards the FP+ factor while permitting entry of the FP* mutant. It would be advantageous to determine conditions which yield phenocopies of *P. aeruginosa* males, thus allowing them to act as good recipients. Superinfection experiments could then be carried out with the wild-type factor in addition to those now possible with the mutant FP*.

At present, the conclusion that multiple copies of FP2 occur in strain PAT donors rests solely on experiments involving the mutant FP*. Several mechanisms have been proposed to account for sex-factor incompatibility, and both host-cell and sex-factor mutations are known to affect this phenomenon (Maas & Gold-

schmidt, 1969; Palchoudhury & Iyer, 1971). It could be that the ability to isolate heterozygous males in *P. aeruginosa* is a property exclusively associated with the mutation carried by FP*. This might be such as to now allow FP* to occupy a different maintenance site to that of the wild-type factor, or alternatively to render it insensitive to a plasmid specific repressor which would normally inhibit its replication. If it were possible to isolate heterozygotes between factors other than FP*, the case for multiple copies as the wild-type situation would be strengthened.

Although no evidence is available to implicate structures analogous to sex pili in conjugation in *P. aeruginosa*, it is tempting to consider the FPd males as strains harbouring mutant sex factors which fail to produce sex pili or produce altered structures. In these circumstances restoration of transfer function in the presence of FP* may be due either to complementation as is observed with defective plasmid mutants in the Enterobacteria (Cooke, Meynell & Lawn, 1970), or simply to FPd using the pairing structure synthesized by FP*. The four FPd mutants examined have behaved similarly in the tests applied to them, but may well have mutations in different genes; in *E. coli* several genes have been found to be required for sex-factor transfer (Willetts, 1971; Ippen-Ihler, Achtman & Willetts, 1972). It may be possible to further classify the FPd mutants using an R factor which interacts with FP2 in a manner apparently analogous to the fertility inhibition of F by fi^+ R factors (Stanisich, 1972).

The precipitation phenomenon found to be FP2-specified in strain PAT is similar to that described between F^+ and F^- strains of *E. coli* (Maccacaro, 1955). However, whether this phenomenon is indeed due to the presence of FP2 pili is as yet unknown, and attempts to demonstrate an antigenic difference between FP^+ and FP^- bacteria have not been successful (V. Krishnapillai, unpublished data).

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