Efficient Transformation of *Erwinia carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica*

JAY C. D. HINTON, MICHEL C. M. PEROMBELON, and GEORGE P. C. SALMOND

Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, and Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom

Received 20 August 1984/Accepted 10 November 1984

We used a modified version of the method of Hanahan (D. Hanahan, J. Mol. Biol. 166:557–580, 1983) to transform *Erwinia carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* with the plasmids pBR322, pBR325, and pAT153. The transformation frequency ranged from 1 × 10^5 to 4 × 10^5 colonies per μg of plasmid DNA. The nature of these transformants was confirmed by plasmid analysis. ColEl-based plasmids make potentially useful cloning vectors for the study of genes involved in the pathogenesis of this species.

*Erwinia carotovora* subsp. *carotovora* is responsible for the soft rot of a number of economically important crops, including potatoes, celery, carrots, green peppers, and cucumbers. The host range of *E. carotovora* subsp. *atroseptica* is confined to potatoes, in which it is the causal agent of blackleg in the field and soft rot of tubers in stores. Various aspects of the epidemiology of the disease caused by these phytopathogens are understood, but no control method is currently available (18).

Genetic and molecular techniques are now being used to study the basis of pathogenicity of these subspecies (17, 20). The development of an efficient transformation system for strains SCR193 (Ecc193) and SCR131 (Eca31) is an important step in this approach. This permits inter- and intragenic transfer of native and recombinant plasmids and facilitates direct shotgun cloning into *Erwinia*.

Transformation procedures have been reported for other *Erwinia* spp.: *E. amylovora* (D. W. Bauer and S. V. Beer, Phytopathology 73:1342, 1983) and *E. herbicola* (13; S. E. Lindow and B. J. Staskawicz, Phytopathology, 71:237, 1981). However, the only method described for the transformation of *E. carotovora* subsp. *carotovora* is inefficient (P. M. Berman, M. S. Mount, and G. H. Lacy, Phytopathology 73:1342, 1983). Extrapolation from the data of Berman et al. gives a frequency of less than 1 transformant per μg of pBR322 DNA (given a transformation mix containing 10^8 cells).

The basis of the induction of competence in gram-negative bacterial cells is poorly understood (3). Consequently, the development of a transformation system in a new species requires an empirical approach.

The bacterial strains and plasmids used in this study are listed in Table 1. pBR322 and pBR325 DNA and restriction enzymes were obtained from Bethesda Research Laboratories. Restrictions were performed as described previously (14), with the addition of 4 mM spermidine (Sigma Chemical Co.). This was required to prevent breakdown of Ecc193-derived plasmids due to the action of nonspecific endonucleases. pKT210 was purified in this laboratory, and pAT153 was a gift from M. Richardson. Preliminary work involved attempts to transform SCR193 with pKT210 and pBR322 DNA by a number of published methods (7–9, 14–16; Bauer and Beer, Phytopathology 73:1342). Of the seven methods, only those of Morrison (15) and Maniatis et al. (14) (modified by substitution of 150 mM CaCl₂ for 100 mM CaCl₂) yielded any transformants. Both methods gave a frequency of 4 transformants per μg of pKT210 DNA. All transformants were Sm^R Cm^R and carried a plasmid which comigrated with authentic pKT210 (plasmid screens were performed by a scaled-down adaptation of the method of Hansen and Olsen [11]). No indigenous plasmids have been detected in Ecc193 or Ecc31 (data not shown).

A more efficient transformation system has recently been developed for both Ecc193 and Ecc31 based on the method of Hanahan (10), but with several modifications. *Erwinia* cultures were routinely grown at 30°C. Samples of competent cells (200 μl) were treated with 100 ng of plasmid DNA, incubated on ice for 30 min, and then heat-shocked at 42°C for 1 min without agitation. After 100 min of expression time (30°C), transformants were selected on LM agar (10) containing 35 μg of ampicillin per ml.

Frequencies of transformation of Ecc193 and Ecc31 with various plasmids are shown in Table 2. A number of Ap^R transformants were screened for the coinheritance of other plasmid-borne antibiotic resistance markers. In all cases, 100% linkage was observed (data not shown).

The effect of various components in the transformation system upon the transformation efficiency of Ecc193 was studied. Growth of cells at 37°C, instead of 30°C, before transformation doubled the transformation efficiency. Removal of dithiothreitol or dimethylsulfoxide from the buffer reduced the transformation efficiency by 10- and 3-fold, respectively. Incorporation of a freeze-thaw step before the heat-shock reduced the transformation efficiency by threefold. Addition of a 30-min incubation on ice after the heat-shock reduced transformation efficiency by fourfold. Storing cells in transformation buffer at 4°C for 18 h before addition of dithiothreitol, dimethylsulfoxide, and plasmid DNA reduced the transformation frequency by 30-fold (data not shown) (the last three modifications were found to increase the transformation efficiency of *E. amylovora* [Bauer and Beer, Phytopathology 73:1342]).

The plasmids carried by the transformants were analyzed by a modification of the boiling method (12). Plasmid bands were found in both Ecc193 and Ecc31 transformants, which comigrated with the appropriate monomeric forms of the three control plasmids (data not shown). Transformants possessed similar pathogenic, pectolytic, and biochemical properties as the parental strain (data not shown).

* Corresponding author.
Plasmids were isolated from DH1(pBR322) and Ecc193 (pBR322) transformants (6) and compared. These plasmids were run with commercial standards by using a Tris-acetate gel system. The pBR322 obtained from Bethesda Research Laboratories was in dimeric form (as was the pBR325 from the same source [data not shown]). When this pBR322 was transformed into DH1, it continued to replicate as a dimer. However, when the same plasmid was transformed to Ecc193, it assumed a monomeric conformation. When plasmid DNA was isolated from Ecc193(pBR322) and transformed into DH1, it continued to replicate as a monomer, as confirmed by comparison with a monomeric sample of pBR322 (data not shown).

In *Escherichia coli*, the recA gene product is required to resolve a multimeric plasmid to its monomeric form (2). This is demonstrated by the fact that dimeric plasmids continue to replicate as dimers in DH1. The observation that dimeric pBR322 is resolved to a monomer upon introduction to Ecc193 suggests that this strain possesses a system analogous to that of recA in *E. coli*.

Plasmid isolated from Ecc193(pBR322) and Eca31 (pBR322) gives a 4.3-kilobase linear fragment after digestion with *Hind*III, which comigrates with that of commercial pBR322 and pBR322 isolated from DH1. Further evidence that the plasmids carried by Ecc193(pBR322) and DH1(pBR322) are similar was obtained by digesting DNA from each, in parallel, with *Hae*III and *Sau*3A (two enzymes which cut pBR322 to give 22 fragments). After electrophoresis on a 3% gel, the same number and size of restriction fragments were produced from both plasmids (data not shown).

These results show that Ecc193 can be efficiently transformed with pBR322 and that this plasmid is probably unaltered in this strain. The utility of transformation in recombinant DNA manipulations involving Ecc193 and Eca31 is apparent. This system has already been used to introduce recombinant plasmids to Ecc193, and to achieve direct complementation of a transposon-induced mutation in this strain (unpublished data).

### TABLE 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant details</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. carotovora</em> subsp. carotovora SCR1193</td>
<td>(Formerly SR44)</td>
<td>17</td>
</tr>
<tr>
<td><em>E. carotovora</em> subsp. atroseptica SCR131</td>
<td>Laboratory collection</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> K-12 DH1</td>
<td>recA hsdR</td>
<td>10</td>
</tr>
<tr>
<td>pAT153</td>
<td>Ap' Tc'</td>
<td>19</td>
</tr>
<tr>
<td>pBR322</td>
<td>Ap' Tc' Cm'</td>
<td>5</td>
</tr>
<tr>
<td>pBR325</td>
<td>Ap' Tc' Cm'</td>
<td>4</td>
</tr>
<tr>
<td>pKT210</td>
<td>Cm' Sm'</td>
<td>1</td>
</tr>
</tbody>
</table>

The technique of Hanahan, which was successfully applied here, may prove applicable to a wide range of gram-negative bacteria which have previously proved recalcitrant to transformation.

We thank K. Derbyshire and C. Oakley for providing bacterial strains, D. Gill and K. Hussain for useful discussion, and C. Alderson for typing the manuscript.

J.H. acknowledges an RCCA cooperative studentship award from the Science and Engineering Research Council in association with the Scottish Crop Research Institute.

### LITERATURE CITED


