HOST SPECIFICITY OF DNA AND CONJUGATION IN *PSEUDOMONAS AERUGINOSA*[1]

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IT is now known that the DNA of different bacterial strains of the same species possesses a specificity characteristic of each particular strain. This specificity can be characterized at present only by a biological test, its chemical basis in most cases being unknown. When DNA of any one specificity enters a bacterium of a different specificity, it is rapidly destroyed by nucleases. This is known as *restriction*. The process by which a bacterial strain confers specificity on any DNA which it synthesises, either bacteriophage, episomal or chromosomal, is known as *modification*. Within any one bacterial strain, it is possible to impose genotypic or phenotypic alterations of restriction or modification and such changes may be recognized either by effects on bacteriophage infection and multiplication, or by changes in bacterial recombination and linkage frequencies.

In *Pseudomonas aeruginosa* the restriction and modification phenotypes are altered following growth of this bacterium at 43°C. While these changes persist so long as the bacterium is grown at 43°C they are retained for only about sixty generations when such 43°C-grown bacteria are cultured at 37°C (HOLLOWAY 1965). The effects of such phenotypic changes in restriction and modification on conjugation have been studied in *P. aeruginosa* (ROLFE and HOLLOWAY 1966) and we have now extended these studies by means of the interrupted mating technique to include genotypic changes of DNA specificity. Similar studies have been made in *Escherichia coli* (BOYER 1964; LEDERBERG 1966; COPELAND and BRYSON 1966).

MATERIALS AND METHODS

Bacterial strains: These are listed in Table 1.

Bacteriophage: The virulent phage E79 was used to kill the male parents in the interrupted mating procedure.

Media: As described previously (ROLFE and HOLLOWAY 1966)

Interrupted mating technique: As described by STANISICH and HOLLOWAY (1969).

The plate mating technique has been previously described, as were the procedures for the isolation and characterization of recombinants (ROLFE and HOLLOWAY 1966).

EXPERIMENTAL

The isolation of mutants with altered restriction and modification properties

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TABLE 1

Bacterial strains

Old strain number	New strain number	Genotype	Restriction and modification phenotype
1–66–4 E79^r	PAO664	*pur-66 pro-4 ese-5* FP$^-$	Res$^+$ Mod$^+$
1–67S E79^r	PAO672	*his-67 str-4 ese-2* FP$^-$	Res$^+$ Mod$^+$
1–60S	PAO602	*ilv-60 str-5* FP$^-$	Res$^+$ Mod$^+$
1–28–2 E79^r	PAO282	*met-28 ilv-2 ese-8* FP$^-$	Res$^+$ Mod$^+$
1–4S FP$^+$	PAO42	*trp-4 str-6* FP$^+$	Res$^+$ Mod$^+$
1–402	PAO402	*trp-4 str-6 res$^-$ mod$^+$* FP$^+$	Res$^-$ Mod$^+$
1–403	PAO403	*trp-4 str-6 res$^-$ mod$^-$* FP$^+$	Res$^-$ Mod$^-$
1–410	PAO410	*trp-4 str-6 fpa-2* FP$^+$	Res$^-$ Mod$^-$
1–615	PAO615	*ilv-60 str-5 res$^-$ mod$^-$* FP$^-$	Res$^-$ Mod$^-$

Genotypic abbreviations used: pur-purine, *pro*-proline, *his*-histidine, *ilv*-isoleucine plus valine, *met*-methionine, *trp*-tryptophan, *str*-streptomycin resistance, *res*-restriction, *mod*-modification. FP + or —, sex factor, *ese*-resistance to virulent phage E79. *fpa-2*-resistance to fluorophenylalanine. *Phenotypic abbreviation used:* Res-restriction, Mod-modification *FPA*-fluorophenylalanine.

New strain numbers are being introduced to conform to the pattern operating in other bacterial genetic systems. For easier reference to earlier papers in this series both numbers are given here.

has already been described (ROLFE and HOLLOWAY 1968). Basically the technique used is the same as that described by WOOD (1966) and involves cross-streaking colonies surviving mutagen treatment against bacteriophage of a different host specificity. Mutants isolated included the two phenotypic classes Res$^-$ Mod$^+$ and Res$^-$ Mod$^-$. In addition it has been found that mutations of *P.aeruginosa* to resistance to p-fluorophenylalanine (FPA) showed concomitant alterations of the restriction and modification properties to give the same phenotypic classes as those obtained by this selection procedure (ROLFE and HOLLOWAY 1968).

We have carried out crosses between male and female derivatives of strain 1 of *P.aeruginosa* (i.e. all strains with the prefix PAO) which vary in their Res and Mod characteristics. Alterations in modification, and hence in DNA specificity, of the male parent can be recognized by the restriction system of the female parent. This restriction system can in turn be altered in various ways. The effects of the restriction are measured by changes in recombination frequency or by changes in linkage of particular markers.

The effects of genotypic DNA-specificity differences on FP$^-$ × FP$^+$ crosses were first demonstrated using plate matings. Different female strains (all *res$^+$ mod$^+$*) were used to enable selection for different markers and these were all crossed to three male strains; PAO42 (*res$^+$ mod$^+$*), PAO402 (*res$^-$ mod$^+$*) and PAO403 (*res$^-$ mod$^-$*), both the latter strains derived by mutation from PAO42. The results are shown in Table 2. It is seen that in general the matings involving the *res$^-$ mod$^-$* genotype in the male parent show a marked reduction in recombinant number, while there is little difference between the *res$^+$ mod$^+$* and *res$^-$ mod$^+$* matings.

Three possible explanations of these results can be suggested. Firstly, that in mutating the male strain PAO42 to the *res$^-$ mod$^-$* form, other changes may have

TABLE 2

Crosses between wild-type females of P. aeruginosa *strain 1 with males in restriction and modification loci*

Recipient strain	Selection for	Number of recombinants per 10^9 FP$^-$ cells		
		Donor strain		
		PAO42 *res*$^+$ *mod*$^+$	PAO402 *res*$^-$ *mod*$^+$	PAO403 *res*$^-$ *mod*$^-$
PAO664	*pro*+*-4*	3,000	3,400	170
PAO672	*his*+*-67*	10,200	10,850	1,000
PAO602	*ilv*+*-60*	700	880	150
PAO282	*ilv*+*-2*	1,810	1,680	330
PAO282	*met*+*-28*	1,620	890	300

The crosses were carried out as plate matings. Each recipient strain has the strain 1 wild-type phenotype of Res+ Mod+. All strains were grown at 37°C prior to mating and all matings carried out at 37°C.

occurred, such as alterations in its surface characteristics, which might impede the ability of this strain to form effective mating pairs. Secondly, the mechanisms controlling the mobilization of the chromosome during conjugation might have been affected and result in lower DNA transfer. The third possibility is that the restriction mechanisms controlled by the *res* locus of the female cells degrade the DNA transferred from the PAO403 donor and hence prevent it from taking part in recombination.

To test the first of these possibilities, the male mutant PAO403 was crossed with two female strains which are Res$^-$. The first was PAO615, a *res*$^-$ *mod*$^-$ mutant obtained by mutating PAO602, and the second was the parent strain PAO602 grown at 43°C and hence phenotypically Res$^-$ Mod$^-$. The results of such matings shown in Table 3 demonstrate that the mutant strain PAO403 can produce recombinants at the same or even greater frequency as the *res*$^+$ *mod*$^+$ strain when mated with these two Res$^-$ female strains. This indicates that the observed reduction in the number of recombinants must be due to the restriction of the male DNA and not to the other reasons listed above. Further evidence supporting

TABLE 3

Bacterial crosses (plate matings) with different strains of P. aeruginosa *showing differences in restriction and modification*

Recipient strain	Selection for	Number of recombinants per 10^9 FP$^-$ cells		
		Donor strain		
		PAO42(37°C)	PAO402(37°C)	PAO403(37°C)
PAO602 (37°C)	*ilv*+*-60*	700	880	150
PAO615 (37°C)	*ilv*+*-60*	600	680	2,200
PAO602 (43°C)	*ilv*+*-60*	800	900	2,190

The parent strains were grown at the indicated temperatures prior to mating. All matings were carried out at 37°C.

TABLE 4

Analysis of linkage data from crosses between parents differing in restriction and modification phenotype using two different mating procedures

Matings method	Unselected marker	Selected marker: *ilv*+-2, Donor strain PAO42	PAO402	PAO403	Selected marker: *met*+-28, Donor strain PAO42	PAO402	PAO403
(a) plate mating	*ilv*+-2	..	..	..	100	96	67
	met+-28	95	95	68	..	..	..
(b) Interrupted mating	*ilv*+-2	..	..	..	96	..	65
	met+-28	91	..	71	..	..	..

The recombinants tested from the interrupted mating were derived from samples taken 60 min after mixing. The recipient strain used was PAO282. (Res+ mod+)

Recombinants from the various crosses were tested for linkage of *met* and *ilv* and the figures given are percent linkage.

this view comes from the analysis of the linkage data of unselected markers (Table 4). In the crosses involving PAO282 (FP$^-$) (Table 2) selection was made for either *ilv*$^+$-*2* or *met*$^+$-*28*, and the unselected markers *met*$^+$-*28* and *ilv*$^+$-*2*, respectively, were examined. (The *met*$^+$-*28* and *ilv*$^+$-*2* markers are cotransduced at about 15% by phage F116 and enter very close together in interrupted matings.) It can be seen that there was a marked decrease in the linkage values of the *ilv*$^+$-*2* *met*$^+$-*28* markers for the crosses with PAO403 compared with the values obtained for the crosses with the other two males. Such effects on linkage in crosses between parents of different DNA specificity have also been recorded for *E. coli* (COPELAND and BRYSON 1966) and these observations are therefore interpreted as manifestations of host-controlled modification and restriction of the *P. aeruginosa* chromosome.

Host specificity effects in FP$^-$ × FP$^+$ crosses using an interrupted mating technique: The effects of restriction and modification can be more quantitatively studied by using an interrupted mating technique as shown by BOYER (1964) and LEDERBERG (1966) for *E. coli*. If male and female strains of *P. aeruginosa* are mixed in broth and the mating interrupted mechanically, an oriented entry of certain chromosomal markers can be demonstrated. The general nature of this process is similar to the entry of markers in an F$^-$ × Hfr cross of *E. coli* although some differences occur.

If the entry of an early marker such as *his-67* is examined (Figure 1), it is found that, with the *res*$^+$ *mod*$^+$ male PAO42 grown at 37°C, the *his*$^+$-*67* marker enters at about 7 min. However, when the *res*$^-$ *mod*$^-$ mutant PAO403 is used as the male parent, the restriction imposed by the female recipient results in a much reduced recovery of recombinants. With the *res*$^-$ *mod*$^+$ mutant PAO402 there is very little difference between this and the parent male strain in the ability to transfer the *his*$^+$-*67* marker.

By contrast, if one selects markers which appear later and which presumably

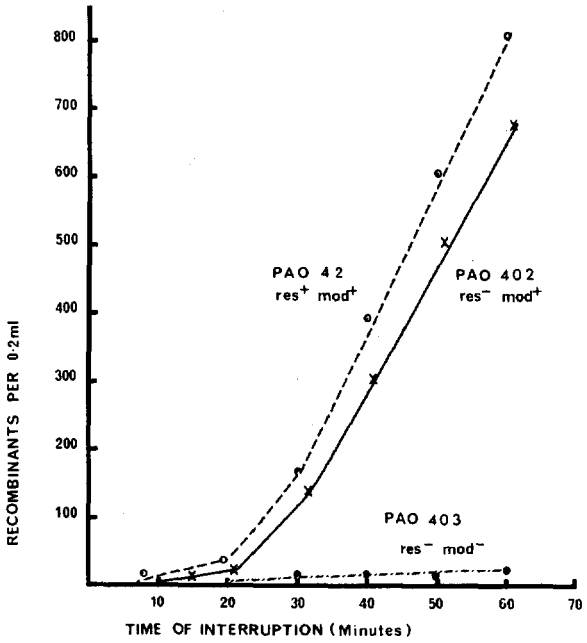

FIGURE 1.—Entry of the *his*$^+$-*67* marker in interrupted matings using males with different restriction and modification genotypes. The female parent PAO672 (*his-67*) is *res*+ *mod*+ and the three male strains used are POA42 (*res*+ *mod*+) PAO402 (*res*$^-$ *mod*+) and PAO403 (*res*$^-$ *mod*$^-$).

require the entry of more chromosomal material and hence more DNA, there is not such a marked effect when a *mod*$^+$ male is compared to a *mod*$^-$ male. These results are shown in Figure 2 where the entry of the markers *ilv*$^+$-*2* and *met*$^+$-*28* is followed. It is seen that there is essentially no difference in the time of entry for *ilv*$^+$-*2* and *met*$^+$-*28* when either PAO403 (*res*$^-$ *mod*$^-$) or PAO42 (*res*$^+$ *mod*$^+$) is used as the male parent while the reduction of recombination frequency is not as marked as that occurring with *his-67* in Figure 1. Similar results have been described in *E. coli* by BOYER (1964) and the explanation advanced by BOYER applies equally well to the Pseudomonas data. With early markers, the chromosome is rapidly degraded and hence the markers are not recovered. With increasing amounts of chromosome entering the cell the restriction enzymes become saturated and less DNA is broken down (BOYER 1964). Thus with later markers there are less extreme effects with only a reduction of about 50% in recovery of recombinants (as against a 99% reduction with early markers).

This effect of the *mod*$^-$ mutation in altering the host specificity of the DNA with the resultant reduction in recombination, can be overcome by using a *res*$^-$ female, or in the present case by growing the female parent at 43°C to give the Res$^-$ phenotype. In Figure 3, the restriction effects evident in Figure 1 with the entry of the *his*$^+$-*67* marker from a *res*$^-$ *mod*$^-$ male have been completely abolished by growing the female parent at 43°C prior to mating.

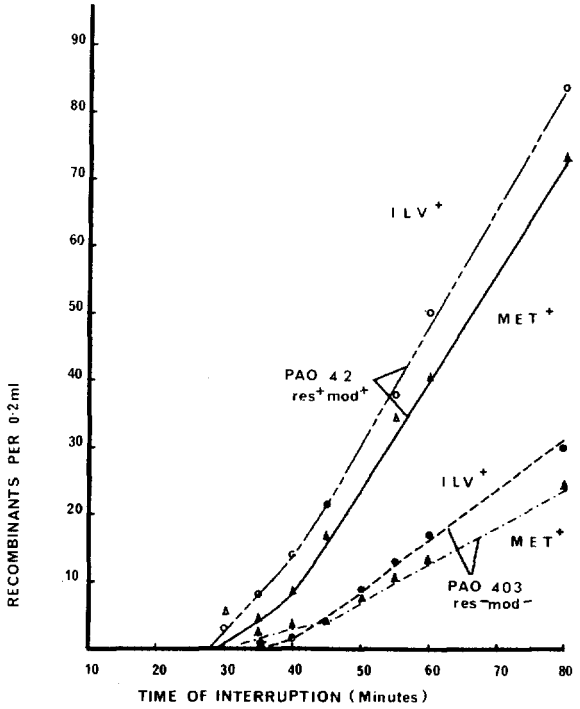

FIGURE 2.—Entry of the *ilv*+*-2* and *met*+*-28* markers in interrupted matings using males with different restriction and modification genotypes. The female parent PAO282 (*met-28*, *ilv-2*) is *res*+ *mod*+ and the male strains are PAO42 (*res*+ *mod*+) and PAO403 (*res*- *mod*-).

Alterations in the Mod phenotype can also be induced by certain mutations to p-fluorophenylalanine resistance (ROLFE 1967) or by growth at 43°C (ROLFE and HOLLOWAY 1966). These results are shown in Figure 4, where a male strain, PAO410, which is FPA-r and has acquired as a result different properties of restriction and modification (Res- Mod-) (curve B), transfers the early marker *his*+*-67* to a *res*+ *mod*+ female parent PAO672 at very low efficiency. Similar results are found when the FP+ male strain PAO42, which is *res*+ *mod*+, is grown at 43°C prior to mating to give the Res- Mod- phenotype (curve C). Growth at 43°C abolishes the restriction function of the female parent and hence, despite the different DNA specificity of the male parent PAO410, evidently due to the *fpa-2* locus, entry of the *his*+*-67* marker is unaffected. (Curve A)

We can thus conclude that, as with phage DNA, the specificity of bacterial DNA can be altered by direct mutation of genes affecting modification as seen in PAO403, by growth of a normal *mod*+ strain at 43°C, or by mutation to *FPA-r* as with PAO410. These alterations can be recognized by the restriction mechanisms of female parental cells and are displayed as alterations in conjugational behaviour.

Two other aspects of these restrictive crosses were also considered. Firstly, it is possible that the reduction in recombinant formation might be related to rate

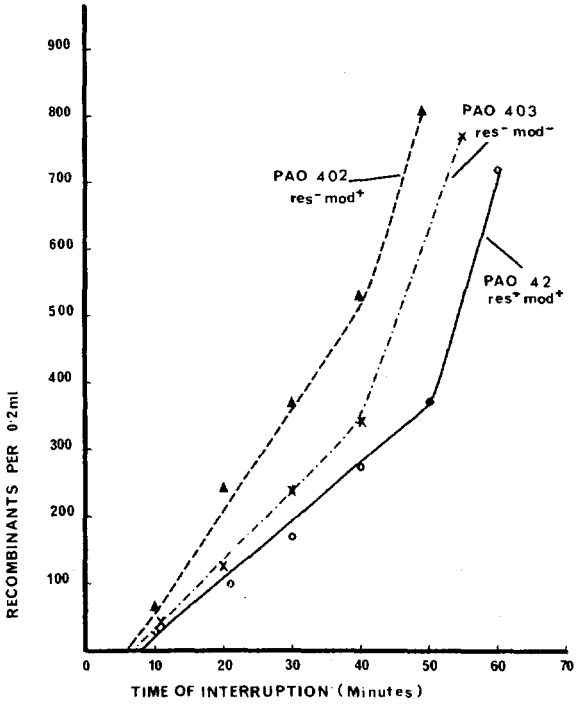

FIGURE 3.—Entry of the *his*$^+$-*67* marker in interrupted matings using parents with different restriction and modification properties. The female parent PAO672 was grown at 43°C prior to mating to make it phenotypically Res$^-$ and the three male parents (grown at 37°C) used were PAO42 (*res*+ *mod*+) PAO402 (*res*$^-$ *mod*+) and PAO403 (*res*$^-$ *mod*$^-$). The matings were carried out at 37°C.

of chromosome transfer from the strains PAO403, PAO410 and PAO42 (grown at 43°C). It is clear from Figures 1–3 that, despite any altered modification characteristics of the male, where the female parent does not show restriction, the kinetics of entry of markers is the same as when *res*$^+$ *mod*$^+$ parents are used and hence it may be concluded that chromosome is being transferred from the Mod$^-$ male strains at the same rate.

In addition, the linkage relations of *ilv*$^+$-*2* and *met*$^+$-*28* were examined. The linkage analysis shown in Table 4 shows that when the marker *met*$^+$-*28* was selected, there was less integration of the earlier marker *ilv*$^+$-*2* in those crosses using the PAO403 male. When selection was made for the *ilv*$^+$-*2* marker, the reduction in linkage of the *met*$^+$-*28* marker was less marked in the PAO403 cross.

We have previously shown (ROLFE and HOLLOWAY 1966), that plate matings between two unrelated strains of *P. aeruginosa* (strain 1 FP$^-$ × strain 2FP$^+$) differing in host specificity, showed increased recombination frequency and higher linkage values when the female strain 1 parent was made restriction-deficient in phenotype by growing it at 43°C. We have attempted to repeat these experiments using the interrupted mating technique, but the recombination fre-

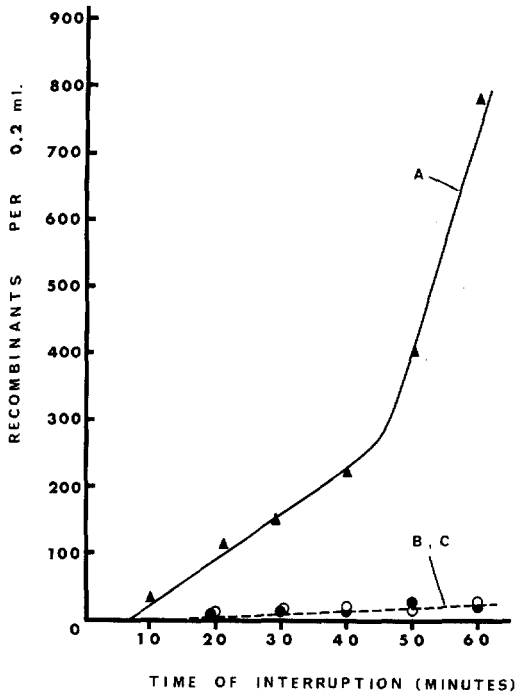

FIGURE 4.—Entry of the *his*$^+$-*67* marker in interrupted matings with parents of various restriction and modification phenotypes. The female parent was PAO672. In curve A, PAO672 was grown at 43°C prior to mating and mated to PAO410 (*fpa-2*, *res*$^-$ *mod*$^-$). In curve B, PAO672 was grown at 37°C prior to mating and mated to PAO410 and in curve C PAO672 was grown at 37°C and mated to PAO42 grown at 43°C prior to mating. All matings were carried out at 37°C.

quency was too low for any significance to be placed on the results. We are not sure why strain 1 × strain 2 matings do not show transfer of chromosome under these conditions, but possibly the aeruginocin released by strain 1 (to which strain 2 is sensitive) may be involved.

DISCUSSION

These effects of restriction and modification on the entry of different markers in interrupted matings clearly support the findings of STANISICH and HOLLOWAY (1969) that an oriented entry of chromosome occurs during conjugation in *P. aeruginosa*. The close similarity of the effects of DNA specificity on bacterial conjugation for both *P. aeruginosa* and *E. coli* also indicates the similarity of the chromosome transfer in these two bacteria.

The lack of recovery of recombinants in the case of say, *E. coli* B × *E. coli* K12 crosses may to some extent be due, not only to restriction, but also to lack of genetic homology and differences in recombinases between the two strains (COPELAND and BRYSON 1966). In at least one of the Pseudomonas examples presented above, this objection does not apply, in that case where the male strain has been

grown at 43°C and the female at 37°C, with subsequent failure of integration of the *his*$^+$-*67* marker (Figure 4, curve C). Here the parents are, for these purposes, isogenic, so it is clear that restriction is responsible for the failure to recover recombinants. This stresses one of the experimental values of the effect of growth at 43°C on DNA specificity, namely that the altered restriction and modification phenotype can be superimposed at will on any genotype without the need to mutate or recombine to get the desired phenotype.

Although it is clear that we can alter the specificity of *P. aeruginosa* DNA by a variety of genotypic and phenotypic means, we do not know if the modification change induced, say by growth at 43°C, is the same as that caused by a *mod*$^-$ mutation, or by a mutation to FPA resistance. In the present system, the only way in which we can detect a DNA specificity different from that of the wild type (strain 1) is its degradation by the wild-type (strain 1) restriction system. We do not know if these Mod$^-$ forms of different origin represent different patterns of modification or merely the absence of a particular modification. These differences can only be investigated by learning something of the chemical basis of modification.

SUMMARY

Differences in bacterial-DNA specificity may be recognized by reductions in recombination frequency and linkage during conjugation. Such differences may be introduced in *P. aeruginosa* by the same genetic mechanisms as those affecting host-controlled modification of bacteriophage infection, namely genes affecting restriction and modification, pleiotropic effects of p-fluorophenylalanine-resistance mutations and growth at 43°C. The nature of recombinant formation in interrupted matings of parents differing in DNA specificity supports the general notion of an oriented entry of chromosome in *P. aeruginosa*.

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