High-frequency Gene Transfer in *Pasteurella pseudotuberculosis*

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(Accepted for publication 21 September 1971)

SUMMARY

A high-frequency donor strain of *Pasteurella pseudotuberculosis* group A was isolated from a strain which had previously accepted an *F'*lac episome from *Escherichia coli*. This donor transferred a variety of genetic markers to *P. pseudotuberculosis* group A recipients. Transfer frequencies of approximately 10^{-2} /donor bacterium were obtained for early markers from membrane matings at 28°; these frequencies were increased to about 2.5×10^{-1} /donor at 37°. Recombinant colonies obtained from membrane matings were free from donor and recipient bacteria but some colonies had more than one recombinant class present. The graded frequencies of marker transfer obtained together with recombinant analysis suggest that markers were transferred linearly.

INTRODUCTION

Strains of *Pasteurella pseudotuberculosis* were initially subdivided into serological types I to V on the basis of agglutination reactions (Thal, 1954). Lipopolysaccharides, specific for each of these types, were chemically characterized by Davies (1958), who redesignated the types I to V as serological groups A to E respectively. Working with strains of group D, Lawton, Morris & Burrows (1968) observed that infection of some strains with the *F'*lac episome, donated by *Escherichia coli* K12 (strain 23.108), permitted gene transfer from them to recipient strains of group D. Early markers were transferred at a frequency of about 10^{-4} /donor bacterium. Rigorous controls were used to prove that gene transfer in fact occurred and that their recombinants were not the consequence of reversion or syntrophic growth of parent strains. This work was extended by Morris & Burrows (1968), who, by unselected marker analysis, produced a tentative ordering of seven gene loci for strains of group D.

A strain of group A which had acquired *F'*lac from *Escherichia coli* K12 (strain 23.108) proved to be a fertile donor which transferred unselected markers more efficiently than the group D donor in crosses with a group D recipient strain. Further tests with this group A donor in crosses with recipients of group A showed it to donate early genes at high frequency (about 2.5×10^{-1} /donor bacterium) as reported in this paper.

METHODS

Strains. *Escherichia coli* K12 strain 23.108 Met⁻ (*F'*lac) was originally supplied to this laboratory by Dr R. C. Clowes. The two starting strains of *Pasteurella pseudotuberculosis* group A, MRE1300 (*met-2*) and MRE1317 (*nic-2*) had been derived in this laboratory by nitrous acid treatment (Kaudewitz, 1959) of the prototrophic strain MRE1224. Strain MRE1224 (synonym PB 1/+) has been described fully by Burrows & Bacon (1960). The isolation and properties of the donor strain MRE4018 are described under Results.

Spontaneously arising auxotrophs were enriched using a modification of the penicillin screening technique (Davis, 1948; Lederberg & Zinder, 1948). Ampicillin was added to cultures to a final concentration of 40 $\mu\text{g./ml.}$ after a 5 h. period of starvation in minimal medium at 28°. After 16 h. incubation in ampicillin at 28°, the bacteria were centrifuged, resuspended in broth and grown to saturation. After a second round of ampicillin treatment the bacteria were plated on complete media. Auxotrophs were characterized by replicating on to defined media containing pools of growth factors (Holliday, 1956). Abbreviations designating genotypes and phenotypes of strains listed in Tables 1 and 2 follow the recommendations of Demerec, Adelberg, Clark & Hartman (1968).

Media. Nutrient broth (NB) no. 2 and blood agar base (BAB) no. 2 (Oxoid Ltd, London) were used as complete media. Minimal agar medium (MA), minimal salts solution (MS) and lactose indicator medium were as described in Lawton *et al.* (1968). Buffer (pH 7.6) was (g./l.) KH_2PO_4 , 67.5; $(\text{NH}_4)_2\text{SO}_4$, 7.5; NH_4Cl , 7.5.

Plate crosses. Donor and recipient strains were inoculated separately from BAB slopes

Table 1. *Genotypes of strains of Pasteurella pseudotuberculosis, group A*

MRE strain nos.		Mutant loci and mutation sites											
Strain	Source	<i>nic</i>	<i>met</i>	<i>his</i>	<i>pro</i>	<i>pyr</i>	<i>pur</i>	<i>lys</i>	<i>arg</i>	<i>ilv</i>	<i>leu</i>	<i>tyr</i>	<i>ser</i>
1300	1224	+	2	+	+	+	+	+	+	+	+	+	+
1317	1224	2	+	+	+	+	+	+	+	+	+	+	+
4018*	1317	2	+	+	+	+	+	+	+	+	+	+	+
4135	1300	+	2	22	+	+	+	+	+	+	+	+	+
4136	1300	+	2	+	11	+	+	+	+	+	+	+	+
4137	1300	+	2	+	+	1	+	+	+	+	+	+	+
4139	1300	+	2	+	10	+	+	+	+	+	+	+	+
4140	1300	+	2	+	+	+	15	+	+	+	+	+	+
4146	1300	+	2	+	+	+	+	7	+	+	+	+	+
4149	1300	+	2	+	+	+	+	+	42	+	+	+	+
4150	1300	+	2	+	+	+	16	+	+	+	+	+	+
4151	1300	+	2	+	+	+	17	+	+	+	+	+	+
4153†	1300	+	2	+	+	+	+	+	+	3	+	+	+
4155†	1300	+	2	+	+	+	+	+	40	+	+	+	+
4157	1300	+	2	+	+	+	19	+	+	+	+	+	+
4161	1300	+	2	+	+	+	+	+	+	+	4	+	+
4162	1300	+	2	+	+	+	+	+	+	+	+	6	+
4168†	1300	+	2	24	+	+	+	+	+	+	+	+	+
4174†	1300	+	2	+	+	+	+	+	+	+	+	+	4
4194	1300	+	2	+	+	+	+	+	+	+	7	+	+
4176	4136	+	2	21	11	+	+	+	+	+	+	+	+
4177	4135	+	2	22	+	+	+	+	+	+	+	7	+
4178	4135	+	2	22	+	+	+	+	+	+	+	+	5
4179	4135	+	2	22	+	+	+	+	+	+	5	+	+
4180	4135	+	2	22	+	+	+	+	+	4	+	+	+
4181	4137	+	2	+	+	1	23	+	+	+	+	+	+
4182	4137	+	2	25	+	1	+	+	+	+	+	+	+
4183	4140	+	2	26	+	+	15	+	+	+	+	+	+
4184	4140	+	2	+	+	+	15	+	+	+	+	8	+
4185	4153	+	2	27	+	+	+	+	+	3	+	+	+
4186	4153	+	2	+	+	+	+	+	+	3	6	+	+
4187	4153	+	2	+	+	+	24	+	+	3	+	+	+
4188	4153	+	2	+	+	+	+	+	+	3	+	+	6
4189	4153	+	2	+	+	+	25	+	+	3	+	+	+
4191	4157	+	2	28	+	+	19	+	+	+	+	+	+

* Infected with (*F'**lac*) from *Escherichia coli* 23.10s and selected for high-frequency donor ability.

† Isolated following u.v. irradiation of strain MRE 1300.

into NB and incubated for 18 h. at 28° with rotation. The cultures were diluted (1 in 9) in fresh NB and incubated with rotation at 28° for a further 90 min. to bring them into log-phase growth. The cultures were diluted in buffer, mixed and plated on selective media. The plates were incubated for 4 days at 28°. Controls consisted of similar numbers of donor and recipient bacteria plated separately on similar media.

Membrane crosses. The log-phase cultures prepared as above, were diluted in NB to give approximately 8×10^7 donor and 2.4×10^8 recipient bacteria/ml. Equal volumes of donor and recipient bacteria were mixed and a 2 ml. sample was filtered through a 13 mm. Millipore membrane (Millipore Corporation, Bedford, Massachusetts, U.S.A.). The membrane was incubated on a BAB plate or, in later experiments, incubated in its holder together with a broth-impregnated disc beneath the membrane. Mating was allowed to proceed for 4 h. at 28° or for 2 h. at 37°. Bacteria were washed from the membrane, diluted in buffer and plated on selective MA so that approximately 1.5×10^4 donor and 4.5×10^4 recipient bacteria were seeded per plate. The plates were incubated at 28° for 48 h. Controls consisted of similar numbers of donor and recipient bacteria plated following their separate impingements on to membranes or from the mating mixture just prior to impingement.

Purification and analysis of recombinants. Colonies appearing on the different selective

Table 2. *Growth responses of mutants*

Mutant locus	Mutant site(s)	Effective growth factors
<i>met</i> *	2	Cystathionine (but not homoserine)
<i>his</i>	21, 22, 24, 25, 26, 27, 28	Histidine
<i>pro</i>	10, 11	Proline
<i>pyr</i> *	1	Uracil + citrulline (but not uracil + ornithine)
<i>pur</i>	17, 25	Adenine
	19, 23, 24	Adenine or hypoxanthine
	16	Adenine or guanine
	15	Hypoxanthine or guanine
<i>lys</i>	7	Lysine
<i>arg</i> *	40, 42	Citrulline (but not ornithine)
<i>ilv</i>	3	Isoleucine + valine
	4	Isoleucine
<i>leu</i>	4, 5, 6, 7	Leucine
<i>tyr</i>	6, 7, 8	Tyrosine
<i>ser</i>	4, 5, 6	Serine

* These mutants were tested on the available intermediates in the biosynthetic pathways; growth was also obtained with the later intermediates.

Table 3. *The numbers of Pur⁺ recombinants obtained from plate crosses of donor MRE4018 and recipient MRE4140 plated in different proportions*

Donor and recipient bacteria were mixed in different proportions, plated on MA + methionine + 0.01 % NB and incubated for 5 days at 28°. 1.7×10^9 donor and 1.5×10^9 recipient bacteria, plated separately on similar media and incubated for 5 days at 28°, gave no reversions.

No. of donor bacteria mixed with 1.5×10^9 recipient bacteria/plate	No. of Pur ⁺ recombinants/plate	No. of recipient bacteria mixed with 1.7×10^9 donor bacteria/plate	No. of Pur ⁺ recombinants/plate
1.7×10^5	Confluent	1.5×10^5	832
1.7×10^4	about 5000	1.5×10^4	112
1.7×10^3	714	1.5×10^3	20
1.7×10^2	164	1.5×10^2	0

Fig. 1. Plate crosses. Effects of numbers of donor bacteria, of the ratios of recipient to donor bacteria plated on selective media and of the period of incubation of the plates on the yields of $\text{Met}^+ \text{Pro}^+$ recombinants from the cross MRE4018 $\times$ MRE4139. Number of donor bacteria per plate: (a) 5.6×10^8 ; (b) 1.9×10^8 ; (c) 9.3×10^2 . Multiplicity of recipient to donor: ○ = 10, □ = 5, △ = 2, ● = 1, ■ = 0.5, ▲ = 0.2.

media from plate crosses were always streaked (or plated at low density) on plates of similar media and single colonies from these streaks examined for their requirements. Initially, colonies from membrane crosses were treated in the same way until it was found that samples of young (40 to 50 h.) colonies, taken with a finely pointed needle under a dissecting microscope, were invariably pure. This latter method was used to purify most of the recombinants obtained from membrane crosses.

Purified recombinants were analysed for their requirements by replication from a BAB master plate to variously supplemented MA media using a multi-point replicator. Where the compositions of selected recombinant colonies were examined, plates from a low-density plating of the recombinant colony on BAB were replicated (Lederberg & Lederberg, 1952) to variously supplemented MA media using a velvet pad.

RESULTS

Isolation of donor strain MRE4018. Cultures of *Escherichia coli* 23.10S and *Pasteurella pseudotuberculosis* MRE1317 were mixed and plated on medium selective for $\text{Lac}^+ \text{Nic}^-$ bacteria. The purified colonies obtained were indistinguishable from MRE1317 except that they were Lac^+ . One colony was retained as a dried gelatin pellet (Stamp, 1947) and when crossed with *P. pseudotuberculosis* recipients was able to transfer a number of genetic markers as well as the Lac^+ episome.

Many cultures of this strain (derived from Lac^+ single colonies) accumulated in this laboratory and when five were re-tested for fertility one was found to be much more fertile than the rest. Four of the 42 Lac^+ isolates tested proved to be significantly more fertile than the rest. One of the four was retained, given the number MRE4018, and used as a donor in the crosses reported here. The highly fertile donor had an enhanced Lac^+ stability; less than 1 in 300 colonies was Lac^- .

Features of the mating process

Plate mating. Mating mixtures containing different proportions of donor (MRE4018) and recipient (MRE4140) organisms were plated on media selective for Pur⁺. The numbers of recombinants obtained are shown in Table 3. Virtually all the donors plated gave rise to a colony from which recombinants could be isolated in crosses in which the donor was the minority parent. Crosses in which the parental ratios were reversed were less fertile.

Crosses in which the parental bacteria were separated by a membrane filter or in which the normal donor was replaced by an F-lac⁻ derivative of it did not give recombinant colonies on selective media.

Mating on selective plates was studied by plating few bacteria so that the number of initial contacts was low (Fig. 1). It is apparent from these results that the greater the multiplicity of recipient to donor, the more recombinants were formed for each of the three levels of donor input; similarly, the greater the donor input for a given multiplicity of recipient,

Table 4. *Fertility of mating mixtures of donor MRE4018 and recipient MRE4139 held on membranes*

Donor (1×10^8) and recipient (3×10^8) bacteria were impinged as a mixture on to membranes then incubated for 4 h. at 28° on BAB. The bacteria were washed from the membranes, diluted and plated on selective media. Similar numbers of donor and recipient bacteria, which had been impinged on to separate membranes and incubated for the same period, were washed from the membranes, mixed, diluted and plated. The recombinants were recorded after 40 h. at 28°.

	No. of donor bacteria (thousands)/plate	No. of recipient bacteria (thousands)/plate	No. of Pro ⁺ recombinants	
			Plate 1	Plate 2
Donor and recipient bacteria impinged together on membrane	9.7	13.7	25	24
	4.9	6.9	10	7
	2.5	3.5	5	5
Donor and recipient bacteria impinged on separate membranes	11.8	15.8	0	0
	5.9	7.9	0	0
	2.9	3.9	0	0

Table 5. *Composition of colonies selected for Pro⁺ in the membrane cross MRE4018 × MRE4139*

Individual selected Pro⁺ colonies were suspended in buffer, serially diluted and plated on BAB. Suitable plates were then replicated with velvet pads to appropriately supplemented MA. In all, 47 colonies were examined, a representative sample of which is shown in the table.

Selected Pro ⁺ colony	No. analysed	Progeny			
		No. of recombinants		No. of parental types	
		Met ⁺	Met ⁻	Nic ⁻	Met ⁻ Pro ⁻
1	380	0	380	0	0
2	328	328	0	0	0
3	206	206	0	0	0
4	129	24	105	0	0
5	119	82	37	0	0
6	163	0	163	0	0
7	168	168	0	0	0
8	112	70	42	0	0
9	109	0	109	0	0
10	163	33	130	0	0

the greater the number of recombinants formed. It had been observed that donor and recipient bacteria, when plated at low density such as in this experiment, would slowly grow into microscopic colonies. The results shown in Fig. 1 are consistent with the idea that recombinants arose on selective plates when microscopic colonies of opposite mating types contacted one another.

Membrane mating. Attempts were made to mimic the close contact of donor and recipient bacteria which was believed to be necessary to produce recombinants. The method which gave the most recombinants was to impinge a mixture of donor and recipient on to a Millipore membrane (13 mm.) so that a total of about 4×10^8 bacteria was deposited (Methods). MRE4018 and MRE4139 were crossed at 28° for 4 h. in this manner and the numbers of donor and recipient bacteria plated were low enough to exclude mating on these plates in 40 h. The results of this cross (Table 4) demonstrate that mating took place exclusively during the 4 h. the donor and recipient were together on the membrane.

Samples were taken at 40 h. from 47 of these selected Pro⁺ recombinants and analysed. Table 5 shows, as examples, the analyses obtained for 10 of them. No donor or recipient bacteria were found in any of the recombinant colonies analysed. Of the recombinants, 23 were Met⁻ and 13 were Met⁺. The remaining 11 recombinants were mixtures of Met⁺ and Met⁻; of these, 6 were between 10 and 29 % Met⁺ and 5 were between 56 and 88 % Met⁺. There was no suggestion of persistent instability in the *met* locus in the bacteria giving rise to these mixed clones; progeny from the mixed clones, when retested in the same manner, were found to be stable. It could be argued that the smaller fraction in these clones segregated from the zygote after the larger fraction had segregated.

Table 6. *Frequencies of recombinants selected for single and for double marker transfer in membrane crosses with donor MRE4018*

Donor (1×10^8) and recipient (3×10^8) bacteria were impinged as a mixture on to membranes and incubated for 4 h. at 28° on BAB. The bacteria were washed from the membranes, 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 obtained are expressed per 10^4 donor bacteria plated. Control platings showed no revertant colonies.

Recipient strains		Selected recombinants/ 10^4 donor bacteria		
MRE no.	Genotype	<i>met-2</i> *	Second marker	Both markers
4135	<i>met-2 his-22</i>	.	0.2	0.0
4136	<i>met-2 pro-11</i>	.	12.8	3.5
4137	<i>met-2 pyr-1</i>	.	10.8	2.3
4139	<i>met-2 pro-10</i>	43.5	4.4	0.5
4140	<i>met-2 pur-15</i>	.	23.7	9.3
4146	<i>met-2 lys-7</i>	41.8	0.0†	0.0
4149	<i>met-2 arg-42</i>	.	61.0	23.0
4150	<i>met-2 pur-16</i>	.	69.3	27.0
4151	<i>met-2 pur-17</i>	.	1.0	0.6
4153	<i>met-2 ilv-3</i>	.	108.0	49.0
4155	<i>met-2 arg-40</i>	104.8	44.0	11.6
4157	<i>met-2 pur-19</i>	15.24	14.2	5.2
4161	<i>met-2 leu-4</i>	.	29.4	6.8
4162	<i>met-2 tyr-6</i>	91.5	0.7	0.7
4168	<i>met-2 his-24</i>	97.4	0.3	0.3
4174	<i>met-2 ser-4</i>	.	0.6	0.0

* Average: 88.6.

† *lys-7*⁺ recombinants were obtained when larger numbers of bacteria were plated on selective media.

Fig. 2. Frequencies of selection per 100 donor bacteria for single markers (abscissa) and for the same markers in conjunction with *met-2* (ordinate) (○). --- represents the expected plot, assuming a 0.5 probability of *met-2* being associated with distal markers in recombinants.

Frequency of gene transfer at 28°

The membrane mating technique was used to assess the frequency of gene transfer to several doubly marked strains. Selections were made for the second marker and also both markers jointly in each strain. The results in Table 6 show that different markers were transferred with different frequencies, the common marker, *met-2*, having the second highest frequency of transfer. If the assumption is made that markers are transferred in a linear order as in *Escherichia coli* (Hayes, 1968), then *ilv-3* is proximal and the remaining markers distal to *met-2*. The frequencies of the single marker selections as a percentage of the donor bacteria plated should indicate the approximate order of these markers on the chromosome (Fig. 2). The frequencies of double selections are shown plotted on the ordinate of Fig. 2 and there is a good correlation between the marker position on the abscissa and its frequency of joint selection with *met-2*. This relationship breaks down for markers more distal than *pro-10*, presumably because the number of recombinants for these markers was very low.

Unselected marker analysis of recombinants from nine of these crosses showed lower percentages of unselected 'second' markers among selected *met-2*⁺ recombinants than of *met-2*⁺ in recombinants selected for the 'second' markers. The single exception was for

selected *ilv-3*⁺, which had 52 % unselected *met-2*⁺ whereas selected *met-2*⁺ showed 69 % unselected *ilv-3*⁺. Of the remaining analyses, the proportion of unselected *met-2*⁺ ranged from 44 % of selected *leu-4*⁺ to 28 % of selected *pyr-1*⁺. These results are consistent with markers being transferred linearly in the order shown in Fig. 2.

Frequency of gene transfer at 37°

When membranes, impinged with mating mixtures, were incubated at 37°, the frequency of transfer of *met-2* was increased from approximately 10^{-2} to 2.5×10^{-1} /donor bacterium; in addition, recombinants could be obtained for most markers after 2 h. mating.

Strains MRE4155, 4194 and 4176 to 4191 (Table 1) were used as recipients in a series of crosses with donor MRE4018 to relate the frequency of transfer of the additional markers to that of the common marker, *met-2*. The method of crossing and the controls used were the same as for determining frequency of gene transfer at 28° except that matings were carried out at 37° and terminated after 2 h. Table 7 shows, in addition to the numbers of recom-

Table 7. *Numbers of recombinants obtained with single selections for different markers and their frequencies relative to that for singly selected met-2 in membrane crosses with donor MRE4018*

Donor (1×10^8) and recipient (3×10^8) bacteria were impinged as a mixture on to membranes and incubated for 2 h. at 37°. The bacteria, washed from the membranes and diluted, were 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 numbers in the table are the sums of colonies on two plates; the results obtained from repeat experiments are shown in sequence.

Recipient strains MRE no.	A		B		C		Marker frequencies relative to <i>met-2</i>	
	Selected marker	No.	Selected marker	No.	Selected marker	No.	B/A	C/A
4155	<i>met-2</i>	214	<i>arg-40</i>	95	.	.	0.44	.
		178		113	.	.	0.64	.
4176	<i>met-2</i>	307	<i>pro-11</i>	75	<i>his-21</i>	12	0.24	0.04
		403		94		12	0.23	0.03
		556		158		30	0.28	0.05
4177	<i>met-2</i>	784	<i>his-22</i>	25	<i>tyr-7</i>	104	0.03	0.13
4178	<i>met-2</i>	620	<i>his-22</i>	22	<i>ser-5</i>	14	0.04	0.02
4179	<i>met-2</i>	954	<i>his-22</i>	25	<i>leu-5</i>	352	0.03	0.37
4180	<i>met-2</i>	571	<i>his-22</i>	19	<i>ilv-4</i>	554	0.03	0.97
4181	<i>met-2</i>	260	<i>pyr-1</i>	184	<i>pur-23*</i>	0	0.71	0
4182	<i>met-2</i>	539	<i>pyr-1</i>	174	<i>his-25</i>	21	0.32	0.04
4183	<i>met-2</i>	262	<i>pur-15</i>	374	<i>his-26</i>	9	1.43	0.03
4184	<i>met-2</i>	172	<i>pur-15</i>	267	<i>tyr-8</i>	84	1.55	0.49
4185	<i>met-2</i>	344	<i>ilv-3</i>	584	<i>his-27</i>	9	1.70	0.03
4186	<i>met-2</i>	716	<i>ilv-3</i>	658	<i>leu-6</i>	274	0.92	0.38
		496		446		149	0.90	0.30
4187	<i>met-2</i>	544	<i>ilv-3</i>	820	<i>pur-24*</i>	0	1.51	0
		614		526		0	0.86	0
4188	<i>met-2</i>	443	<i>ilv-3</i>	532	<i>ser-6*</i>	0	1.20	0
		458		465		0	1.02	0
4189	<i>met-2</i>	386	<i>ilv-3</i>	662	<i>pur-25</i>	179	1.72	0.46
		484		470		126	0.97	0.26
4191	<i>met-2</i>	770	<i>pur-19</i>	291	<i>his-28</i>	106	0.38	0.14
		128		58		41	0.45	0.32
4194	<i>met-2</i>	350	<i>leu-7</i>	181	.	.	0.52	.

* Recombinants were obtained for these markers when larger numbers of bacteria were plated on selective media.

Fig. 3. Frequencies of selected markers relative to selected *met-2* recombinants. Recombinants were not obtained in these experiments for markers shown inside brackets.

binants obtained, second and third marker selections expressed as a ratio of the number of selected *met-2*⁺ within each cross.

The number of selected *met-2*⁺ obtained varied from one cross to another and also between repeats of the same cross. This was presumably due in part to the variation between recipient strains but mostly to the variation in the numbers of donor and recipient plated. However, this variation was usually reflected in the other selections so that the ratios obtained for the same marker in different strains or from repeat estimates in the one cross were in general reasonably consistent. Provided there is no restriction on *met-2*⁺ recombination, then these ratios should indicate the relative order of markers to *met-2* on the chromosome. The ratios from Table 7 are shown plotted on a log scale in Fig. 3; average ratios are plotted for markers having more than one estimate. Fig. 3 could be regarded as constituting a qualitative map of the chromosome of *Pasteurella pseudotuberculosis* group A.

DISCUSSION

The method by which the highly fertile donor strain (MRE4018) was derived suggests that a rare event, possibly an integration of F into the chromosome, occurred during its storage at 4°. Its higher fertility, together with an enhanced Lac⁺ stability, distinguished this derivative from other *F'*lac bacteria of the same strain. Unlike the donor strain of *Pasteurella pseudotuberculosis* (MRE2027) used by Lawton *et al.* (1968), treatment of MRE4018 with ultraviolet light did not enhance its fertility, a situation analogous to that for *Hfr* strains of *Escherichia coli* (Hayes, 1953). The highly fertile derivative strain grew less rapidly than the original *F'*lac strain on MA supplemented with nicotinamide, having become, in addition, partially dependent on methionine. This situation is similar to that reported for *Hfr* derivatives of *E. coli* strain C (Sasaki & Bertani, 1965). The same authors defined *Hfr* derivatives of *E. coli* strain C as those donating their lead markers at frequencies of not less than 1/100 donor bacteria plated. Donor strain MRE4018 would satisfy this definition of *Hfr* in that *met-2*⁺ was transferred at approximately this frequency at 28° and at about 1/25 donor bacteria at 37°.

Throughout this work tests were conducted to ensure that the colonies obtained on selective media from crosses were true recombinants. It seemed improbable that spontaneous reversion of the genes in the donor or recipient strains could have accounted for the large numbers of altered phenotypes recovered from a variety of crosses since no revertants were observed when these strains were separately plated at the concentrations used in the crosses. Likewise, mixed growth or cross-feeding could not account for the occurrence of pure recombinant colonies since these were readily obtained from membrane crosses where mixed growth was minimal.

Increasing the number of contacts between donor and recipient bacteria increased the yields of recombinants per donor bacterium. If the number of contacts were reduced, the yield of recombinants was also reduced. Normal mating mixtures in different liquid media, held statically or gently rotated, proved to be completely infertile; recombinants were, however, obtained from high-density mating mixtures (of the order of 10^{10} bacteria/ml.) but the yield per donor bacterium was very low. These observations leave little doubt that in *Pasteurella pseudotuberculosis* group A mating takes place when donor and recipient bacteria are held in close contact. The need for this contact and the absence or instability of mating in liquid media suggests that gene transfer in *P. pseudotuberculosis* group A occurs by a conjugation process analogous to that described for *Escherichia coli* (Matney & Achenbach, 1962) and for *Salmonella typhimurium* (Sanderson & Demerec, 1965).

The analyses of selected Pro⁺ recombinants obtained from a membrane cross suggested that approximately half the zygotes segregated Met⁻ clones, a quarter segregated Met⁺ clones and a further quarter segregated clones which were mixtures of Met⁺ and Met⁻. These mixed clones were of two distinct types, one type tending to 25 % Met⁺, 75 % Met⁻ and the other to 75 % Met⁺, 25 % Met⁻. These mixed clones could be explained, in terms of the observations on pedigree analysis of *Escherichia coli* zygotes (Anderson, 1958), by the minor component segregating about one division after the major component had segregated.

The proximal marker, *met-2*, has a probability of less than 0.5 of being associated with distal markers in recombinants; the more distal the marker is from *met-2*, the lower is its probability of being associated with it (Fig. 2). Reduction of proximal marker incorporation in recombinants has also been observed for *Salmonella typhimurium* (Johnson, Krauskopf & Baron, 1965; Dubnau & Stocker, 1967). Reasonable conformity was obtained for the order of transfer of certain markers when crosses were conducted at 28° and 37°. Of the eight markers which were common to both tests, *ilv-3*, *met-2*, *arg-40*, *pur-19*, *pro-11*, *his-22* transferred in the same relative order in the two experiments. Mutants with similar requirements tended to map in the same region (see Figs. 2, 3). All the His mutants, except *his-28*, map as near distal markers and *ser-4* (Table 6), *ser-5* and *ser-6* (unplaced) map as distal markers. The main exception is *tyr-7* and *tyr-8*, which would appear to be well separated.

The fertility system reported here is one in which a high-frequency donor strain transfers its genetic information in an orientated linear manner to recipient bacteria. Since the proximal marker *met-2* was inherited with a variety of other markers and recombinants could be obtained for all the genes examined, it is probable that these genes are on one linkage group and their frequencies of transfer, as shown in Fig. 3, can be used to formulate a qualitative map of the genome. These conclusions differ from those of Lawton & Stull (1971), who, because they did not obtain proximal markers in association with other markers in their recombinants, concluded that there were two or more linkage groups in *Pasteurella pseudotuberculosis* group D. It is possible that groups A and D differ in this respect.

Preliminary results from interrupted mating experiments suggest that *met-2* has an intercept at about 30 min. on the time scale and unselected *ser-6* (among selected *met-2*⁺) an intercept at about 100 min.

I am indebted to Dr T. W. Burrows for most helpful discussion and to Mr P. E. Stone for his excellent technical assistance.

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