

Genetic control of DNA specificity in *Pseudomonas aeruginosa*

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1. INTRODUCTION

Host-controlled modification (HCM) has now been studied in several bacterial genera. In all cases it involves two distinguishable processes: (a) *restriction* in which DNA introduced into the cell is identified as acceptable or non-acceptable, the latter alternative resulting in enzymic degradation and biological impotence of the introduced DNA; an endonuclease with the properties of the restriction enzyme has recently been isolated from *Escherichia coli* (Meselson & Yuan, 1968); (b) *modification*, in which the bacterium imposes a characteristic host specificity on any DNA, bacterial or episomal, which it synthesizes. Restriction provides the means by which this specificity is recognized.

While it is apparent that one role of HCM is to prevent the entry of 'foreign' DNA into the bacterium, it is possible that it may be also important for other cellular functions. One approach to this problem is through the study of variations in HCM phenotype and genotype. Previous studies (Holloway & Rolfe, 1964; Holloway, 1965) have indicated that the genetic control of HCM in *Pseudomonas aeruginosa* showed important differences from the situation found in *Escherichia coli*. When two interfertile strains of *P. aeruginosa* were crossed, differences in restriction and modification genotype were demonstrated by the segregation of a number of genes affecting these characteristics. Unlike the situation in *E. coli*, these genes are unlinked, so that recombinants can be isolated which have HCM properties quite unlike either parent (Rolfe, 1967).

In addition, growth of some *P. aeruginosa* strains at 43 °C results in a semi-permanent alteration of the HCM properties (Holloway, 1965). The important features of this phenomenon are: (a) growth at 43 °C and not merely exposure to that temperature is necessary to induce the effect; (b) once the effect is established (by as few as five cell divisions at 43 °C) it is not lost by such 43 °C grown bacteria on return to growth at 37 °C until after about sixty cell divisions; (c) both restriction and modification properties are altered by growth at 43 °C. For convenience, we shall refer to this phenomenon as the '43° effect'. This phenomenon apparently does not occur in *E. coli* (Dunn, personal communication).

Thus, in at least these two ways, the genetic basis of HCM in *P. aeruginosa* differs from that in *E. coli*. However, it has now been shown that the general phenotypic characteristics of HCM in *P. aeruginosa* are the same as those established for the enterobacteria. This has been demonstrated with respect to enzymic degradation of host-modified bacteriophage DNA, multiplicity reactivation, heat inactivation of restriction, and modification changes following single-cycle infection of a modifying host (Rolfe, 1967). We have further investigated the control of HCM in *P. aeruginosa* and have established additional genetic means of affecting this characteristic.

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2. METHODS AND RESULTS

Media and cultural procedures used have been described previously (Holloway, 1965; Rolfe & Holloway, 1966). The procedure for mutation by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was to treat exponential-phase bacteria suspended in citrate buffer at pH 5.4 for 20 min at 37 °C with 25 µg NG/ml. The mutants were isolated from various derivatives of *P. aeruginosa* strain 1. The HCM phage system used was phage B3 with bacterial strains PAO 1 (strain 1) and PTS 271 (strain 271) acting as the alternate hosts having different DNA specificity. The reciprocal nature of this system can be seen in Table 1.

Restriction-deficient strains were isolated by cross-streaking colonies surviving NG treatment against phage B3.271. Isolates with an impaired restriction function produced lysed areas at the intersection of the phage and bacterial streaks.

3. RESULTS

(a) Characterization of restriction-deficient (*Res*⁻) mutants

Res⁻ mutants were isolated from PAO 42 (tryptophan-requiring, streptomycin-resistant male) and PAO 602 (isoleucine-plus-valine-requiring, streptomycin-resistant female). Some mutants showed complete loss of restriction (*Res*⁻), others only a partial loss (*Res'*). With either of these types, concomitant changes in modification were also found

Table 1. *Restriction and modification mutants of Pseudomonas aeruginosa*

Strain	Restriction*		Modification†		Phenotypes
	Plating efficiency on each strain for		Plating efficiency of B3 grown on each strain when plated on		
	B 3.1	B 3.271	PAO 1	PTS 271	
PAO 1 (grown at 37 °C)	1.0	10 ⁻⁵	1.0	10 ⁻⁵	<i>Res</i> ⁺ <i>Mod</i> ⁺
PAO 1 (grown at 43 °C)	1.0	1.0	10 ⁻¹	10 ⁻⁵	<i>Res</i> ⁻ <i>Mod'</i>
PTS 271	10 ⁻⁵	1.0	10 ⁻⁵	1.0	<i>Res</i> ⁺ <i>Mod</i> ⁺
PAO 402	1.0	1.0	1.0	10 ⁻⁵	<i>Res</i> ⁻ <i>Mod</i> ⁺
PAO 403	1.0	1.0	10 ⁻⁶	10 ⁻⁴	<i>Res</i> ⁻ <i>Mod</i> ⁻
PAO 615	1.0	1.0	10 ⁻²	10 ⁻⁵	<i>Res</i> ⁻ <i>Mod'</i>
PAO 617	1.0	10 ⁻²	1.0	10 ⁻⁵	<i>Res'</i> <i>Mod</i> ⁺

* Restriction is measured as the plating efficiency (free particle assay) of B3 grown on PAO 1 (B 3.1) and PTS 271 (B 3.271).

† Modification is examined by plating B 3.271 in turn onto each of the strains in the left-hand column. Plaques from each plating were then suspended in broth and assayed for plating efficiency (free particle assay) on PAO 1 and PTS 271. The efficiency of plating was calculated with respect to the titre on the yielder strain in each case. All phage assays were carried out at 37 °C and, except for PAO 1 where indicated, all strains were grown at 37 °C.

which can be described as *Mod*⁻ or *Mod'* and the distinction between *Mod*⁻ and *Mod'* is determined by the degree of restriction expressed by the *Res*⁺ *Mod*⁺ parent PAO 1 for phage grown on the particular modification mutant. The characteristics of some representative mutants are shown in Table 1. Isolation of such HCM mutants was achieved at a frequency of about 0.2–0.5 % of the colonies tested.

It is seen that the range of mutants isolated affecting the *Res* and *Mod* phenotypes is

essentially the same as that observed for *E. coli* (Lederberg, 1965; Wood, 1966), so that in this respect *P. aeruginosa* and *E. coli* show a similar genetic control of the HCM phenotype.

(b) *p*-fluorophenylalanine (FPA) resistant mutants with altered restriction and modification properties

We have previously shown that while *P. aeruginosa* is resistant to a large variety of antimetabolites it is sensitive to FPA and that mutants resistant to this substance can be isolated (Waltho & Holloway, 1966). Three loci for FPA resistance have been identified in *P. aeruginosa* PAT 2, (Waltho, 1968), two of these mapping close to the locus for streptomycin resistance. The resistance allele of one of these loci is suppressed by the resistance allele of the streptomycin locus.

P. aeruginosa PAO 1 (prototrophic female) was mutated with NG and the survivors plated on minimal medium containing 1 mg/ml FPA. Resistant colonies appeared at a frequency of 10^{-3} to 10^{-4} viable cells. When such FPA-r colonies were tested for their restriction properties by cross-streaking against phage B 3.271 it was found that over 50% of the resistant clones showed some increase in their ability to plate the host-modified phage. The change in restriction phenotype varied from only a 2- to 5-fold increase in plating ability for B 3.271 to complete loss of restriction. When representative types of such Res⁻ and Res['] mutants were examined for their modification properties, the same range of restriction and modification phenotypic combinations were found as for the mutants isolated by direct selection and listed in Table 1.

Because of the high frequency of association between FPA resistance and altered HCM properties, control experiments were carried out to see if the relationship occurred with any other mutations. As described above, the direct isolation of res⁻ mutants occurs at a frequency of about 0.5%, which on occasions may rise to 1%. Selection for streptomycin-resistant mutants from the NG-treated PAO 1 was made on complete medium plus 250 µg./ml streptomycin and less than 0.5% of such isolates showed any loss at all in their restriction abilities. When PAO 38 (leucine-requiring streptomycin-sensitive female) was mutated with NG and selection made for back mutation to prototrophy, only 2 to 4% of the prototrophs showed any loss of restriction. It is apparent then that in *P. aeruginosa* there is a real relationship between mutation to FPA resistance and changes in HCM properties. Certainly the easiest way of obtaining mutants with altered Res or Mod phenotypes in this bacterium is by mutation to FPA resistance.

(c) Specificity of the 43 °C effect and FPA-r mutations for changes in HCM properties

It is seen that both growth at 43 °C and mutation to FPA-r have singular effects on HCM. It is important to know if any other changes in the bacterial phenotype are produced by this growth temperature or this type of mutation. To date we have been unable to find any changes comparable to those occurring with HCM, although the list of phenotypic characteristics examined is by no means complete. Growth at 43 °C does produce changes in phenotype (for example, conjugation at 43 °C does not result in formation of recombinants) but we have been unable to demonstrate any persistence of such effects after return to growth at 37 °C for the following characteristics: recombination, host-cell reactivation, lysogeny, radiation sensitivity, and resistance to a range of inhibitory agents. Similarly, no changes from the wild type in any of these characteristics were found for FPA-r mutants, although it was found that many FPA-mutants cannot grow at 43 °C on minimal medium (Waltho, 1968). Growth at 43 °C of the Res['] and Mod['] forms obtained above, either by direct selection or by FPA resistance, did not change their HCM characteristics; Res['] did not become Res⁻, nor did the Mod['] become Mod⁻. It seems that growth at 43 °C and mutation to FPA-r are both specifically affecting some component of the cell which is concerned with HCM.

DISCUSSION

There is no doubt that there is a greater diversity of genetic controls of HCM in *P. aeruginosa* than in other bacteria so far examined. We think it unlikely that the different controls observed act solely through structural chromosomal genes coding for restriction and modification enzymes. The kinetics of persistence at 37 °C of HCM changes following growth at 43 °C are quite incompatible with a chromosomal control mechanism. It is possible that some autonomously replicating plasmid with some temperature-sensitive function is involved in the 43 °C effect, but our attempts to transfer, cure or mutate such a genetic element have been unsuccessful.

The mechanism of FPA resistance involved in the three loci examined to date does not involve overproduction of phenylalanine or impermeability to FPA (Waltho & Holloway, in preparation). As FPA can be incorporated into protein, FPA-r mutants may have lost the ability to incorporate FPA into protein by a change either in the structure of the ribosome or in *t*-RNA function. Lederberg (1965) suggested that the ribosome may be involved in the processes of restriction and modification, and if *fpa-r* mutants do affect the ribosome, our data support his hypothesis. One additional piece of evidence is the observed genotypic interaction between *fpa-r* and *str-r* mutants in *P. aeruginosa*, it being known that *str-r* mutants in *E. coli* influence both ribosome structure and function. If this relationship can be substantiated, not only will this be significant for our knowledge of the mechanism of HCM, but selection for FPA resistance may provide a ready method of obtaining ribosomal mutants in *P. aeruginosa*.

4. SUMMARY

Genotypic and phenotypic changes of HCM in *P. aeruginosa* are due to a variety of causes. In addition to interstrain genetic differences and the semi-permanent effects of growth at 43 °C, mutations affecting both restriction and modification have been isolated by direct selection and through the pleiotropic effects of *p*-fluorophenylalanine resistance mutations. It is suggested that some of these changes affecting HCM may be taking place through alterations of the ribosomes.

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