Mapping the Chromosome of *Yersinia pseudotuberculosis* by Interrupted Mating

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SUMMARY

By interrupting matings at different times, the sequential transfer of selected and unselected markers to recipient bacteria was followed in *Yersinia pseudotuberculosis*. Under the best mating conditions employed, the earliest marker was transferred within 13 min and the latest within 98 min of impinging mating mixtures on membranes. All tested donor markers appeared in recombinants. A genetic map based on the entry times of markers indicated a single linkage group showing much similarity to that of *Escherichia coli*. The high frequency donor transferred markers in a clockwise direction, its origin corresponding to a point near 60 min on the *E. coli* map.

INTRODUCTION

Other workers have demonstrated fertility in *Yersinia* (formerly *Pasteurella*) *pseudotuberculosis* group D (Lawton, Morris & Burrows, 1968; Morris & Burrows, 1968) and shown that genes could be transferred at a frequency of about 10^{-4} /donor bacterium mediated by the F lac episome which had been introduced from *Escherichia coli* K12 (strain 23.105). A study of the same system led Lawton & Stull (1971) to conclude that there was possibly more than one chromosome in *Y. pseudotuberculosis* group D.

Later, a high frequency donor strain (MRE 4018) was obtained in *Yersinia pseudotuberculosis* group A (McMahon, 1971) which could transfer early markers at a frequency of about 1/25 donor bacteria. Furthermore, genes were transferred with different frequencies suggesting a linear order and recombinants were obtained for all genes examined.

The present paper is an extension of that work and presents quantitative results and map positions for a number of markers obtained by the interrupted mating technique (Wollman & Jacob, 1955, 1958). A genetic map of *Yersinia pseudotuberculosis* was constructed from the entry times of markers and is compared with that of *Escherichia coli*.

METHODS

Strains. The genotypes and origins of the strains of *Yersinia pseudotuberculosis* group A used were as described by McMahon (1971); the same high frequency donor, MRE 4018, was used in all crosses in the present work. In addition, the following strains were used: MRE 4200, a colonially opaque derivative of MRE 4176 which had the same genotype as MRE 4176; MRE 4197, *str-23* from MRE 4018; MRE 4198, *str-24* from MRE 4018. Abbreviations designating genotypes and phenotypes of strains follow the recommendations of Demerec, Adelberg, Clark & Hartman (1968).

The media and buffer were as described by McMahon (1971). Selective media consisted of minimal agar (MA) supplemented with the growth factors of the recipient strain, other

Table 1. *Comparison of agitation methods for effective separation of mating pairs in the cross MRE 4018 × MRE 4176*

Donor (1×10^8) and recipient (3×10^8) bacteria were impinged as a mixture on to a number of membranes and incubated for different periods at 28 °C. The bacteria were removed from the membranes by hand-shaking in buffer, diluted and plated on different selective media so that approximately 1.5×10^4 donor and 4.5×10^4 recipient bacteria were seeded per plate; the suspensions were then agitated with a Towers Vibrator and plated in a similar fashion. The recombinants were recorded after 40 h at 28 °C.

Sample time (min)	Agitation method	Recombinants/plate selected marker			% Unselected markers among selected Met ⁺	
		Met ⁺	Pro ⁺	His ⁺	Pro ⁺	His ⁺
100	Hand	126	21	2	6	1
100	Towers	43	0	0	0	0
110	Hand	523	127	12	17	7
110	Towers	85	0	0	0	0

than that of the selected marker(s). The final concentrations ($\mu\text{g/ml}$) of the growth factors were: bases, 10; L-amino acids, 20; DL-amino acids, 40.

Interrupted mating. Crosses were carried out on membranes (McMahon, 1971), a separate one being used for each sample. Except for earlier experiments at 28 °C, all matings were carried out in a 37 °C hot room. The media, glassware and membrane holders were warmed in a hot room prior to use.

Mixtures of donor and recipient bacteria (4×10^7 donor and 1.2×10^8 recipient) were incubated with rotation in a 37 °C hot room for 1 h to bring them into log-phase growth; mating does not take place during this pre-incubation period (McMahon, 1971). Samples (2 ml) were impinged on to a number of membranes in the shortest practicable time, the time of impingement being taken as time 0. At different intervals, individual membranes were blended for 1 min in 10 ml buffer containing five large glass beads using a Towers Vibrator (J. W. Towers & Co Ltd, Widnes, Lancashire). The blended samples were diluted so that approximately 1.5×10^4 donor and 4.5×10^4 recipient bacteria were seeded/plate from each sample on to the different selective media. The plates were incubated at 28 °C for 2 to 3 days. Controls consisted of plating similar numbers of donor and recipient (taken from the mating mixture just prior to impingement) on similar selective media.

The number of recombinants obtained from each sample (for a particular marker) was plotted against time. The linear part of the plot was extrapolated on to the time axis to give an intercept value, which was taken as the earliest time of entry of the marker.

Purification and analysis of recombinants. The methods were those described by McMahon (1971). Samples of purified selected recombinants were routinely checked on appropriate media, and where possible, a minimum of 100 were analysed for unselected markers.

RESULTS

Separation of mating pairs

It has already been established (McMahon, 1971) that donor and recipient bacteria when plated on selective media in the same numbers as outlined under Methods, did not mate on the plate. Recombinant-free platings from interrupted mating experiments were only obtained when either a Waring blender or a Towers Vibrator was used to separate the mating bacteria. Met⁺, Pro⁺ and His⁺ recombinants were obtained from hand-shaken samples in the

Table 2. *Analysis of recombinants obtained from interrupted matings in the cross MRE 4018 × MRE 4176*

Donor (1×10^8) and recipient (3×10^8) bacteria were impinged as a mixture on to 13 membranes and incubated for different periods in a 37 °C hot room. The bacteria after removal from membranes and blending were diluted and plated on different selective media so that approximately 1.5×10^4 donor and 4.5×10^4 recipient bacteria were seeded per plate. The recombinants were recorded after 40 h of incubation at 28 °C. The control was a sample of the mating mixture taken just prior to impingement, diluted and plated on the three selective media (2.36×10^4 donors/plate). The number of recombinants obtained on the control plates (plate matings) per 10^4 donors plated were Met⁺ 3.39, Pro⁺ 1.98, His⁺ 0.28; the recombinants shown in the Table have been corrected for these control values.

Time of blending (min)...	11	20	30	40	50	60	70	81	90	100	110	120	130
Donors/plate $\times 10^4$ ...	2.15	2.10	2.40	1.86	2.31	1.77	1.62	2.05	1.49	1.38	1.41	1.84	1.06
Recombinants obtained on selection for Met ⁻	{ No. Met ⁺ /10 ⁴ donors plated												
	{ No. analysed												
	{ Recombinant classes												
	{ % Unselected markers												
Recombinants obtained on selection for Pro ⁺	{ No. Pro ⁺ /10 ⁴ donors plated												
	{ No. analysed												
	{ Recombinant classes												
	{ % Unselected markers												
Recombinants obtained on selection for His ⁺	{ No. His ⁺ /10 ⁴ donors plated												
	{ No. analysed												
	{ Recombinant classes												
	{ % Unselected markers												

Fig. 1. Percentages of unselected Pro⁺ (○—○) and His⁺ (△—△) markers among selected Met⁺ markers relative to time of mating.

cross MRE 4018 × MRE 4176 (Table 1). Blending these samples with a Towers Vibrator reduced the number of Met⁺ obtained and prevented the transfer of *pro-11* and *his-21*. Since no loss in viability was detected following this treatment, it was inferred that blending could disrupt mating pairs and prevent the transfer of the more distal markers, *pro-11* and *his-21*.

Interruption of mating at 37 °C

As reported previously (McMahon, 1971), marker transfer frequencies were increased about fourfold when matings were carried out at 37 °C instead of 28 °C. In addition to obtaining these higher frequencies from interrupted matings at 37 °C, selected Met⁺ was recovered about 50 min earlier than from matings at 28 °C. When all operations (except blending and plating), were carried out in a 37 °C hot room, entry times were further reduced by about 15 min. Under the same conditions, selected Pro⁺ was recovered about 80 min earlier and selected His⁺ about 115 min earlier.

An interrupted mating experiment was carried out under these improved conditions using MRE 4018 and MRE 4176; mating was allowed to proceed up to 130 min, an individual membrane being taken every 10 min for analysis (Table 2). It is apparent from these results that the three donor markers were transferred into the recipient at different times, the approximate times of entry being 30 min for Met⁺, 60 min for Pro⁺ and 80 min for His⁺. The recombinant classes obtained were those that would be expected from a linear transfer of genes: an increase with time for markers more distal than the selected marker and a fairly constant number of markers which were proximal to that selected. The percentages of unselected distal markers among selected Met⁺ are shown as a function of time in Fig. 1, the intercepts on the time axis being in good agreement with the estimates of the times of entry of these markers deduced from direct selection. None of the recombinants examined inherited Lac⁺.

Table 3. *Time intercept values obtained from interrupted matings with donor MRE4018*

Donor (1×10^8) and recipient (3×10^8) bacteria were impinged as a mixture on to membranes and incubated for different periods in a 37°C hot room. The bacteria after removal from membranes and blending were diluted and plated on different selective media so that approximately 1.5×10^4 donor and 4.5×10^4 recipient bacteria were seeded per plate. The recombinants were recorded and picked for analysis after 2 days of incubation at 28°C . Time intercepts were obtained for selected markers by plotting their increase in number against time and extrapolation on to the time axis; intercepts were obtained for unselected markers by plotting and extrapolating the numbers of unselected markers, associated with a selected marker, against time.

Recipient strain (MRE no.)	Selected marker	Timed marker	Intercept value of timed marker (min)	
			Selected	Unselected
4177	<i>met-2</i>	<i>tyr-7</i>	—	67
	<i>tyr-7</i>	<i>tyr-7</i>	71	—
4177	<i>tyr-7</i>	<i>his-22</i>	—	77
4178	<i>his-22</i>	<i>his-22</i>	81	—
4179	<i>leu-5</i>	<i>his-22</i>	—	78
	<i>his-22</i>	<i>his-22</i>	81	—
4179	<i>leu-5</i>	<i>leu-5</i>	44	—
	<i>met-2</i>	<i>leu-5</i>	—	41
	<i>leu-5</i>	<i>leu-5</i>	46	—
4180	<i>met-2</i>	<i>met-2</i>	28	—
	<i>ilv-4</i>	<i>met-2</i>	—	P
4183	<i>met-2</i>	<i>met-2</i>	31	—
	<i>pur-15</i>	<i>met-2</i>	—	28
4155	<i>met-2</i>	<i>met-2</i>	32	—
4194	<i>met-2</i>	<i>met-2</i>	34	—
	<i>met-2</i>	<i>met-2</i>	26	—
4185	<i>ilv-3</i>	<i>met-2</i>	—	P
4188	<i>met-2</i>	<i>met-2</i>	32	—
4179	<i>met-2</i>	<i>met-2</i>	31	—
4186	<i>met-2</i>	<i>met-2</i>	31	—
4200	<i>met-2</i>	<i>met-2</i>	29	—
4180	<i>ilv-4</i>	<i>ilv-4</i>	36	—
	<i>met-2</i>	<i>ilv-4</i>	—	P
4181	<i>met-2</i>	<i>pyr-1</i>	—	56
4182	<i>met-2</i>	<i>pyr-1</i>	—	54
4200	<i>met-2</i>	<i>pro-11</i>	—	54
	<i>pro-11</i>	<i>pro-11</i>	54	—
	<i>pro-11</i>	<i>pro-11</i>	55	—
4200	<i>pro-11</i>	<i>his-21</i>	—	82
	<i>his-21</i>	<i>his-21</i>	79	—
	<i>his-21</i>	<i>his-21</i>	80	—
4183	<i>met-2</i>	<i>pur-15</i>	—	P
	<i>pur-15</i>	<i>pur-15</i>	25*	—
4184	<i>met-2</i>	<i>pur-15</i>	—	P
	<i>pur-15</i>	<i>pur-15</i>	25*	—
4184	<i>met-2</i>	<i>tyr-8</i>	—	66
4183	<i>met-2</i>	<i>his-26</i>	—	78
4155	<i>arg-40</i>	<i>arg-40</i>	48	—
	<i>met-2</i>	<i>arg-40</i>	—	44
	<i>met-2</i>	<i>arg-40</i>	—	43
4194	<i>met-2</i>	<i>leu-7</i>	—	44
4191	<i>met-2</i>	<i>pur-19</i>	—	44
	<i>met-2</i>	<i>pur-19</i>	—	41
4191	<i>met-2</i>	<i>his-28</i>	—	46
	<i>met-2</i>	<i>his-28</i>	—	45
4185	<i>ilv-3</i>	<i>ilv-3</i>	29*	—
4186	<i>ilv-3</i>	<i>ilv-3</i>	30*	—
4188	<i>met-2</i>	<i>ilv-3</i>	—	P
4187	<i>met-2</i>	<i>ilv-3</i>	—	P
4185	<i>met-2</i>	<i>his-27</i>	—	79
4186	<i>ilv-3</i>	<i>leu-6</i>	—	46
4188	<i>met-2</i>	<i>ser-6</i>	—	98

P, Unselected markers which had reached high plateau values (80 to 100 %) indicative of early transfer or of close linkage to the selected marker.

* These markers are proximal to *met-2* and appear to be closely linked; the intercept values were difficult to determine and are only approximate.

Mapping by interrupted mating

Using the technique of interrupted mating, the times of entry of 19 markers carried by 18 different recipient strains were determined. Both selected and unselected intercept values (Methods) were used in determining the earliest times of marker entry, which are shown in Table 3. Selected and unselected intercept values for markers were in good agreement, as were intercept values for the same marker in different strains. The two markers *pur-15* and *ilv-3* appeared to be proximal to *met-2* in that their intercepts obtained from direct selection were earlier (although this was not significantly so for *ilv-3*), but also in that 100 % of selected Met^+ were also Pur^+ or Ilv^+ in the appropriate crosses; in addition, the only unselected intercept for *met-2* was obtained from a *pur-15*⁻ selection. The marker *ilv-4* (which determined a requirement for isoleucine only unlike *ilv-3* which determined a requirement for isoleucine + valine) appeared to be distal to *met-2* although closely linked to it. Six markers showed entry times in a cluster around 45 min among which was *his-28*; this was distinct from the remaining four *His* markers which had entry times around 80 min. The latest entering marker was *ser-6* at 98 min.

In uninterrupted matings between MRE 4018 and MRE 4188 the ultimate terminal marker, integrated F, could also be transferred. In a selection for Ser^+ recombinants, 28 % were found to have inherited Lac^+ . The only class among these recombinants which could be tested for fertility was *ser-6*⁺ *met-2* *ilv-3* Lac^+ and of these 80 % proved to be fertile donors. A high proportion of recombinants, which proved to be donors, could also be obtained by selecting Ser^+ and Lac^+ jointly. The frequency of recombinants obtained for *ser-6* was about $1/10^5$ donor bacteria, and the frequency with which integrated F could be transferred, was about $1/3 \times 10^5$ donor bacteria.

Transfer of the streptomycin marker

Four independently isolated SmR derivatives of MRE 4176 (*met-2*, *pro-11*, *his-21*) were used as recipients in interrupted matings with the SmS donor MRE 4018. The numbers of selected Met^+ , Pro^+ and His^+ recombinants recovered from the four recipients in the presence of streptomycin were about 50 % of the numbers recovered in the absence of streptomycin from all interrupted samples. Analysis of Met^+ progeny for the co-inheritance of SmS (selected in the absence of streptomycin) showed that 53 % to 58 % were SmS over a sampling range of 30 to 75 min. These findings suggested that *str* was proximal to *met-2*.

When two SmR isolates (MRE 4197, 4198) of donor MRE 4018 were used in crosses with an SmS recipient (MRE 4200), it was found that 6 to 8 h of incubation in the absence of streptomycin was necessary for the segregation of SmR recombinants. Unselected analysis

Table 4. *Analysis of SmR recombinants obtained from the cross MRE 4198 × MRE 4200*

Donor (1×10^8) and recipient (3×10^8) bacteria were impinged as a mixture on to five membranes and incubated from 15 to 75 min at 37 °C. The bacteria, after removal from the membranes, were blended and spread on to 4 cm Oxoid membranes on plates supplemented with methionine, proline and histidine and incubated for 8 h at 28 °C. The 4 cm membranes were then transferred on to the same media plus streptomycin and incubated for a further 36 h at 28 °C. SmR recombinants were analysed for the unselected auxotrophic markers.

Time (min) . . .		15	30	45	60	75
% of selected <i>str-24</i> which were	$\left\{ \text{Met}^+ \right.$	0	0	10.8	26.8	34.1
	Pro^+	0	0	1.2	4.7	3.5
	His^+	0	0	0	0	2.4

Fig. 2. The chromosome map of *Yersinia pseudotuberculosis* group A based on marker entry times (min) at 37 °C. The broken part represents a postulated lag period prior to the onset of transfer. The inner map shows the positions of some of the equivalent loci in *Escherichia coli* (taken from the data of Taylor, 1970).

of such recombinants (Table 4) showed that *met-2*, *pro-11* and *his-21* were distal to *str-24*. The intercept values obtained from direct selection for *str-24* was 13 min and for *str-23*, 15 min.

Map construction

The data contained in Table 3 together with that obtained for the transfer of *str-23* and *str-24* were used to construct a chromosome map of *Yersinia pseudotuberculosis* based on the entry times of the different markers at 37 °C. The map is shown in circular form in Fig. 2 and by way of comparison, the map of *Escherichia coli* (constructed from data of Taylor, 1970) is also shown.

DISCUSSION

It was previously established (McMahon, 1971) that mating in crosses between strains of *Yersinia pseudotuberculosis* did not take place in broth culture at bacterial densities of 3×10^8 /ml but that cell to cell contact on membranes was necessary. The present work shows that once mating had commenced, very vigorous methods of blending were necessary in order to separate mating pairs. By obtaining graded numbers of recombinants following the separation of mating pairs at different times, confirmation of the linear transfer of genes by a conjugation process in *Yersinia pseudotuberculosis* was obtained, a process analogous to that described by Matney & Achenbach (1962) for *Escherichia coli* and by Sanderson & Demerec (1965) for *Salmonella typhimurium*.

The map of *Yersinia pseudotuberculosis* (Fig. 2) resembles that previously constructed from the relative frequencies of marker transfer (McMahon, 1971). The most difficult markers to position accurately were *pur-15*, *ilv-3* and *ilv-4* because positioning depended on intercepts from direct selection only and although the intercept for *pur-15* was different from *met-2*, that for *ilv-3* was virtually identical. The conclusion that *pur-15* and *ilv-3* were proximal to *met-2* was based on the fact that the numbers of recombinants relative to time was always greater for these markers than for *met-2*. By similar reasoning, *ilv-4* was thought to be distal to *met-2*.

Although the enzyme deficiencies for the mapped genes are not known, it is reasonable to deduce the probable loci involved from the growth responses of the mutants (McMahon, 1971) bearing them. Thus, *met-2* was considered to be a *metA* or *B*, *arg-40* to be an *argF*, *pyr-1* (a one step mutation resulting in requirements for arginine + uracil) to be a *cap* mutant (Pierard, Glansdorff, Mergeay & Wiame, 1965) and classified as a *pyrA* (Taylor, 1970). Strains bearing *pur-15* and *pur-19* have different growth requirements suggesting that the loci involved are different; likewise *ilv-3* and *ilv-4* are probably non-allelic. A comparison of the two maps in Fig. 2 shows that all the markers in *Yersinia pseudotuberculosis*, with the exception of *pur-19* and *pyr-1*, are comparable with those of *Escherichia coli*. It would appear that *his-28* is a second *his* locus which is not present in *E. coli*.

No direct evidence was obtained to confirm the circularity of the chromosome since transfer was promoted from one site only and donors, having different sites of integration, were not obtained (unpublished). However, the results indicate both a single linkage group and the transfer of the fertility factor as an integrated terminal marker, a situation analogous to that found in *Escherichia coli*. These similarities indicate that the chromosome of *Yersinia pseudotuberculosis* may also be circular. Pemberton & Holloway (1972) have reported a single linkage group for *Pseudomonas aeruginosa* but, unlike the present work, found no similarities to the *E. coli* map.

If the assumption is made that the chromosome of *Yersinia pseudotuberculosis* is circular and the comparison with *Escherichia coli* as shown in Fig. 2 is meaningful, then the origin of donor MRE4018 should lie between 57 and 64 min in terms of the *E. coli* map. Donor MRE4018, when used with a group D recipient (MRE2291), was able to transfer a presumed *argG* as a lead marker and a presumed *metC* as a terminal marker (unpublished). This would position the origin between 59 and 61 min. Since the estimated position of the origin is approximately 11 min from time 0 on the map, it was postulated that this constituted a lag period before transfer commenced following impingement on membranes.

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