Physical Properties of Plasmid Mor174, Which Determines Bacteriocin Production in Proteus morganii 174

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Plasmid Mor174 has a molecular weight of 3.6 × 10⁶ and a buoyant density of 1.6994 g/cm³. The covalently closed circular form has a sedimentation coefficient of 22S. There are 30 to 40 plasmid copies per genome equivalent, but growth in chloramphenicol results in amplification of the copy number to 600. In Proteus morganii 174, Mor174 coexists with a cryptic plasmid of molecular weight 15.8 × 10⁶ and a buoyant density of 1.7170 g/cm³.

Proteus morganii 174 produces a bacteriocin active against a number of P. morganii strains (11). This bacteriocin, morganocin 174, interferes with the energy metabolism of sensitive cells. Characteristics of this interference include inhibition of macromolecular synthesis and intracellular accumulation of glutamine and proline, efflux of K⁺, and rapid depletion of intracellular ATP (12). Although the genetic determinant of morganocin 174, plasmid Mor174, is nonconjugative, conjugal transfer may be mediated by a mobilizing plasmid, kanamycin resistant factor R772 (11). Transfer of Mor174 to a susceptible indicator strain renders it both morganocinogenic and immune to homologous bacteriocin (12). The purpose of this investigation was to determine physical characteristics of Mor174.

Plasmid DNA was initially isolated from P. morganii 174. Cells were lysed by a lysozyme-sodium dodecyl sulfate procedure (8), and the "cleared" lysate was centrifuged to equilibrium in cesium chloride (CsCl)-ethidium bromide (EtBr). The satellite band obtained was subjected to a second equilibrium banding in CsCl-EtBr. After removal of EtBr with isopropanol (8), the DNA was banded in CsCl (2) in a Spinco model E analytical ultracentrifuge equipped with a photoelectric scanner. Two DNA bands with buoyant densities of 1.6994 and 1.7170 g/cm³ and molecular weights of 2.4 × 10⁶ and 16.9 × 10⁶ (2), respectively, were observed (Table 1). When plasmid DNA isolated as described above was sedimented through a 15 to 50% neutral sucrose gradient, two discrete bands were again resolved (Fig. 1), which on analysis in the analytical ultracentrifuge corresponded to the two buoyant density bands initially observed. DNA from the two peaks from the sucrose gradient were mounted for electron microscopy by the aqueous method (6) and shadowed with Pt-Pd at an angle of 8°. Circular DNA molecules were observed. DNA from the slower-sedimenting band, corresponding to ρ = 1.6994 g/cm³,

TABLE 1. Physical properties of plasmid DNA isolated from P. morganii 174 and P. morganii 165 mor"(Mor174)

<table>
<thead>
<tr>
<th>P. morganii strain</th>
<th>Plasmid</th>
<th>Contour length* (µm)</th>
<th>Mol wtb (×10⁶)</th>
<th>Sccc</th>
<th>Mol wtc (×10⁶)</th>
<th>Buoyant density (g/cm³)</th>
<th>Mol wtd (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>174</td>
<td>Mor174</td>
<td>1.85</td>
<td>3.6</td>
<td></td>
<td></td>
<td>1.6994</td>
<td>2.408</td>
</tr>
<tr>
<td></td>
<td>Cryptic</td>
<td>8.1</td>
<td>15.8</td>
<td></td>
<td></td>
<td>1.7170</td>
<td>16.89</td>
</tr>
<tr>
<td>165 mor&quot;(Mor174)</td>
<td>Mor174</td>
<td>1.85</td>
<td>3.6</td>
<td>22</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Average of seven molecules each; ColE1 DNA (contour length, 2.15 µm [1]) was mounted on the same grids as a standard.

b Calculated from contour length, assuming 1.96 × 10⁶ daltons per µm (1).

c Sedimentation constant of CCC form.

d Determined from the equation Sccc = 0.034 M₀⁰.⁴⁰⁸ (1).

e Computed from the width of the band at equilibrium in a CsCl gradient in the analytical ultracentrifuge (2).
had a contour length of 1.85 μm, equivalent to a molecular weight of $3.6 \times 10^6$. The other DNA species had a contour length of 8.1 μm, which corresponds to a molecular weight of $15.8 \times 10^6$ (Table 1).

As either of these DNA species could have been the Mor174 plasmid, a kanamycin-sensitive defective segregant of a *P. morganii* 165 *mor* (Mor174 R772) transconjugant (11) was selected for further study, since DNA isolated from *P. morganii* 165 did not yield a satellite band on centrifugation in a CsCl-EtBr gradient. Plasmid DNA from *P. morganii* 165 *mor* (Mor174) was examined by electron microscopy. A single plasmid species with a contour length of 1.85 μm was observed, identifying the morganocinogenic plasmid Mor174 as the smaller of the two plasmids isolated from *P. morganii* 174. No function has yet been found associated with the larger plasmid.

Differentially labeled Mor174 $[3H]$DNA and ColE1 $[14C]$DNA were isolated from *P. morganii* 165 *mor* (Mor174) and *Escherichia coli* W3110(ColE1) by sedimentation of cleared lysates through 15 to 50% neutral sucrose gradients (4). Portions from the respective plasmid peaks were cosedimented through a 5 to 20% neutral sucrose gradient (4) to calculate Mor174 DNA S values relative to those of ColE1 DNA (5). The covalently closed circular (CCC) DNA sedimented at 22S, whereas the open circular (OC) DNA sedimented at 16S (Fig. 2). This agrees with the $S_{ccc}/S_{soc}$ ratio of 1.33 to 1.35, as expected for a plasmid of $2 \times 10^6$ to $6 \times 10^6$ daltons (5). The molecular weight of Mor174 calculated from the $S_{ccc}$ value (1), $3.7 \times 10^6$, agrees with the molecular weight obtained from the contour length rather than that obtained from the equilibrium centrifugation.

Colicinogenic plasmids have been divided into two groups according to their molecular weight and number of copies per genome equivalent (7). Group I plasmids have molecular weights in the region of $5 \times 10^6$ and exist as multiple copies in the cell; group II plasmids have molecular weights of $62 \times 10^6$ to $94 \times 10^6$ and are found as one to two copies per genome equivalent. To determine the copy number of Mor174,
P. morganii 165 mor*(Mor174) was grown in the presence of [3H]thymidine, and lysates were banded in CsCl-EtBr. The plasmid copy number was determined from the relative amounts of radioactivity in plasmid and chromosomal DNA bands, assuming a chromosome mass of 2 × 10^9 daltons for P. morganii. Cells in both exponential (Fig. 3A) and stationary (not shown) growth phases were found to contain 30 to 40 plasmid copies per genome equivalent. Growth of cells in the presence of chloramphenicol resulted in amplification of the plasmid to 600 copies per genome equivalent (Fig. 3B). Amplification of the plasmid copy number after growth in chloramphenicol has also been demonstrated for bacteriocinogenic plasmids ColE1 (3) and CloDF13 (9).

The physical data on Mor174 agrees with the nonconjugative nature of this plasmid. A plasmid of molecular weight 3.6 × 10^9 could only code for approximately six proteins of average molecular weight 50,000, and this would be insufficient to meet the requirements for transfer (10). The small size of Mor174, as well as its multiple copy number, identifies it with the group I colicinogenic plasmids.

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LITERATURE CITED