Genome Size of the Rickettsia Coxiella burnetii

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The genome size of Coxiella burnetii Nine Mile strain was determined by the method of initial rate of deoxyribonucleic acid renaturation. The mean value obtained was 1.0 ± 10^8 daltons.

We have determined recently (7) that the genome sizes of the typhus group of rickettsiae, Rickettsia prowazekii (epidemic typhus) and Rickettsia mooseri (R. typhi) (murine typhus), are approximately 10^8 daltons. A similar value has been described by others for Rickettsia rickettsii, the etiological agent of Rocky Mountain spotted fever (3), although we found a slightly higher value for this organism (unpublished results). Schramek (8) described the isolation and characterization of DNA from Coxiella burnetii, strain L-35, the etiological agent of Q fever. In that study the DNA was subjected to buoyant density gradient centrifugation in cesium chloride. An analysis of the sedimentation pattern indicated a rather large distribution of particle size in the final isolation product. Its average molecular weight was 1.8 × 10^7 daltons. Although Schramek (8) had taken care to minimize shear of the DNA in his procedure, some undoubtedly occurred, and no claim was made that the fragment length obtained necessarily represented that of the intact genome. Since the DNA fragment size reported by Schramek (8) was almost 2 orders of magnitude below what we had found as the genome size of the typhus group of rickettsiae (7), it seemed worthwhile to resolve the question of genome size in C. burnetii. Our procedure is based on the initial rate of renaturation of heat-denatured DNA (2) and does not require isolation of the intact genome.

C. burnetii Nine Mile strain phase one (type strain) was grown in the yolk sac cells of embryonated chicken eggs, and the rickettsial cells were purified from the host tissue by a standard procedure which included centrifugation through 30 to 60% (wt/wt) linear sucrose gradients (11). The rickettsial DNA was purified by the procedures of Thomas et al. (10) with slight modifications which involve the sequential use of pronase, sodium dodecyl sulfate, phenol, RNase, and hydroxyapatite. The procedure is described fully in a recent publication (7). The purified DNA was divided into two equal lots, A and B, and each DNA sample was sheared in a Ribi cell fractionator to a size of approximately

<table>
<thead>
<tr>
<th>Organism</th>
<th>Guanine plus cytosine content (mol%)</th>
<th>DNA preparation</th>
<th>No. of expts.</th>
<th>Genome size (mol wt (10^6) ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental* + Published*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. burnetii</td>
<td>42.7 ± 0.05</td>
<td>A</td>
<td>5</td>
<td>1.04 ± 0.04</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. prowazekii Breinl</td>
<td>29.0 (7)</td>
<td>29.0 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. prowazekii Madrid</td>
<td>28.5 (7)</td>
<td>29.3 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. mooseri Wilmington</td>
<td>29.0 (7)</td>
<td>29.3 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. quintana Fuller</td>
<td>39.3 (7)</td>
<td>38.8 (12)</td>
<td>6</td>
<td>1.01 ± 0.03 (7)</td>
</tr>
<tr>
<td>E. coli K-12</td>
<td>51.7 (7)</td>
<td>50.0 (5)</td>
<td>6</td>
<td>2.41 ± 0.13 (7)</td>
</tr>
</tbody>
</table>

* Our data.

† Data from the literature. Numbers within parentheses indicate references.

‡ Standard deviation.

§ Each experiment was done in triplicate.


460
2.5 \times 10^6\text{ daltons} \ (7).\ The\ purified\ DNA\ had
been sheared as two separate lots as an internal
control on the procedure, since the genome size
values obtained are, to a slight extent, a function
of DNA fragment length.

The DNA product obtained showed an ab-
sorption spectrum characteristic of DNA. The
260\text{ nm}/280\text{ nm} and 260\text{ nm}/230\text{ nm} absorbancy
ratios were 1.69 and 1.88, respectively. The de-
gree of DNA hyperchromicity obtained when
the sample was denatured was 1.37 to 1.38. The
levels for protein and ribonucleic acid were be-
low the sensitivity of the chemical methods em-
yployed (4, 6), and, in any case, were less than 1% of
the DNA content.

The base composition of the DNA was deter-
mined from a measurement of its thermal de-
naturation temperature (5), and the value ob-
tained compares well with values obtained by
others (see Table 1).

The genome size was determined by the initial
rate (30 \text{ min}) of DNA renaturation, which was
measured optically as previously described (7).
Table 1 shows that the mean values for lots A
and B were identical (1.04 \times 10^6\text{ daltons}).

Thus, the genomes of a significant number of
rickettsial species (\textit{R. prowazekii}, \textit{R. mooseri},
\textit{R. rickettsii}, and \textit{C. burnetii}) are well within
the size range of genomes of certain free-living bac-
teria and are, in fact, significantly larger than
the genomes of members of the genus \textit{Mycoplasma}
(5 \times 10^6\text{ daltons}) (1) which, unlike the
rickettsiae, can be cultivated in cell-free media.
The failure of rickettsiae to grow extracellularly
is evidently not due to a genome too small to
code for the essentials of extracellular growth,
but, rather, may be due to a relatively small
number of evolutionary adaptations to a host
cell dependency.

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