Isolation of a *Legionella pneumophila* Restriction Mutant with Increased Ability To Act as a Recipient in Heterospecific Matings

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The ability of *Legionella pneumophila* to act as a recipient of IncP and IncQ plasmids in matings with *Escherichia coli* varies widely from strain to strain. We found that the low efficiency of mating of the Philadelphia-1 strain is due to a type II restriction-modification system, and we isolated and characterized a Philadelphia-1 mutant that lacks the restriction enzyme activity.

*Legionella pneumophila*, the causative agent of Legionnaires disease, is a bacterial facultative intracellular pathogen (5). It invades phagocytic cells by attachment to complement receptors and subsequent coating phagocytosis (4, 9). The bacteria survive inside the phagocyte by preventing phagosome-lysosome fusion (3) and grow exponentially, eventually causing destruction of the cell (5).

We are using a genetic approach to study the interaction of this gram-negative bacterium with phagocytes as a model for understanding the molecular basis of intracellular survival and growth of parasites in general. It is therefore desirable to efficiently transfer DNA between *L. pneumophila* and *Escherichia coli*. The virulent Philadelphia-1 strain is of particular interest because its interactions with human peripheral blood monocytes have been best characterized (3, 5). However, this strain is not amenable to genetic manipulation because it is a very poor recipient. In order to facilitate genetic analysis, we isolated a derivative of Philadelphia-1 that mates better with *E. coli*.

Table 1 shows the frequencies of transfer of plasmid RK2 from *E. coli* to the Philadelphia-1 and Bloomington-2 strains of *L. pneumophila*. Plasmid RK2 transferred efficiently to the Bloomington-2 strain; half of the recipient cells received and maintained the plasmid. In contrast, RK2 transconjugants of Philadelphia-1 were rare.

We reasoned that among cells of strain Philadelphia-1 that had successfully received plasmid RK2, there may be mutants with an increased capacity to act as recipients. As RK2 is quite stable in *L. pneumophila* (8), to test our hypothesis we used an easily cured, temperature-sensitive derivative of RP1, namely, pMR5 (RP1 and RK2 are probably identical) (10). Plasmid pMR5 was transferred to strain CS1 (Philadelphia-1 Sm') at 30°C, and a single kanamycin-resistant exconjugant was obtained. The growth properties of CS1(pMR5) at 30 and 37°C are shown in Table 2. Growth of CS1(pMR5) at 37°C without antibiotic selection did result in the efficient loss of the plasmid; kanamycin-sensitive colonies were obtained. One of these, AM170, was characterized in detail.

Plasmid transfer was much higher to strain AM170 than to strain CS1 (Table 1). We performed two experiments to verify that strain AM170 was indeed a derivative of strain Philadelphia-1 and not an unrelated strain (e.g., Bloomington-2). First, we used a fluorescein-labeled serogroup 1-specific antibody to label strain AM170 (data not shown). Bloomington-2 is not labeled by this antibody because it is a member of serogroup 3. We also examined the patterns of DNA fragments produced with the restriction endonucleases *NotI* and *SfiI* by pulsed-field gel electrophoresis (12). The AM170 restriction pattern was found to be indistinguishable from that of Philadelphia-1 and different from that of Bloomington-2 (data not shown). We conclude that strain AM170 is a derivative of Philadelphia-1 with an increased ability to act as a recipient.

To find out if strain AM170 was a more efficient recipient because it had lost a restriction endonuclease, we prepared crude extracts and assayed them for restriction endonuclease activity (11). Chen et al. have recently reported a restriction endonuclease activity called *LpnII* in Philadelphia-1 (1). We also found that extracts of the Philadelphia-1 strain were able to cleave bacteriophage lambda DNA into discrete fragments. Similar extracts prepared from strain AM170 lacked this activity (data not shown). To find out if this nuclease activity was indeed part of a restriction-modification system, we introduced plasmid pMMB33 (2) into strains CS1, AM170, and Bloomington-2 Sm'. The frequency of transfer of pMMB33 into these strains indicated that the potential restriction barrier operates on pMMB33 (Table 1).

Plasmid DNA (pMMB33) was then prepared (6) from the *L. pneumophila* hosts as well as from an *E. coli* host (DH5). Figure 1 shows the results obtained when the different plasmid preparations were treated with partially purified extracts from strains Philadelphia-1, Bloomington-2, and AM170, respectively.

![Figure 1](http://jb.asm.org/)

**TABLE 1. Frequency of plasmid transfer between *E. coli* and *L. pneumophila***

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>X7131(RK2) (IncP)</td>
<td>CS1</td>
<td>4.0 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>AM170</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Bloomington-2 Sm'</td>
<td>0.50</td>
</tr>
<tr>
<td>DH5(pRK2073, pMMB33)*</td>
<td>CS1</td>
<td>1.8 × 10⁻⁸</td>
</tr>
<tr>
<td>(IncQ)</td>
<td>AM170</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Bloomington-2 Sm'</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Frequency of plasmid transfer is reported as the number of drug-resistant exconjugants per total number of recipients at the end of the mating.
* Plasmid pRK2073 was used to mobilize plasmid pMMB33.

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TABLE 2. Growth properties of strains containing pMR5, a temperature-sensitive derivative of RK2

<table>
<thead>
<tr>
<th>Strain</th>
<th>CFU at:</th>
<th>30°C</th>
<th>37°C</th>
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<tbody>
<tr>
<td></td>
<td>+Km</td>
<td>−Km</td>
<td>+Km</td>
</tr>
<tr>
<td>CS1</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>CS1(RK2)</td>
<td>8.4 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2.7 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>3.2 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>CS1(pMR5)</td>
<td>5.0 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3.0 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>2.6 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cultures were plated on ABCYE (5) agar plates with (+) or without (−) kanamycin (Km) at the indicated temperature. All three cultures were grown to saturation in the absence of kanamycin at 30°C before being plated.

AM170. The extract prepared from strain Philadelphia-1 cleaved pMMB33 DNA prepared in an E. coli host (Fig. 1A and B), while similar extracts prepared from AM170 or Bloomington-2 did not (Fig. 1B). None of the extracts cleaved pMMB33 DNA prepared in the L. pneumophila Philadelphia-1 or AM170 hosts (Fig. 1C and D); however, pMMB33 DNA from Bloomington-2 was susceptible to cleavage by the LpnII activity present in the Philadelphia-1 extract (Fig. 1E). All four DNA preparations were equally susceptible to cleavage by an unrelated restriction endonuclease, PstI. These results show that wild-type Philadelphia-1 contains a restriction-modification system and that strain AM170 is defective in the restriction activity but is still able to carry out modification. The Bloomington-2 strain contains neither component of the LpnII restriction-modification system.

The frequency of RK2 transfer to wild-type Philadelphia-1 (Table 1) was about the same as the spontaneous mutation rate of a given gene. For example, we find that trimethoprim-resistant thy mutants occur spontaneously at a frequency of 10<sup>−7</sup> (7). Although we have not measured the frequency of restriction mutants among a large number of RK2 exconjugants, we have used a procedure similar to the one described here to isolate restriction mutant derivatives of other Philadelphia-1 strains. This approach relies on plasmid pRK212, which carries a Mu cts prophage. Strains containing the plasmid die at 37 or 42°C due to induction of Mu DNA replication and transposition (7, 8). Growth of Legionella strains that contain pRK212 at 37°C in the absence of selection for the plasmid allows the isolation of cells which have lost the plasmid and the Mu cts62 genome. These cured strains also show increased mating frequency when restested in matings with RK2, and they lack LpnII activity (A. Marra, unpublished results).

The restriction-deficient strains of L. pneumophila have been useful in a wide variety of genetic experiments, such as transposon mutagenesis with plasmid suicide vehicles, gene disruption experiments with cloned fragments of Legionella DNA, and complementation studies for the identification of cloned fragments of Legionella DNA (L. Szeto and A. Marra, unpublished results). The approach that will be used to obtain the strains with an increased mating frequency should be applicable to any type of bacterium into which plasmid RK2 can be introduced.

This research was supported by Public Health Service grant AI23549 from the National Institute of Allergy and Infectious Diseases and by the John D. and Catherine T. McArthur Foundation Consortium on Parasitic Diseases. H.A.S. is a Career Scientist of the Irma T. Hirschl Charitable Trust.

We thank David Figurski for his ongoing interest in our work. We thank Cassandra Smith and the members of her laboratory for considerable assistance in the performance of pulsed-field gel electrophoresis. We thank Ralph Isberg for giving us plasmid pMR5 and Michael Bagdasarian for plasmid pMMB33. We gratefully acknowledge the cheerful and conscientious technical assistance of Carmen Rodriguez.

LITERATURE CITED