Gene Transfer in *Pasteurella pestis* Harboring the F'Cm Plasmid of *Escherichia coli*

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A strain of *Pasteurella pestis*, harboring the F'Cm plasmid from *Escherichia coli*, was able to donate its chromosome to auxotrophic recipient strains of *P. pestis*. The frequency of gene transfer in *P. pestis* was approximately \(10^{-4}\) per donor cell, 100 times less efficient than gene transfer in *Pasteurella pseudotuberculosis*, but efficient enough to determine entry times for the markers histidine, threonine, and tryptophan and to show linkage to the markers arginine and pigmentation. An attempt to extend the conjugation system to different serotypes of *P. pseudotuberculosis* and to *Yersinia enterocolitica* did not succeed.

Although a large amount of information exists about specific virulence factors in *Pasteurella pestis* (2), attempts to use this pathogen as a model for investigating the genetics of virulence have been hampered because of the lack of a gene transfer system. Several F' factors of *Escherichia coli* have been transferred into the closely related species *Pasteurella pseudotuberculosis*, resulting in strains of *P. pseudotuberculosis* F' that were sensitive to male-specific phage and that could donate both the F' plasmid and several chromosomal markers to other strains of *P. pseudotuberculosis* (9). In contrast, when F' plasmids were transferred from *E. coli* F' or from *P. pseudotuberculosis* F' strains into *P. pestis*, the strains of *P. pestis* F' isolated could donate their F' plasmid to recipient strains of *E. coli*, *P. pseudotuberculosis*, or *P. pestis*, but could not donate chromosomal markers to any other strains. Correlated with this inability to transfer chromosomal genes was the inability of *P. pestis* F' strains to plaque male-specific phages (10). By use of a sib-selection technique on *P. pestis* strain YpA-17 carrying the *E. coli* plasmid F'Cm (6), Molnar and Lawton (10) isolated a single clone that was sensitive to male-specific phage MS2. Using this MS2-sensitive strain, we have extended the conjugation system described for *P. pseudotuberculosis* (8, 9, 11) and report here our results of conjugation in *P. pestis*.

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**MATERIALS AND METHODS**

The procedure for obtaining auxotrophic mutants, the growth media, and the mating procedure were the same as previously described for *P. pseudotuberculosis* (9), except that the donor strain of *P. pestis* was not irradiated. The selective medium was the same except that the following factors, essential or stimulatory to *P. pestis* (4), were added (final concentration in \(\mu g/ml\)): L-phenylalanine (100), L-cystine (100), L-methionine (50), L-valine (50), L-isoleucine (50), and glycine (50). All selective plates contained 10 \(\mu g\) of nalidixic acid per ml to counterselect the nalidixic acid-sensitive donor strain. Measurement of pigmentation and fibrinolysin was performed as described by Surgalla et al. (12).

**RESULTS**

**Chromosome transfer.** A comparison of the donating ability of MS2-resistant *P. pestis* strain YpA-17 and MS2-sensitive *P. pestis* strain YpA-38 is shown in Table 1. Strain YpA-38 donated the F'Cm plasmid to the nalidixic acid-resistant recipient strain (YpA-23) more efficiently than did strain YpA-17, and, with YpA-38, the transfer of a chromosomal gene (*his*\(^+\)) was observed for the first time.

**Variation of mating condition.** By measuring the number of *his*\(^+\) nalidixic acid-resistant recombinants 3 hr after mating YpA-38 with nalidixic acid-resistant recipient strain YpA-23, we were able to determine the effect of varying the system, hoping to improve on the poor efficiency of transfer. Most of the factors that were important in the *P. pseudotuberculosis* mating system (9) made no difference in the *P. pestis* mating system. Some of the conditions that we found which did not sig-
nificantly alter the expected number of his\(^*\) recombinants were: (i) the F pilus expression time at 34 °C between 3 and 7 hr; (ii) the source of gelatin used; (iii) ultraviolet irradiation of the donor cells prior to mating; (iv) the temperature of mating between 30 and 37 °C; and (v) increasing the concentrations of gelatin, glucose, or recipients in the mating mixture.

**Mapping of chromosomal markers.** Although the low efficiency of the *P. pestis* mating system did not improve by varying conditions, it was efficient enough to attempt to map chromosomal markers. Figure 1 shows the result of mating the donor strain YpA-38 with recipient strain YpA-23. The plasmid F' Cm was transferred at high frequency beginning in less than 5 min after mating. The entry time of the chromosomal gene his was 55 min. By scoring his\(^*\) recombinants on minimal medium containing 0.01% Congo red, the unselected marker pig\(^*\) could be determined by the color of each his\(^*\) recombinant colony. Since approximately 35% of the his\(^*\) colonies were pig\(^*\) regardless of the sampling time, the pig\(^*\) gene appeared to be linked to the his\(^*\) gene and to enter the recipient earlier than the his\(^*\) gene. This mating also provided information about the arg locus. The donor strain YpA-38 was arg\(^-\) and the recipient strain YpA-23 was arg\(^*\). Since arg and his were closely linked in the *P. pseudotuberculosis* mating system, we looked for a similar linkage in *P. pestis* by scoring for his\(^*\) recombinants with and without arginine in the selective media. We observed six to eight times more his\(^*\) recombinants in the presence of arginine than we did in its absence, indicating that arg and his were closely linked in *P. pestis* and that most of the recombinants that integrated the donor his\(^*\) gene also integrated the donor arg\(^-\) gene. This observation was confirmed by picking his\(^*\) colonies (from a selective plate containing arginine) and finding that 87% were arg\(^-\). Therefore, arginine was included in the selective media for all subsequent experiments.

Our goal was to determine the chromosome location (or plasmid location) of genes determining properties associated with virulence in *P. pestis*. With this in mind, we isolated, by repeated passage at 4 °C (3), a clone of *P. pestis* that did not produce fibrinolytic factor. This mutant also did not produce pesticin or coagulase, an unexpected observation since these three properties have been shown to be either all present or all absent in several strains of *P. pestis* (1). This strain, YpA-63, was also pig\(^*\) and was made auxotrophic for threonine and tryptophan. Mating the donor strain YpA-38 with YpA-63 permitted us to determine linkages among the markers thr, trp, pig, and the fibrinolytic factor (fib). The results (Table 2) indicated that: (i) thr (entry time = 42 min) and trp (entry time = 60 min) were not on the

![Fig. 1. Kinetics of transfer of the F'Cm plasmid and the his\(^*\) gene from YpA-38 to YpA-23.](http://jb.asm.org/)

**Table 2. Linkage of genes in mating *P. pestis* YpA-38 and YpA-63**

<table>
<thead>
<tr>
<th>Minutes of mating</th>
<th>Purified recombinant clones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trp(^<em>)pig(^</em>)/total trp(^*)</td>
</tr>
<tr>
<td>50</td>
<td>2/5</td>
</tr>
<tr>
<td>60</td>
<td>2/4</td>
</tr>
<tr>
<td>75</td>
<td>3/6</td>
</tr>
<tr>
<td>90</td>
<td>3/11</td>
</tr>
<tr>
<td>105</td>
<td>3/5</td>
</tr>
<tr>
<td>120</td>
<td>2/3</td>
</tr>
<tr>
<td>180</td>
<td>4/16</td>
</tr>
<tr>
<td>230</td>
<td>19/34</td>
</tr>
</tbody>
</table>

**Table 1. Frequency of transfer of F'Cm and his\(^*\) into Pasteurella pestis strain YpA-23**

<table>
<thead>
<tr>
<th><em>P. pestis</em> donor</th>
<th>Sex type</th>
<th>MS2 phage</th>
<th>Transfer of marker per donor cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>YpA-17</td>
<td>F'Cm</td>
<td>Resistant</td>
<td>4 x 10(^{-4}) (120 min)</td>
</tr>
<tr>
<td>YpA-38</td>
<td>F'Cm</td>
<td>Sensitive</td>
<td>&lt;3 x 10(^{-8}) (180 min)</td>
</tr>
<tr>
<td>YpA-17</td>
<td>F'Cm</td>
<td>Resistant</td>
<td>4 x 10(^{-4}) (120 min)</td>
</tr>
<tr>
<td>YpA-38</td>
<td>F'Cm</td>
<td>Sensitive</td>
<td>6 x 10(^{-3}) (120 min)</td>
</tr>
</tbody>
</table>

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same linkage group, because no thr+trp+ double recombinants were detected during 180 min of mating time; (ii) pig entered the recipient before trp and was linked to trp, but pig was not linked to thr; (iii) among the 79 trp+ and 87 thr+ purified recombinants tested, none was fib+. Our initial screening appeared to show that many recombinants were fib+, but it soon was evident that all the recombinants that accepted the F'Cm plasmid gave a positive reaction in the fibrinolytic assay, though not as positive as the donor fib+ strain. The E. coli F'Cm strain (K57) that we used to transfer F'Cm into P. pestis gave a completely negative reaction in the fibrinolytic assay. To test the possibility that the F'Cm plasmid had recombinated with the fib+ gene in P. pestis YpA-38, we transferred F'Cm from the original E. coli strain directly into P. pestis YpA-63. Again, all YpA-63 cells harboring F'Cm appeared to be fib+. These YpA-63 F'Cm cells as well as the recombinant YpA-63 cells carrying F'Cm were all pesticin-negative. We considered the positive reaction of YpA-63 F'Cm cells to be unrelated to the presence of the P. pestis donor fib gene, and we concluded that fib+ was not transferred to any of the thr+ or trp+ recombinants that we examined.

Other donor strains of P. pestis. In a search for a better donor of P. pestis, we have tried eight single-colony isolates of YpA-38, one pig− strain of YpA-38, and five isolates of YpA-38 that had been spontaneously cured of F'Cm and were reinfectected with the plasmid F'lac. The latter technique was used in the hope that the plasmid F'lac in an MS2-sensitive strain might mobilize the chromosome of P. pestis better than did the plasmid F'Cm. Since YpA-38 was the only MS2-sensitive strain available, we picked chloramphenicol-sensitive clones and reinfectected them with F'lac. None of these potential chromosome donors was better than the parent YpA-38 strain.

Conjugation in other Pasteurella. The original work on conjugation in Pasteurella was accomplished in P. pseudotuberculosis type IV (8, 9, 11). To determine the possibilities of F'-mediated conjugation within the genus Pasteurella, we infected with F'Cm and, separately, with F'lac a representative strain of P. pseudotuberculosis types I, II, III, IV, and V, and of Yersinia (Pasteurella) enterocolitica strains 65, 69, 70, and Winblad. By use of the mutagen N-methyl-N'-nitro-N-nitrosoguanidine, we prepared nalidixic acid-resistant auxotrophs of each strain and then attempted mating with all homologous donor and recipient strains, following the mating procedure developed for P. pseudotuberculosis type IV (9). Although all donor strains transferred F'Cm or F'lac, only P. pseudotuberculosis type IV donated chromosomal markers to its homologous recipient.

DISCUSSION

Our results demonstrate, for the first time, chromosome transfer in P. pestis. The relationship between the ability to transfer chromosomal genes and sensitivity to male-specific phage MS2 remains unclear.

Since the F'Cm or F'lac plasmids were transferred at high frequency by P. pestis, the necessary mechanisms of F pili production and pair formation were functioning. We believe that the most probable explanation for the lack of chromosome transfer—or low frequency in the case of our only donor strain—is poor homology between the F' plasmids of E. coli and the chromosome of P. pestis. If this were the reason, it would follow that Y. enterocolitica and some of the serotypes of P. pseudotuberculosis may also lack enough homology with the F factor to permit the necessary integration and subsequent mobilization of the chromosome (5). P. pseudotuberculosis type IV, in which chromosome transfer can be shown, may have a unique area (or areas) permitting F to mobilize its chromosome. Alternatively, the conditions developed for chromosome transfer in type IV might not work for other types or species. Altering many conditions did not significantly influence chromosome transfer in P. pestis, but the only conditions tried with the other P. pseudotuberculosis serotypes and with Y. enterocolitica were those found to be optimal with P. pseudotuberculosis type IV. If lack of homology prevents better chromosome transfer in P. pestis, it might be possible to transfer the necessary region of homology from P. pseudotuberculosis type IV into P. pestis.

In P. pestis, there appeared to be more than one linkage group transferred, which agreed with the data obtained by us with P. pseudotuberculosis (9). In that paper, we stated that our data could be explained by the hypothesis that two linkage groups were present in the donor strain. Early-entering single markers from either linkage group could be recovered during the first hour of mating (due to different donor cells) but double recombinants of markers on different linkage groups could only be recovered after long periods of mating (due to a single donor cell transferring one linkage group and subsequently the second linkage group to
the same recipient cell). In *P. pestis*, since *thr* entered in 42 min and *trp* entered in 60 min but no *thr trp* double recombinants were detected until 230 min of mating, we concluded that *thr* and *trp* may be on different linkage groups. Since the genes *his* and *arg* were linked to *pig*, and *trp* was linked to *pig*, and *thr* was not linked to *pig* during mating for 180 min, we concluded that the genes *pig*, *arg*, *his*, and *trp* appeared to be on one linkage group and the gene *thr* appeared to be on a separate linkage group. The entry times were not comparable to those obtained with *P. pseudotuberculosis*, but this was not to be expected as the auxotrophs were derived separately and may be located in different areas, and the origin and direction of transfer may be different in each species.

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LITERATURE CITED


