Bacteriophage \( \phi KP \) mediated generalized transduction in *Erwinia carotovora* subspecies *carotovora*

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(Received 22 June 1993; accepted 13 July 1993)

The bacteriophage \( \phi KP \) is capable of generalized transduction in *Erwinia carotovora* subspecies *carotovora* (Ecc) strains SCRI193 and ATCC 39048. \( \phi KP \) is a virulent phage containing double stranded DNA of approximately 46 kb. The frequencies of transduction were established for a number of chromosomal markers and plasmid pHCP2, and conditions for transduction optimized after exposure of the phage lysate to UV irradiation.

**Introduction**

The soft rot erwinias, *Erwinia carotovora* subspecies *carotovora* (Ecc), *Erwinia carotovora* subspecies *atroseptica* (Eca) and *Erwinia chrysanthemi* (Echr), are plant pathogens responsible for large crop losses throughout the world, estimated to be around $50-100 million annually (Perombelon & Kelman, 1980). Of the soft rot erwinias, Ecc has probably the widest host range, causing soft rot in a number of crops from both temperate and subtropical regions (Perombelon, 1985).

Ecc strain SCRI193 is presently being used in this laboratory to investigate the role of extracellular enzymes in the economically important soft rot disease of potato. Within the last 5 years this strain has become amenable to genetic manipulation by techniques such as transposon mutagenesis (Hinton & Salmond, 1987; Hinton et al., 1989) conjugation and transformation (Hinton, 1985). Transduction via a generalized transducing phage, however, has not yet been achieved.

To date, generalized transducing phages have been isolated for only a small number of *Erwinia* species, e.g. Erch-12 on *Echr* strain EC183 (Chatterjee & Brown, 1981), \( \phi EC-2 \) on *Echr* strain 3937 (Resibois et al., 1984) and phages \( \phi 49 \) and \( \phi 59 \) on both *Ecc* strain 268 and *Erwinia horticola* strain 450 (Mukvich et al., 1987).

Generalized transduction can be used to carry out a range of genetic manipulations including fine structure mapping, localized mutagenesis, construction of isogenic strains, transposon mutagenesis and plasmid transfer (Masters, 1985). In this report we describe the isolation of a generalized transducing phage for our genetically amenable strain SCRI193 to complete the range of genetic tools available for this strain.

**Methods**

**Bacterial strains.** Bacterial strains are listed in Table 1. All *Erwinia* strains were grown at 30 °C, unless stated otherwise.

**Media.** Luria broth (LB) and phage buffer (PB) were as described by Maniatis et al. (1982). Minimal medium (MM) was as described by Chatterjee et al. (1985). Ampicillin, for the selection of pHCP2, and kanamycin, for the selection of Tn5 induced mutants, were used at 50 μg ml\(^{-1}\) final concentration.

**Isolation of \( \phi KP \).** Untreated sewage, activated sludge and effluent (500 ml each) were collected from Finham sewage works, Warwickshire, UK, and pooled. The resulting mixture was centrifuged at 9000 r.p.m. for 15 min at 4 °C. The supernatant was collected and the constituents of LB added. Chloroform (0.5 ml) was added to destroy

<table>
<thead>
<tr>
<th>Table 1. Strains used in this study</th>
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<tbody>
<tr>
<td><strong>Bacterial strain</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>SCRI193</td>
</tr>
<tr>
<td>HC131</td>
</tr>
<tr>
<td>HCS19</td>
</tr>
<tr>
<td>HCS50</td>
</tr>
<tr>
<td>HCS512</td>
</tr>
<tr>
<td>HCS(8.11)</td>
</tr>
<tr>
<td>HCS(12.19)</td>
</tr>
<tr>
<td>HCS(17.5)</td>
</tr>
<tr>
<td>SCRI1043</td>
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Abbreviation: PB, phage buffer.

\( \phi \)CP2, and conditions for transduction optimized after exposure of the phage lysate to UV irradiation.

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bacterial contaminants and the centrifugation step repeated. Supernatant (250 ml) was transferred to a 2 litre flask and 5 ml of an overnight culture of SCR1193 added. After 24–48 h incubation at 25 °C in a shaking waterbath at 250 r.p.m., the culture had reached stationary phase. Chloroform was added and the culture was then shaken for 5 min. The supernatant was recentrifuged and stored over chloroform.

After serial dilution the lysate was incubated at 25 °C for 15 min with 300 µl of a culture of SCR1193 and a 0.7 % agar overlay made on an LB agar plate which was incubated overnight at 25 °C.

Plaque purification and preparation of high titre lysates. A phage lysate was serially diluted, and incubated with 300 pl of a culture of SCR1193 and 300 pl of a culture of SCR1193 and a 0.7 % agar overlay made on an LB agar plate containing 10 µl chloroform. For the preparation of high titre lysates, the soft agar overlay was removed from the single plate showing semi-confluent lysis and resuspended in 0.5 ml sterile PB containing 4 °C. The mixture was added to 3 ml of 0.7 % agar and an overlay made on an LB agar plate. After overnight incubation at 25 °C, individual plaques were removed from the bacterial overlay with a Pasteur pipette and resuspended in 200 µl sterile PB containing 10 µl chloroform. For the preparation of high titre lysates, the soft agar overlay was removed from a single plate showing semi-confluent lysis and resuspended in 0.5 ml sterile PB containing chloroform. Phages were eluted at 4 °C for 4–6 h and residual agar was removed from the lysate by centrifugation at 1000 r.p.m. Lysates of φKP for transduction assays were prepared on wild-type SCR1193 and lysates for plasmid phCP2 transduction assays were prepared on HCl131 (Table 1).

 Screening for transduction. A φKP lysate made on SCR1193 was tested for its ability to transduce the cysB marker from the auxotoxic mutant HCS500 (cysB::Tn5). After overnight incubation at 30 °C, 10 ml of bacterial culture (approximately 1×10^8 c.f.u. ml⁻¹) was centrifuged at 5000 r.p.m. for 10 min at room temperature and the supernatant was discarded. Lysates of φKP were centrifuged at 3000 r.p.m. for 5 min at 4 °C to remove bacterial cells and adsorbed phage, then the supernatant was diluted and the phage titrated. After incubation at 25 °C, at time intervals of approximately 10 min up to 60 min, further samples were removed, diluted and titrated. Phage adsorption assays were performed in triplicate.

Results and Discussion

Isolation and characterization of φKP

Phage φKP was isolated after enrichment on the host Ecc SCR1193. On a 0.7% soft agar overlay after overnight incubation at 25 °C the plaques appeared turbid, had irregular edges, and were approximately 1–2 mm in diameter. The morphology of φKP was determined by electron microscopy. The phage possessed an isometric head of approximately 60 nm and a contractile tail of approximately 120–135 nm. φKP therefore fell into the most commonly isolated phage type, group B of the Bradley classification system (Bradley, 1967). The nucleic acid of φKP was shown to be double-stranded DNA by restriction with restriction endonucleases (Fig. 1). From restriction fragment analysis the genome size was estimated to be approximately 46 kb.

![Fig. 1. Restriction endonuclease digestion of φKP DNA and electrophoresis through a 0.7% agarose gel. Lane 2, HindIII; lane 3, ClaI; lane 4, BamHI; lane 5, BglII; lane 6, EcoRI; lanes 1 and 7, λ DNA restricted with HindIII.](image-url)
**Table 2.** \( \phi KP \) mediated transduction of various markers from Ecc SCR1193, and plasmid pHCP2 from HC131

Frequencies of transduction and standard deviations of transduction frequencies are given per p.f.u., while reversion/spontaneous resistance frequencies are given per c.f.u.

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>Transduced marker</th>
<th>Reversion frequency (c.f.u.(^{-1}))</th>
<th>Transduction frequency (p.f.u.(^{-1}))</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCR1193</td>
<td>Ap(^{+})(pHCP2)</td>
<td>&lt; 10(^{-8})</td>
<td>2 \times 10^{-6}</td>
<td>2.6 \times 10^{-7}</td>
</tr>
<tr>
<td>HC519</td>
<td>lac(^{-})</td>
<td>2 \times 10^{-6}</td>
<td>2.8 \times 10^{-6}</td>
<td>1.9 \times 10^{-7}</td>
</tr>
<tr>
<td>HC500</td>
<td>cysB(^{+})</td>
<td>2 \times 10^{-7}</td>
<td>1 \times 10^{-6}</td>
<td>3.4 \times 10^{-7}</td>
</tr>
<tr>
<td>HC512</td>
<td>lac(^{-})</td>
<td>&lt; 10^{-8}</td>
<td>2 \times 10^{-7}</td>
<td>1.2 \times 10^{-7}</td>
</tr>
<tr>
<td>HC(8.11)</td>
<td>trp(^{-})</td>
<td>7 \times 10^{-8}</td>
<td>1 \times 10^{-6}</td>
<td>6.2 \times 10^{-7}</td>
</tr>
<tr>
<td>HC(12.19)</td>
<td>arg(^{-})</td>
<td>7 \times 10^{-8}</td>
<td>1 \times 10^{-6}</td>
<td>9.7 \times 10^{-7}</td>
</tr>
<tr>
<td>HC(17.5)</td>
<td>pur(^{-})</td>
<td>5 \times 10^{-7}</td>
<td>2 \times 10^{-6}</td>
<td>1 \times 10^{-6}</td>
</tr>
</tbody>
</table>

From over 100 colonies tested for lysozyme after infection with \( \phi KP \), none showed signs of lysozyme, indicating that \( \phi KP \) is probably a virulent phage.

From 49 Ecc strains tested \( \phi KP \) was able to plaque on only two, SCR1193 (on which the phage was originally isolated) and ATCC 39048 (a strain presently being studied in this laboratory for its carbapenem antibiotic production). From the range of other *Erwinia* strains tested no susceptible strains were found.

**Screening for transduction**

Preliminary crude screening for transduction of the cysB mutant HC500 to prototrophy suggested that \( \phi KP \) was capable of transduction. In an attempt to optimize the transduction system we investigated the effects of the multiplicity of infection (m.o.i.), UV irradiation, temperature, and adsorption time on the efficiency of transduction.

At an m.o.i. of 1, \( \phi KP \) adsorbed rapidly to SCR1193 over the first 45 min, resulting in adsorption of up to 90% of the phage population. At 30 min, up to 85% of the phage population had adsorbed, and this time period was therefore chosen for further assays, since it allowed a high proportion of the phage population to adsorb and at the same time minimized the time required for the assay. \( \phi KP \) did not adsorb to Eco strain SCR11043, another genetically amenable strain used to study *Erwinia* virulence (Hinton et al., 1989). Although some phages require the presence of divalent cations for adsorption, e.g. \( \lambda \) requires Mg\(^{2+}\) for efficient plaque formation (Arber et al., 1983), the addition of Mg\(^{2+}\) (10 mM-MgSO\(_4\)) did not increase the rate of \( \phi KP \) adsorption to SCR1193. The effects of other possible adsorption factors, e.g. Ca\(^{2+}\) were not tested.

The efficiency of transduction at different temperatures was studied. After initial phage adsorption at 25 °C, \( \phi KP \) failed to produce plaques on SCR1193 at 37 °C. This suggested that post-adsorption incubation could only be carried out at 37 °C, thereby reducing host killing due to co-infection or superinfection of transductants with wild-type phage. In practice, however, fewer transductant colonies were produced at 37 °C (2 \times 10^{-7} p.f.u.\(^{-1}\)) than at 30 °C (6.8 \times 10^{-6} p.f.u.\(^{-1}\)). A combination of healthy cell growth and a 65% reduction in the efficiency of plating (o.p.) at 30 °C gave the best transduction frequencies.

Transduction frequencies at m.o.i. values of 10, 1 and 0.1, and at temperatures of 25 °C, 30 °C and 37 °C were studied. The results indicated that an m.o.i. of 1 at 30 °C produced the highest transduction frequencies (6.8 \times 10^{-6} p.f.u.\(^{-1}\)). A reduced transducing efficiency at an m.o.i. of 10 and 30 °C (1.8 \times 10^{-7} p.f.u.\(^{-1}\)) was probably a result of excess phage killing.

**Generalized nature of transduction**

The ability of \( \phi KP \) to transduce the cysB\(^{+}\) marker was insufficient evidence for generalized transduction. A number of other markers were selected in SCR1193 derivatives and their transduction, via \( \phi KP \), was assessed. Recipients were Tn5-induced mutants of SCR1193, i.e. five auxotrophs and one lactose utilization mutant. In the case of plasmid transduction, \( \phi KP \) lysates were prepared on HC131 with SCR1193 as a recipient (Table 1). All experiments were carried out in triplicate and an average transduction frequency taken together with a standard deviation for each marker. Frequencies of transduction, spontaneous ampicillin resistance and reversion to prototrophy and sugar utilization are given in Table 2. All chromosomal markers tested and the plasmid pHCP2 were transduced at similar frequencies to the cysB\(^{+}\) marker, with the exception of the lac\(^{-}\) marker which transduced at a frequency 13 times lower. Transducing frequencies of \( \phi KP \) ranged from 2.1 \times 10^{-7} p.f.u.\(^{-1}\) to 2.8 \times 10^{-6} p.f.u.\(^{-1}\) without UV stimulation. (Table 2). These values are generally higher than those of Echr-12 (2 \times 10^{-6} to 1.0 \times 10^{-7} p.f.u.\(^{-1}\)), \( \Phi EC2 \) (1.0 \times 10^{-8} to 1.0 \times 10^{-7} p.f.u.\(^{-1}\)) and \( \Phi 59 \) (1.1 \times 10^{-7} to 1.8 \times 10^{-6} p.f.u.\(^{-1}\)). The above results showed that \( \phi KP \) was a generalized transducing phage. Transduction of a number of Tn5 insertion mutations, e.g. cysB::Tn5 into wild-type SCR1193, allowed the percentage of secondary transposition to be calculated for each marker. This was done by comparing the number of kanamycin resistant prototrophic colonies with the number of kanamycin resistant cysB auxotrophs. From these studies, the percentage of secondary transposition for each marker was found to be highly variable, ranging from undetectable to 80%, suggesting that secondary transposition of Tn5 into different areas of the *Erwinia* chromosome...
possibly reaching a maximum in both cases. Similar determining variation in marker transduction frequencies irradiated P1 lysates in E. coli, suggesting a stimulation in recombination efficiency, de Bruijn et al., 1984; Newman & Masters, 1980).

Conclusions

A generalized transducing phage, 4KP, has been isolated from sewage and characterized. A number of chromosomal markers and the plasmid pHCP2 have been transduced and conditions for transduction optimized. Generalized transduction, via 4KP, is now being used extensively in this laboratory for construction of transposon-tagged strains and co-transductionsal linkage mapping. It also provides a powerful tool for the creation of novel mutations, by localized mutagenesis techniques, as our current studies demonstrate (J. N. Housby & G. P. C. Salmond, unpublished). Recent data show that 4KP is also able to transduce in Ecc ATCC 39048 (unpublished).

This work was funded as part of a SERC/CASE studentship with the Scottish Crop Research Institute, Dundee, to I.K.T., and by AFRC awards (PG88/511 and PG88/513) to G.P.C.S. We also acknowledge the technical assistance of Lauren Kinnersley and Graham Pavitt funded by the University of Warwick Research and Innovations Fund.

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Generalized transduction in Erwinia


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