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Investigation of the mating system of *Pseudomonas aeruginosa* strain 1

IV. Mapping of distal markers

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1. INTRODUCTION

The genetic material of *Pseudomonas aeruginosa* strain 1 has been shown to be transferred in a definite order from the FP^+ donor cells to the FP^- recipients (Loutit, Pearce & Marinus, 1968) and this information has been used to develop methods for mapping ten of the early markers (Loutit & Marinus, 1968). With these markers it was possible to measure their times of entry into FP^- recipients and to confirm their order by linkage analysis.

This paper is concerned with the mapping of thirteen markers more distal to the ten previously reported. Many of them gave so few recombinants that they could not be mapped by determination of their times of entry.

2. MATERIALS AND METHODS

The original strains of *P. aeruginosa*, the methods of isolation of strains and the media used have been described previously (Loutit, Pearce & Marinus, 1968; Loutit & Marinus, 1968). The strains used in the present study together with their relevant characteristics are listed in Table 1.

Mapping by gradient of transmission

Mating cell suspensions were prepared as described by Loutit, Pearce & Marinus (1968). Equal volumes (0·5 ml) of log phase donor and stationary phase recipient suspensions were mixed and incubated at 37 °C for 90 min when 9 ml of minimal medium were added. The mixture was shaken on a Mickle shaker and appropriate dilutions were plated in soft agars on various selective media. Streptomycin (500 μg/ml) was added for contra-selection of the male cells. After 2–3 days' incubation at 37 °C the recombinants were scored.

Mapping by linkage analysis

The mating procedures were essentially those described above except that it was necessary to use logarithmic phase recipient cell suspensions to obtain reproducible results with the extreme distal markers. Mating time was generally 90 min but mating for 120 min or even longer was sometimes necessary to obtain

enough recombinants. Contra-selection of both donor and recipient cells usually depended on an auxotrophic mutation.

The methods for examining the selected classes of recombinants for unselected markers have been described previously (Loutit & Marinus, 1968).

Table 1. *Strains used and their relevant characteristics*

Number	FP	*str*	Auxotrophic markers	Derivation of new strains
OT 1	+	*r*	*trp-1*	—
OT 2	−	*s*	*leu-1*	—
OT 15	+	*s*	prototrophic	—
OT 47	−	*r*	*ilvB112* leu-1*	—
OT 51	+	*s*	*pro-1*	—
OT 77	+	*s*	*ser-1 leu-1*	Donor from OT 10
OT 108	−	*r*	*ilvB112 ade-4 leu-1*	Mutant of OT 47
OT 109	−	*r*	*ilvB112 pro-5 leu-1*	Mutant of OT 47
OT 110	−	*r*	*ilvB112 trp-5 leu-1*	Mutant of OT 47
OT 112	−	*r*	*ilvB112 ser-7 leu-1*	Mutant of OT 47
OT 113	−	*r*	*ilvB112 met-11 leu-1*	Mutant of OT 47
OT 114	−	*r*	*ilvB112 his-8 leu-1*	Mutant of OT 47
OT 115	−	*r*	*ilvB112 ade-5 leu-1*	Mutant of OT 47
OT 116	−	*r*	*ilvB112 met-12 leu-1*	Mutant of OT 47
OT 117	−	*r*	*ilvB112 pro-6 leu-1*	Mutant of OT 47
OT 118	−	*r*	*ilvB112 hom-1 leu-1*	Mutant of OT 47
OT 119	−	*r*	*ilvB112 arg-6 leu-1*	Mutant of OT 47
OT 124	+	*r*	*ilvB112 met-9 leu-1*	Donor from OT 104
OT 128	+	*r*	*ilvB112 ade-4 leu-1*	Donor from OT 108
OT 129	+	*r*	*ilvB112 pro-5 leu-1*	Donor from OT 109
OT 135	+	*r*	*ilvB112 pro-6 leu-1*	Donor from OT 117
OT 136	+	*r*	*ilvB112 arg-6 leu-1*	Donor from OT 119
OT 238	−	*r*	*ade-4 leu-1*	Revertant from OT 108
OT 239	−	*r*	*trp-5 leu-1*	Revertant from OT 110
OT 240	−	*r*	*his-8 leu-1*	Revertant from OT 114
OT 241	−	*r*	*ade-5 leu-1*	Revertant from OT 115
OT 242	−	*r*	*met-12 leu-1*	Revertant from OT 116
OT 243	−	*r*	*pro-6 leu-1*	Revertant from OT 117
OT 244	−	*r*	*hom-1 leu-1*	Revertant from OT 118
OT 245	−	*r*	*arg-6 leu-1*	Revertant from OT 119
OT 248	−	*r*	*ser-7 leu-1*	Revertant from OT 112
OT 249	−	*r*	*met-11 leu-1*	Revertant from OT 113
OT 250	−	*s*	prototrophic	Revertant from OT 2
OT 254	+	*r*	*ilvB112 his-8 leu-1*	Donor from OT 114
OT 256	−	*r*	*pro-5 leu-1*	Revertant from OT 109
OT 272	+	*r*	*ilvB112 arg-4 leu-1*	Donor from OT 96

* Previously described as *ilvA12* (Loutit, Pearce & Marinus, 1968).

(Abbreviations used: *ade*, adenine; *arg*, arginine; *his*, histidine; *hom*, homoserine; *met*, methionine; *pro*, proline; *ser*, serine; *trp*, tryptophan; *str*, streptomycin sensitivity or resistance.)

Mapping by time of entry

The methods are essentially those described by Loutit & Marinus (1968) except that the cells were paired for 5 min and diluted 10^{-2} in nitrate nutrient broth rather than 10^{-3}. For early markers a further 10^{-1} dilution was necessary before the mated cells were plated.

3. RESULTS

(i) *Mapping by gradient of transmission*

This was first attempted with strains which did not carry the *ilvB112* marker and, to allow a comparison to be made, the number of pairs formed in each mating was determined by measuring the transfer of sex factor as described by Loutit, Marinus & Pearce (1968). The results were then expressed as the number of recombinants per 10^7 pairs.

Table 2. *Gradient of transmission of 12 selected markers in* Pseudomonas aeruginosa *expressed as the number of prototrophic recombinants obtained for each marker per 1000* ilvB112$^+$ *recombinants in the same cross**

Recipient	Marker	Recombinants per 1000 *ilvB112*$^+$
OT 108	*ade-4*	31
OT 109	*pro-5*	20
OT 110	*trp-5*	20
OT 112	*ser-7*	11
OT 113	*met-11*	8
OT 114	*his-8*	7
OT 115	*ade-5*	5
OT 116	*met-12*	1·6
OT 117	*pro-6*	1·3
	leu-1	1·0
OT 118	*hom-1*	1·0
OT 119	*arg-6*	0·65

* Matings were carried out for 80 min using OT 15 (prototrophic) as donor and streptomycin was added for contra-selection.

The method was relatively successful but was time-consuming and was subsequently replaced when a large number of auxotrophic mutants was isolated from strain OT 47 which carried the early marker *ilvB112*. With these strains the number of recombinants for any new marker could be compared with the number of *ilvB112*$^+$ recombinants in the same cross. The results were recorded as the number per 1000 *ilvB112*$^+$ colonies. Many auxotrophic mutants were tested in this way and 11 strains were finally selected for further study. They ranged from the one producing the least recombinants to the one next to the most distal of the markers already reported (*arg-4*). The results are shown in Table 2.

This gives a preliminary indication of the order but it was not considered reliable for markers close together. Nevertheless, it provided a number of hypotheses which could be investigated by linkage analysis.

(ii) *Linkage analysis*

For convenience the genetic markers were investigated in two groups within each of which there was a reasonable degree of linkage. The first included the markers from *pro-5* to *his-8* and the second included those from *ade-5* to the

bottom of Table 2. One further point which should be made at the outset is that the order of markers in *P. aeruginosa* apparently can be established by selecting recombinants for distal markers and looking at proximal ones unselected (Loutit & Marinus, 1968). This method has been used in the present work and the order obtained in this way has been confirmed by carrying out a more orthodox analysis by isolating proximal markers and looking at the distal ones unselected (Hayes, Jacob & Wollman, 1963).

Table 3. *Linkage of selected distal markers to* arg-4 *expressed as the percentage of prototrophic recombinants of the selected markers which have also incorporated the proximal* arg-4 *donor marker*

Cross	Selected marker	Double recombinants (%)*
OT 272 × OT 238	*ade-4*	0
OT 272 × OT 239	*trp-5*	43
OT 272 × OT 249	*met-11*	25
OT 272 × OT 256	*pro-5*	24
OT 272 × OT 241	*ade-5*	13

* Approximately 200 recombinants from two experiments were examined for each cross.

Table 4. *Linkage of certain proximal markers to* pro-5 *expressed as the percentage of prototrophic recombinants of the markers which have also incorporated the distal* pro-5 *donor marker*

Cross	Selected marker	Double recombinants (%)*
OT 129 × OT 239	*trp-5*	14
OT 129 × OT 248	*ser-7*	59
OT 129 × OT 240	*his-8*	76
OT 129 × OT 249	*met-11*	97

* Aproximately 150 recombinants from two experiments were examined for each cross.

The first task was to determine which marker was most closely linked to *arg-4* (the most distal one reported by Loutit & Marinus, 1968). This was done by mating selected recipients with the donor strain OT 272 and examining the recombinants for the *arg-4* donor marker. The results are shown in Table 3 and it is obvious that *trp-5* and not *pro-5* was closest to *arg-4*. The results differ from those in Table 2 and one further important difference was the lack of any linkage of *ade-4* to *arg-4*.

It was eventually shown that *pro-5* was the last marker in the first group and the order of the group is shown in Table 4. These results are based on the assumption that *pro-5* was the most distal and this was investigated as the unselected marker in the appropriate recombinants. The order shown in Table 3 is confirmed and additional markers have been included.

The order of the remaining markers was determined by crossing suitable

recipients with the donor strain OT 254 and looking at the selected recombinants for the proximal *his-8* donor marker. The results are shown in Table 5 and again there are departures from the original order shown in Table 2. Two markers were of particular interest. *Met-12* was not linked to *his-8* and *arg-6* had to be moved from the most distal position to one next to *pro-5*.

Table 5. *Linkage of distal markers to* his-8 *expressed as the percentage of prototrophic recombinants of the markers which have also incorporated the proximal* his-8 *donor marker*

Cross	Selected marker	Double recombinants (%)*
OT 254 × OT 256	*pro-5*	82
OT 254 × OT 245	*arg-6*	43
OT 254 × OT 243	*pro-6*	34
OT 254 × OT 244	*hom-1*	26
OT 254 × OT 242	*met-12*	0

* Approximately 200 recombinants from two experiments were examined for each cross except the one with OT 243 when 120 were examined.

Table 6. *Linkage between adjacent distal markers expressed as the percentage of prototrophic recombinants which have incorporated the adjacent proximal donor marker*

Cross	Selected marker	Unselected marker	Double recombinants (%)
OT 129 × OT 245	*arg-6*	*pro-5*	45
OT 136 × OT 250*	*str-2*	*arg-6*	89
OT 77 × OT 245	*arg-6*	*str-2*	92
OT 136 × OT 256	*pro-6*	*arg-6*	43
OT 135 × OT 241	*ade-5*	*pro-6*	62
OT 1 × OT 241	*leu-1*	*ade-5*	95
OT 1 × OT 241*	*ade-5*	*leu-1*	79
OT 51 × OT 244	*hom-1*	*leu-1*	78
OT 51 × OT 244*	*leu-1*	*hom-1*	67

* These crosses involved the selection of recombinants of the proximal marker and their examination for the unselected distal marker. Reciprocal crosses were necessary to establish the order of the *str-2* and *leu-1* markers. At least 200 recombinants were examined.

These results have also been checked by selecting for recombinants of the proximal markers and looking for the incorporation of the most distal marker (*hom-1*) and an identical order to that shown in Table 5 was found. In addition, a wide variety of other crosses has been carried out and the linkage data confirm the order of the markers. All markers except two can be shown to be linked to each other and presumably constitute one linkage group. Two other markers are shown to be on this same linkage group. *Leu-1* which was present in all the recipient strains was investigated and found to be between *ade-5* and *hom-1*. The other marker was that concerned with streptomycin resistance which has not been fixed unequivocally. It was shown to be very closely linked to *arg-6* which

accounts for the very low number of recombinants recorded in Table 2 from experiments in which streptomycin was used for contra-selection. The results of experiments to show the linkage between the extreme distal markers as well as to place the additional markers within the group, are shown in Table 6.

Table 7. *Linkage between markers on the second linkage group expressed as the percentage of recombinants of the distal marker which have incorporated the proximal donor marker*

Cross	Selected marker	Unselected marker	Double recombinants (%)
OT 124 × OT 238	*ade-4*	*met-9*	10
OT 128 × OT 242	*met-12*	*ade-4*	8

More than 200 recombinants were examined for each cross.

Table 8. *Distance of selected markers from* ilvB112 *measured as the number of minutes between their respective times of entry into a suitable recipient*

Cross	Marker	Distance in minutes
OT 15 × OT 96	*arg-4*	18
OT 15 × OT 110	*trp-5*	20
OT 15 × OT 112	*ser-7*	22
OT 15 × OT 114	*his-8*	23
OT 15 × OT 113	*met-11*	24
OT 15 × OT 109	*pro-5*	25
OT 15 × OT 108	*ade-4*	18*

* This distance is recorded as the distance from *pro-4* which enters at 4 min and is the first marker on the same linkage group as *ade-4*. The *ilvB112* marker was used as a control and entered at nine minutes as it did when the *pro-4* time of entry was determined.

Two markers need further discussion because they do not show linkage to any of the markers on the main linkage group. These are *ade-4* and *met-12* now known to be linked to *pro-4* and *met-9* which were reported to be on an apparent separate linkage group by Loutit & Marinus (1968). The linkage between *met-9*, *ade-4* and *met-12* is shown in Table 7. It is interesting that no linkage has been demonstrated between *met-12* and any of the distal markers of the main group.

(iii) *Time of entry analysis*

Certain of the markers could be mapped in this way provided that the cells were diluted 10^{-2} instead of 10^{-3}. For the linkage group with the smaller number of markers only the time of entry of *ade-4* could be determined at 23 min compared with the entry of *ilvB112* at nine minutes. The last marker (*met-12*) gave so few recombinants that they could not be detected following a dilution of 10^{-2}. It was not felt that any reliable information would be obtained if the mated cells were diluted only 10^{-1} in view of the results of Loutit, Pearce & Marinus (1968). They

demonstrated that at that concentration there was a delay in the appearance of recombinants and it was greatest for more distal markers.

For the main linkage group it was not possible to determine the time of entry of any markers beyond *pro-5*, which entered approximately 25 min after *ilvB112*. The results of the time of entry experiments are shown in Table 8 and again the order of the earlier markers is confirmed.

(iv) *Linkage maps of* Pseudomonas aeruginosa

From the data as well as that provided by Loutit & Marinus (1968) it is possible to prepare linkage maps for at least twenty-three genetic markers of *P. aeruginosa*. Sixteen of these can be mapped by determination of their times of entry into suitable recipients. The total span of these markers is 57 min, which probably

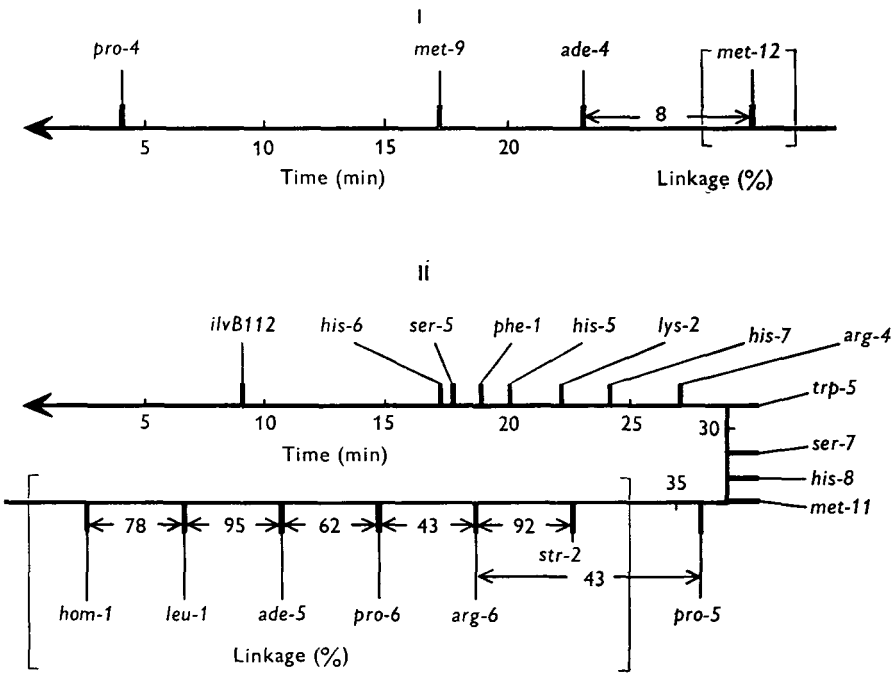

Fig. 1. Maps of two linkage groups (I and II) of *Pseudomonas aeruginosa* determined by the times of entry of certain of the markers into suitable recipients as well as linkage between adjacent markers. The markers shown in the brackets cannot be mapped by the time of entry studies since they produce so few recombinants. The first two markers of group I and the first eight of group II were mapped previously by Loutit & Marinus (1968).

means about 49 min allowing four from each of the two linkage groups for the initiation of transfer. The remaining seven markers cannot yet be mapped in this way and the linkage data do not provide any satisfactory quantitative estimate. The information is summarized in Fig. 1.

4. DISCUSSION

The order of thirteen distal genetic markers has been determined and quantitative estimates have been provided for the distances separating six of them. The remainder, which yielded few recombinants, were ordered by linkage analysis alone. The donor strains available should make it possible to establish the order of any further strains since they appear to be well dispersed on the genetic material.

With the ten markers previously investigated (Loutit & Marinus, 1968) maps of two distinct linkage groups have been prepared and it is interesting that no significant linkage has been demonstrated between the distal markers of the two groups. This could mean that there are two independent linkage groups in each cell, but the system is complex and it would be just as easy to account for the results assuming only one circular linkage group with the sex factor having two main sites of attachment, one near *ilvB* and the other near *pro-4*. If *ilvB* and *pro-4* were close together on a circular chromosome, the distal markers of both linkage groups would also be close and could be expected to show some linkage. This was not the case, but the other possibility that *pro-4* and *hom-1* are close together and *ilvB* and *met-12* also together can not be proved or disproved at present. Recombinants which might show such linkage would be submerged in the much larger number from the other donor. The possibility of such linkage could be investigated with the isolation of a donor strain which transferred only one linkage group.

SUMMARY

Thirteen selected distal markers have been mapped by time of entry studies and linkage analysis. They appear to fall on two distinct linkage groups and with the ten previously described there are nineteen markers on one group and four on the other. There is as yet no clear evidence that the two groups are linked, but this possibility has not been excluded.

Of the thirteen markers described, six can be mapped by measuring their time of entry into suitable recipients. It should now be possible to establish the order of any further marker and to provide more accurate information for any marker transferred within 35 min from the time of mixing the cells.

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