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SHORT PAPER

Chromosomal location of a prophage in *Pseudomonas aeruginosa* strain PAO

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SUMMARY

Segregation of the prophage of bacteriophage 90 has been observed in reciprocal crosses between lysogenic and non-lysogenic parents of *Pseudomonas aeruginosa* strain PAO. Linkage of the prophage was shown to three genes determining histidine biosynthesis in that region of the chromosome 7–13 min from the site on the chromosome at which the sex factor FP2 promotes chromosome mobilization.

1. INTRODUCTION

Different prophages may either be integrated into the continuity of the bacterial chromosome, for example coliphage λ, or extrachromosomally maintained, probably attached to a membrane site as has been shown with P1 (Ikeda & Tomizawa, 1968). Single and unique chromosomal locations for ultraviolet (u.v.) inducible and non-inducible *Escherichia coli* phages (including λ) were determined from segregation data in conjugation crosses, the lysogeny/non-lysogeny character segregating just like any bacterial gene (Jacob & Wollman, 1961). Unique integration sites in the chromosome have now been well established for many *Escherichia* and *Salmonella* phages (Taylor, 1970; Sanderson, 1970). By contrast the *E. coli* phages P2 and Mu–1 have three or many different sites, respectively (Wiman *et al.* 1970; Martuscelli *et al.* 1971).

This paper documents the first instance of chromosomal location for a prophage in *Pseudomonas aeruginosa*.

2. RESULTS AND DISCUSSION

The temperate phage 90 was isolated by plating a wild-type lysogenic strain on strain PAO of *P. aeruginosa* (Holloway, Krishnapillai & Stanisich, 1971). It formed plaques of about 1 mm in 0·6% soft-agar, was chloroform resistant, non-inducible by UV but zygotically inducible (Carey & Krishnapillai, in preparation) and was unrelated to any of the serological groups A–F (Holloway *et al.* 1960). The phage was used to lysogenize appropriate genetically marked PAO derivatives.

In plate-mating conjugation experiments (for methods see Stanisich & Holloway, 1969) between PAO242 (genotype *his 4 lys 56 met 28 trp 6* FP2–*90*–) as recipient and PAO381 (*leu 38 str 7* FP2+ *90*+) as donor, *his 4*+, *lys 56*+, *met 28*+ and *trp 6*+ recombinants were selected by plating on appropriately supplemented minimal media. Purified recombinants (48) were scored for co-inheritance of the other markers by replica-plating and for the lysogeny/non-lysogeny phenotype by testing for spontaneous release of phage (by streaking out on soft-agar overlay seeded with an indicator). The linkage data so obtained are shown in Table 1. It is seen (Table 1 A) that there is very high linkage

Table 1. *Reciprocal conjugation crosses using PAO242 as recipient*

A. PAO381 lysogenic (for phage 90) donor × PAO242 non-lysogenic recipient

Selected marker	Co-inheritance of unselected donor alleles or lysogeny/non-lysogeny (%)				
	Lysogeny	*his 4*	*lys 56*	*met 28*	*trp 6*
his 4	65	—	29	0	0
lys 56	48	46	—	2	2
met 28	22	16	31	—	31
trp 6	8	8	12	29	—

B. PAO381 non-lysogenic donor × PAO242 lysogenic (for phage 90) recipient

	Non-lysogeny	*his 4*	*lys 56*	*met 28*	*trp 6*
his 4	81	—	33	0	0
lys 56	92	94	—	10	0
met 28	56	63	67	—	35
trp 6	41	47	50	69	—

Table 2. *Reciprocal conjugation crosses using PAO300 as recipient*

A. PAO381 lysogenic (for phage 90) donor × PAO300 non-lysogenic recipient

Donor allele selected	Co-inheritance of unselected donor alleles or lysogeny/non-lysogeny (%)				
	Lysogeny	*arg 1*	*his 12*	*ilv 202*	*met 28*
arg 1	38	—	4	15	15
his 12	2	2	—	0	0
ilv 202	52	88	6	—	83
met 28	31	42	6	90	—

B. PAO381 non-lysogenic donor × PAO300 lysogenic (for phage 90) recipient

	Non-lysogeny	*arg 1*	*his 12*	*ilv 202*	*met 28*
arg 1	72	—	8	13	13
his 12	7	4	—	2	2
ilv 202	50	71	6	—	75
met 28	40	52	0	98	—

(65 %) between *his 4* and lysogeny and very low linkage (8 %) to *trp 6*. The reciprocal cross of PAO381 (*90*−) × PAO242 (*90*+) gave the linkage result shown in Table 1B. Again there was high linkage (81 %) between *his 4* but this time to non-lysogeny and low linkage (41 %) to *trp 6*. These linkage values are consistent with the view that the lysogeny/non-lysogeny character is segregating in bacterial crosses as are the other markers. Similar reciprocal conjugation experiments, where one parent was lysogenic at a time, were performed with another recipient, PAO300 (*arg 1 his 12 ilv 202 met 28 str 2* FP2−), and the same PAO381 donor, giving the results shown in Table 2. The anomalously low linkage of *his 12* to the other markers is not understood despite its 13 min location on the chromosome (from interrupted-mating data) and, more importantly, its co-transduci-

Table 3. *Conjugation cross of PAO8 and PAO831*

PAO8 lysogenic (for phage 90) donor × PAO831 non-lysogenic recipient

Donor allele selected	Co-inheritance of unselected donor alleles or lysogeny/non-lysogeny (%)					
	Lysogeny	*pro 71*	*thi 1*	*his 151*	*pyr 21*	*pur 66*
pro 71	2	—	35	2	0	0
thi 1	0	8	—	0	0	0
his 151	71	8	69	—	0	0
pyr 21	2	6	19	10	—	0
pur 66	2	4	13	4	0	—

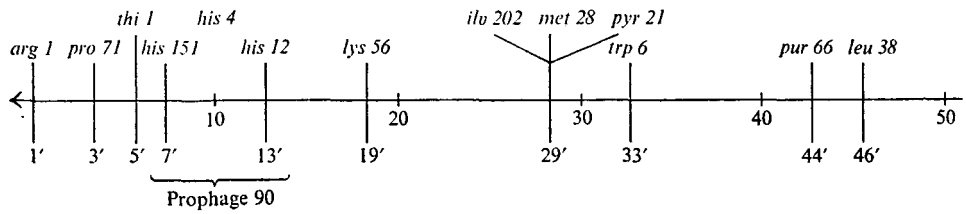

Fig. 1. Chromosomal location of prophage 90. *arg* = arginine; *pro* = proline; *his* = histidine; *lys* = lysine; *ilv* = isoleucine plus valine; *pyr* = pyrimidine (uracil); *met* = methionine; *trp* = tryptophan; *pur* = purine (adenine); *leu* = leucine; *thi* = thiamine. *his 12* was formerly *his 2* (Stanisich & Holloway, 1969). The three *his* alleles were independently defined mutations; *his 4* and *his 12* are unlinked on the basis of co-transduction tests (Stanisich & Holloway, 1969); so are *his 151* and *his 12* (Pemberton, 1971). The possibility that *his 4* is in the same transducing fragment as *his 151* has not been excluded. The numerals refer to chromosomal location in minutes (obtained from interrupted mating data – Stanisich & Holloway (1969), Holloway *et al.* (1971), Pemberton (1971), Pemberton & Holloway (1972).

bility with other markers in this vicinity (Stanisich & Holloway, 1969; Holloway *et al.* 1971; Pemberton & Holloway, 1972). Perhaps structural idiosyncracies in the PAO300 chromosome (probably induced by the multiple mutagenic steps employed in its derivation) could have influenced conjugational recombination over stretches of DNA much larger than is involved in transductional recombination. Whatever the explanation the relevance of *his 12* in the context of prophage 90 mapping was that the lysogeny/non-lysogeny character also showed the anomalous very low linkage with *his 12* in unselected marker co-inheritance (Table 2A and 2B). A final experiment involved a conjugation cross between another donor-recipient combination: PAO8 lysogenic donor (genotype = *met 28 ilv 202 str 1* FP2+ *90*+) with PAO831 non-lysogenic recipient (genotype = *pur 66 his 151 pyr 21 thi 1 pro 71* FP2− *90*−) with the result shown in Table 3. Again it was observed that the lysogeny/non-lysogeny character was highly linked (71%) with the *his 151* gene. The reciproval cross using a lysogenic PAO831 was not possible because PAO831 was already resistant to adsorption by the virulent phage E79 which fortuitously makes it also cross-resistant to phage 90.

Despite the linkage anomalies in conjugation, such as the differential percentage co-inheritance of unselected markers in reciprocal crosses (Table 1), it is concluded that prophage 90 has a chromosomal location in the 7–13 min region of the chromosome (see Fig. 1). This is especially reinforced by the consistency in the linkage relationship between the lysogeny/non-lysogeny character and the other genetic markers in reciprocal

crosses. Moreover and importantly zygotic induction has recently been shown to occur with prophage 90 in interrupted mating experiments as indicated by a 5–10-fold reduction in recovery of *his151+* and *pyr21+* recombinants, but not those of *pro71+* or *thi1+* recombinants, in crosses between PAO381 (= donor lysogenic for phage 90) and PAO831 (= non-lysogenic recipient). Additionally there was a 20 min delayed entry time for *his151* and *pyr21*. From these data prophage 90 has been more precisely located at 5–7 min of the chromosome (Carey & Krishnapillai, in preparation). Although zygotic induction occurred in interrupted mating crosses this was not obvious, even though there was suggestive evidence, in the lysogenic donor × non-lysogenic recipient crosses reported here presumably due to the use of the plate mating method. Preliminary transduction experiments have failed to show any closer linkage between prophage 90 and one of the three *his* alleles: *his151*, *his4* or *his12*.

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