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Chromosome mapping in *Pseudomonas aeruginosa*

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SUMMARY

A more extensive linkage map of *Pseudomonas aeruginosa* strain PAO has been compiled from data obtained by both conjugation and transduction procedures. All the markers examined are located on one linkage group and the available evidence suggests that the sex factor FP2 promotes transfer of the chromosome in a polarized manner from only one site on this linkage group.

1. INTRODUCTION

There can be no question that the experimental potential for genetic studies on any organism is determined by the extent and precision of the genetic map available and continued studies on *Pseudomonas aeruginosa* have indicated the demand for a more extensive map than those currently available (Loutit, 1969; Stanisich & Holloway, 1969*a*). The techniques of transduction and conjugation in this bacterium have been studied in some detail (Holloway, 1969; Holloway, Krishnapillai & Stanisich, 1971), although the lack of a complete understanding of several features of the mechanism of conjugation has tended to delay the use of this technique for some aspects of the establishment of a complete genetic map of this bacterium. These uncertainties include the question as to whether chromosome transfer by the sex factor FP2 is initiated from only one or a number of origins, whether there is one or more chromosomes in this bacterium and whether all or any of the linkage groups are linear or circular. While some of these questions still remain, the data obtained in this paper have established the relative location of a large number of markers on one linkage group and will serve as a basis for the solution of the other outstanding problems.

2. MATERIALS AND METHODS

Bacterial strains. The strains used are shown in Table 1. All are derived from strain PAO (Holloway, 1969). In general the nomenclature and abbreviations used follow those of Demerec, Adelberg, Clark & Hartman (1966).

Bacteriophage strains. F116 (Holloway, Egan & Monk, 1960); F116-L – a derivative of F116 (Krishnapillai, 1971); G101 (Holloway & van de Putte, 1968).

Methods. In general the methods described by previous workers from this

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Table 1

Strain no.	Genotype	Reference
PAO1	Prototrophic, *chl-2*, FP-	Holloway (1955)
PAO2	*ser-3*, FP-	Rolfe & Holloway (1966)
PAO8	*met-28*, *ilv-202*, *str-1*, FP-	Isaac & Holloway (1968)
PAO12	*pur-136*, *leu-8*, *chl-2*, FP-	Holloway Collection
PAO38	*leu-38*, FP-	Stanisich & Holloway (1969*a*)
PAO39	*ilv-260*, *pur-108*, *str-5*, FP-	Holloway Collection
PAO68	*trp-54*, *chl-13*, *str-6*, FP-	Stanisich & Holloway (1972)
PAO76	*pur-66*, *str-38*, FP-	Holloway Collection
PAO129	*pyr-29*, *chl-2*, FP-	Isaac & Holloway (1968)
PAO174	Prototropic, *chl-2*, *str-8*	Holloway Collection
PAO246	*met-28*, *trp-6*, *lys-56*, *his-3*, FP-	Holloway Collection
GMA253	*cys-5605*, *his-5075*	Mee & Lee (1969)
PAO280	*met-28*, *trp-6*, *lys-56*, FP-	Holloway Collection
PAO286	*met-28*, *trp-6*, FP-	Stanisich & Holloway (1969*a*)
PAO287	*met-28*, *ilv-202*, *arg-1*, *his-12*, *ese-4*, *str-1*, FP-	Stanisich & Holloway (1969*a*)
PAO303	*arg-18*, *chl-2*, FP-	Isaac & Holloway (unpublished)
PAO307	*arg-54*, *chl-2*, FP-	Isaac & Holloway (unpublished)
PAO362	*arg-32*, *chl-2*, FP-	Isaac & Holloway (unpublished)
PAO381	*leu-38*, *str-7*, FP2+	Stanisich & Holloway (1969*a*)
PAO664	*pro-64*, *pur-66*, *ese-5*, FP-	Stanisich & Holloway (1969*a*)
PAO824	*pur-66*, *his-151*, *arg-163*, *ese-14*, FP-	This paper
PAO826	*pur-66*, *his-151*, *lys-52*, *ese-14*, FP-	This paper
PAO831	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *pro-71*, *ese 14*, FP-	This paper
PAO832	*pur-66*, *his-151*, *pyr-21*, *ilv-261*, *ese-14*, FP-	This paper
PAO833	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *lys-53*, *ese-14*, FP-	This paper
PAO834	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *arg-161*, *ese-14*, FP-	This paper
PAO838	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *ese-14*, FP-	This paper
PAO839	*pur-66*, *his-151*, *pyr-21*, *trp-153*, *ese-14*, FP-	This paper
PAO841	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *trp-50*, *ese-14*, FP-	This paper
PAO842	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *met-60*, *ese-14*, FP-	This paper
PAO843	*pur-66*, *his-152*, *phe-60*, *ese-40*, FP-	This paper
PAO844	*pur-66*, *his-152*, *arg-162*, *ese-40*, FP-	This paper
PAO850	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *arg-160*, *ese-14*, FP-	This paper
PAO851	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *trp-150*, *ese-14*, FP-	This paper
PAO852	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *cys-61*, *ese-14*, FP-	This paper
PAO853	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *pro-70*, *ese-14*, FP-	This paper
PAO855	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *met-63*, *ese-14*, FP-	This paper
PAO858	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *met-66*, *ese-14*, FP-	This paper
PAO862	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *arg-166*, *ese-14*, FP-	This paper
PAO872	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *ser-204*, *ese-14*, FP-	This paper
PAO873	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *thr-1*, *ese-14*, FP-	This paper
PAO877	*pur-136*, *leu-8*, *his-154*, *ese-16*, FP-	This paper
PAO879	*pur-136*, *leu-8*, *pro-73*, *ese-20*, FP-	This paper
PAO886	*pur-66*, *his-151*, *pyr-21*, *thi-1*, *pro-71*, *leu-41*, *ese-14*, FP-	This paper
PAO1227	*trp-54*, *chl-13*, *str-6*, FP2+	This paper
PAO1376	*pur-154*, *leu-38*, FP-	This paper

The following genotypic abbreviations have been used: *arg*, arginine; *chl*, chloramphenicol; *cys*, cysteine; *ese*, resistance to virulent phage E79; *his*, histidine; *ilv*, isoleucine plus valine; *leu*, leucine; *lys*, lysine; *met*, methionine; *phe*, phenylalanine; *pro*, proline; *pur*, purine; *pyr*, pyrimidine; *ser*, serine; *str*, streptomycin; *thi*, thiamine; *trp*, tryptophan; *thr*, threonine.

laboratory have been followed. The principal media used include Nutrient Yeast Broth (NYB), Heart Infusion Broth (HIB), Nutrient Agar (NA) (Stanisich & Holloway, 1969*a*, *b*) and Minimal Medium (Vogel & Bonner, 1956). Any variations of these media are described in the text.

A modification of the technique for interrupted mating (Stanisich & Holloway, 1969*a*) was developed in which recipient strains are used in stationary growth phase while the donor is in exponential phase. Matings are carried out in liquid minimal medium rather than HIB plus glucose as before.

Strains with various numbers of auxotrophic markers were prepared by treatment with methyl-nitro-nitroso-guanidine (NG) (Holloway, 1966).

Two modifications of existing mapping procedures have enabled more precise location of markers to be made on the chromosome.

(1) *Minimal medium interrupted mating technique.* The procedure followed in this technique is set out above. There are two advantages in using this method compared to the broth method (Stanisich & Holloway, 1969*a*). First, there is a fivefold increase in the level of recovery of recombinants which enhances the accuracy of mapping by the interrupted mating technique. Secondly, markers having a low requirement for growth, e.g. thiamine, can be mapped using this method.

(2) *Multiply marked auxotrophic recipients.* In general the recipient strains used were so constructed as to contain three reference markers. These map in the early (*his-151*), middle (*pyr-21*), and late (*pur-66*) regions of the map. Any additional markers occurring between 0 and 45 min from the FP2 origin were mapped relative to one of these reference markers. In addition, the recombinants formed after 120 min of mating in liquid media were plated on to appropriately supplemented media to select for various markers and the segregation of other non-selected markers determined.

Transduction was used in two ways. In some instances mutants with mutations presumably in the same gene were isolated independently in different recipient strains and this was confirmed using the transduction method of reduction of prototroph frequency (Fargie & Holloway, 1965). Where different markers showed much the same time of entry, linkage was confirmed in most instances by measuring co-transduction with phages F116, F116-L or G101.

3. RESULTS

It is convenient to discuss the data obtained in terms of various regions of the chromosome, measured on a time scale from the origin of chromosome transfer promoted by the sex factor FP2. The list of strains used in this study is given in Table 1.

The 0–10 min region

The origin (0′) is arbitrarily defined as the origin of transfer of the donor chromosome by the FP2 sex factor. In practical terms it is that region of the donor chromosome which enters 1 min before *arg-1* and 3 min before *pro-71* (Fig. 1) in

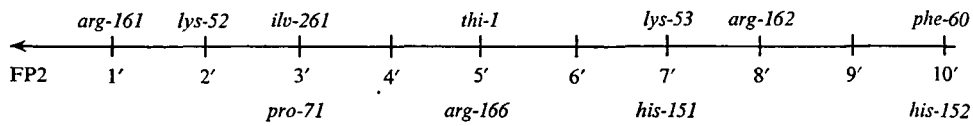

Fig. 1. The position of markers in the 0–10 min region of the map.

Table 2. *The location of markers in the 0–10 min region of the map*

(The reference markers in this region are *his-151* and *his-152*.)

		Linkage		
		Conjugational	Transductional	
Selected marker	Time of entry (min)	% inheritance of unselected reference marker *his-151*	% co-transduction with surrounding markers	Closely linked markers by prototroph reduction transduction
arg-161	1	21	.	*arg-18*, *arg-1*, *arg-31*
lys-52	2	16	.	.
ilv-261	3	15	87 (*pro-71*)	.
pro-71	3	12	90 (*ilv-261*)	*pro-64*
thi-1	5	25	.	.
arg-166	5	40	30 (*thi-1*)	*arg-160*
lys-53	8	70	37 (*his-151*)	.
his-154	8	.	.	*his-67*, *his-151*
		his-152		
arg-162	9	78	29 (*his-152*)	*arg-163*
phe-60	10	80	60 (*his-152*)	.

crosses between PAO 1227 FP2+ and recipient strains possessing these markers. The arrangement of genes for the 0–10 min region of the PAO chromosome is given in Fig. 1. The earliest marker mapped is *arg-161* which has an entry time 1 of min (Table 2, Fig. 1). From transduction data (Table 2) the markers *arg-18*, *arg-31*, *arg-161*, and *arg-1* appear to be closely linked. It is known that *arg-31* and *arg-18* are mutations in different genes involved in the biosynthesis of arginine (J. Isaac & B. W. Holloway, manuscript submitted for publication). B. W. Holloway & A. Ong (unpublished data) have shown that *arg-18* shows 60% linkage to *pro-71* in interrupted matings. However, these two markers do not show detectable co-transduction using F116-L. The position of *lys-52* has been determined solely by conjugational data and shows high co-transfer with *his-151* (Table 2).

Prototroph reduction transduction tests (Table 2) show that *pro-64* (formerly *pro-4* of Stanisich & Holloway, 1969) and *pro-71* are closely linked. The markers *pro-71* and *ilv-261* have similar entry times, and transduction tests show that they are closely linked (Table 2). The markers *thi-1* and *arg-166* have similar entry times and show close linkage in interrupted matings. In addition, they are co-transducible to a level of 30% (Table 2). The markers *arg-160* and *arg-166* are closely linked. Mee & Lee (1967) have shown that *his-151* (formerly *his-42*) and *his-67* are

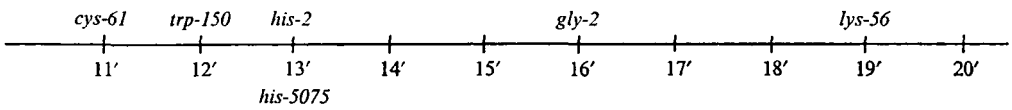

Fig. 2. The position of markers in the 11–20 min region of the map.

Table 3. *The location of markers in the 11–20 min region of the map using donor strains carrying the FP2 sex factor*

(The reference marker is *his-151*.)

		Linkage		
		Conjugational	Transductional	
Selected marker	Time of entry (min)	% inheritance of unselected reference marker, *his-151*	% co-transduction with surrounding markers	Closely linked markers by prototroph reduction transduction
cys-61	12	10	90 (*trp-150*)	*cys-5605*
trp-150	13	44	82 (*cys-61*)	*trp-50*
trp-50	13	45	.	*trp-150*
his-5075	13	.	95 (*cys-5605*)	*his-3*
gly-2	16	12	.	.

closely linked, whilst *his-152* (formerly *his-47*) is not closely linked to either of these mutations. The markers *lys-53* and *arg-163* enter at the same time as *his-151*, and both of these markers are closely linked to *his-151* (Table 2). A similar situation exists with *arg-162* and *phe-60* which enter at the same time as *his-152* (Table 2).

The 11–20 min region

Only a few markers have been located in this region (Fig. 2). From transduction data it is found that the *cys-61* marker is closely linked to the *cys-5605* marker of Mee & Lee (1969) (Table 3). The *cys-5605* marker is closely linked to *his-5075* (Mee & Lee, 1969) the two loci being 95% co-transducible (Table 3). The prototroph reduction transduction tests show that *his-3* and *his-5075* are closely linked. The independently isolated markers *trp-50* and *trp-150* have similar entry times and show close linkage (Table 3). In addition, it is found that *trp-150* is co-transducible with *cys-61* to a level of 90% (Table 3). The position of *lys-56* on the map (formerly *lys-12*, Stanisich & Holloway, 1969*a*) has been determined solely by interrupted mating. The *gly-2* marker enters at 16 minutes and is not co-transducible with either *lys-56* or with any of the genes of the *cys his trp* group.

The region from 21 to 30 min

The location of markers in this region is given in Fig. 3. Prototroph reduction transduction tests reveal that the following pairs of markers are closely linked mutations: *ser-3* and *ser-204*; *pyr-21* and *pyr-29*; *ilv-202* and *ilv-260*; and *met-28* and *met-60* (Table 4). The relative positions of *ser-204*, *ilv-202*, *pro-71*, *met-60* and

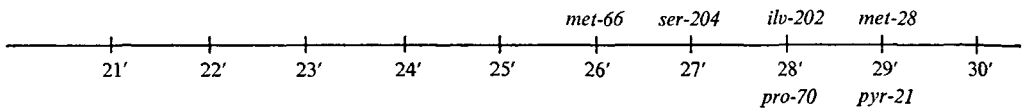

Fig. 3. The position of markers in the 21–30 min region of the map.

Table 4. *The location of markers in the 21–30 min region of the map using donor strains carrying the FP2 sex factor*

(The reference marker in this region is *pyr-21*.)

		Linkage		
		Conjugational	Transductional	
Selected marker	Time of entry (min)	% inheritance of unselected reference marker, *pyr-21*	% co-transduction with surrounding markers	Closely linked markers by prototroph reduction transduction
met-66	26	59	.	.
ser-204	28	68	.	*ser-3*
ilv-202	28	80	28 (*met-28*)	*ilv-260*
			67 (*ser*-204)	.
			5 (*pyr-21*)	.
			44 (*pro-70*)	.
met-28	29	90	30 (*ilv-202*)	*met-60*
			70 (*pro-70*)	.
			57 (*pyr-21*)	.
			5 (*ser-204*)	.
pyr-21	30	.	55 (*pro-70*)	*pyr-29*

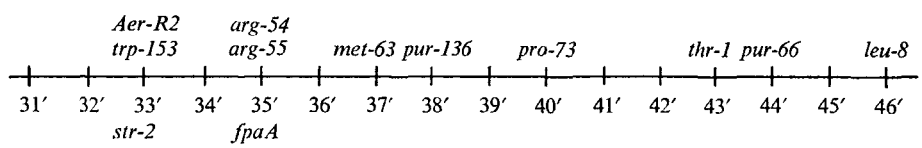

Fig. 4. The position of markers in the 31–46 min region of the map.

pyr-21 have been determined by transduction analysis after reference to entry times and linkage analysis studies. It is clear from Table 4 that all the markers so far identified in this region, with the exception of *met-66*, are closely linked.

The 31–46 min region

The entry times for markers which enter after 30 min are less accurate than those determined for earlier markers due mainly to a reduced number of recombinants. To some extent this has been overcome by treating the data by the method of de Haan, Hoekstra, Verhoef & Felix (1969) which uses the observed gradients of transmission. The positions of *trp-153*, *pur-136* and *met-63* and *pur-66* in Fig. 4 have been determined using this method. Determinations of times of entry for earlier markers by this method gave values very close to those observed by inter-

Table 5. *The location of markers in the 31–46 min region of the map using donor strains carrying the FP2 sex factor*

(The reference markers in this region are *pyr-21*, *pur-66* and *leu-8*)

		Linkage		
		Conjugational	Transductional	
Selected marker	Time of entry (min)	% inheritance of unselected marker	% co-transduction with surrounding markers	Closely linked markers by prototroph reduction transduction
		pyr-21		
trp-153	33	56	.	*trp-6*, *trp-54*
met-63	36	18	.	.
		leu-38		
pur-136	38	14	.	.
pro-73	40	10	.	.
leu-8	46	.	.	*leu-38*, *leu-41*
		pur-66		
thr-1	45	68	.	.
pur-66	.	.	40 (*leu-38*) 29 (*thr-1*)	*pur-154*

rupted mating, indicating the validity of the procedure for *P. aeruginosa*. It will be noted that by this method the entry time for *pur-66*+ is about 44 min, a reduction of 16 min on the previously published time of entry of 60 min (Stanisich & Holloway, 1969*a*). It has been shown by B. W. Holloway (unpublished data) that *pur-66* and *leu-38* are 15% cotransducible using phage F116. Transductional analysis (Table 5) shows that *pur-154* and *pur-66*, and *leu-41*, *leu-38* and *leu-8* are closely linked mutations.

The data in Table 5 show that two more genes can be placed in this region. The *thr-1* marker enters at 43 min and is closely linked to *pur-66*. In transduction studies it is found that *thr-1* is cotransducible with *pur-66*, but not with *leu-38* (Table 5) which suggests that *thr-1* should be placed proximal to *pur-66*. *Aer-R2* is an aeruginocinogenic determinant mapped by Kageyama (1970*a*, *b*). The terminology previously used by Kageyama for this locus (pyR2) has been changed to avoid confusion with the *pyr* locus. The position of the *fpaA* locus was shown by Waltho & Holloway (1966) by transduction with phage F116. From Table 5 it is seen that *trp-6*, *trp-54* and *trp-153* are closely linked mutations. The order of the genes *trp-6*, ... *arg-54 fpa-A* ... *str-2* has been determined by transductional analysis using phage F116. (A. Ong & B. W. Holloway, unpublished observations; Waltho & Holloway, 1966.)

How many linkage groups?

All the markers described individually for the various time span regions above show linkage to at least one other marker and hence it can be concluded that they lie on the one linkage group. This has been confirmed by the examination of marker

Table 6. *Comparison of single and double selection for early and late markers in the cross PAO1227 FP2 × PAO886 FP-*

(Mating was allowed to proceed in liquid medium for 120 min.)

Selection for	Entry time (min)	Genotype of recombinants						% in each class
		pro-71	*thi-1*	*his-151*	*pyr-21*	*pur-66*	*leu-41*	
pro-71+	3	+	+	+	+	.	.	3
		+	+	+	.	.	.	3
		+	+	.	.	.	.	7
		+	.	.	.	.	.	87
pur-66+	44	+	+	+	+	+	+	3
		.	+	+	+	+	+	3
		+	+	+	.	+	+	7
		+	+	.	.	+	+	4
		.	+	+	.	+	.	4
		.	+	.	.	+	.	40
		.	+	.	+	+	+	20
		.	+	.	+	+	.	10
		.	.	.	+	+	.	9
pro-71+ *pur-66*+	44	+	+	+	+	+	+	30
		+	+	+	.	+	+	7
		+	+	.	.	+	+	35
		+	+	+	.	+	.	16
		+	+	.	.	+	.	10
		+	.	.	.	+	.	2

segregation when an early and a late marker are selected together (double selection). If the two co-selected markers fall on the one linkage group the apparent entry time of each marker should be that of the later marker. In addition, markers located between the two co-selected markers should show a higher degree of inheritance when compared to single selection for either marker.

An example of the type of data obtained in this way is shown in Table 6 for the cross PAO 1227 FP2+ × PAO 886 FP-. It is seen that when selection is made for both *pro-71*+ and *pur-66*+ the apparent entry time for each marker is 44 minutes, the entry time of *pur-66*. It is seen that the frequency of recombinants which have inherited one or more of the markers located between *pro-71* and *pur-66* rises considerably when double selection is imposed, providing additional support for the view that all five markers in the cross lie on a single linkage group. This is important since three of the markers, *pur-66*, *pyr-21* and *his-151* have been used as reference markers in the interrupted matings described above.

In an earlier paper, Stanisich & Holloway (1969*a*) suggested that two histidine markers, *his-2* and *his-3* were on a separate linkage group to the majority of markers mapped in their study. The results indicate that these markers are closely linked to markers on the main linkage group so that there is now no discordant evidence from this laboratory with respect to the view that there is only one linkage group in *P. aeruginosa*. However Loutit and his co-workers (Loutit, 1969)

have suggested that a few markers do not show linkage to the main linkage group and have postulated at least one minor linkage group. As yet this difference in the number of postulated linkage groups has not been resolved.

4. DISCUSSION

It can be expected that continued studies on *P. aeruginosa* will not only add to the map described above but also resolve the outstanding problem of the nature of conjugation in this species. A search for other sex factors has revealed two other types (J. M. Pemberton & B. W. Holloway, in preparation) but they have not enabled a determination of circularity to be made. The present data suggest that FP2 promotes transfer of chromosome from only one origin, the 0 min region of the map, none of the results in any way suggesting that there may be alternative or even less preferred sites. If this is generally true for other sex factors, and for the R factors which promote chromosome transfer in *P. aeruginosa* (Stanisich & Holloway, 1971), it will make any determination of circularity much easier.

The linkage data obtained confirm the nonclustering of markers of individual biosynthetic pathways but as yet any overall pattern of marker distribution is not apparent. While most of the biosynthetic markers have not been identified with respect to the deficient enzyme lesion, no overall similarity between gene distribution in *P. aeruginosa* and *E. coli* or *Salmonella typhimurium* is obvious.

It is clear that more markers including conditional lethal mutants and more sex factors must now be isolated and studied to further extend the mapping of *P. aeruginosa*.

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