Cloning and Expression in *Escherichia coli* of the Polysaccharide Depolymerase Associated with Bacteriophage-Infected *Erwinia amylovora*

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The bacteriophage-encoded polysaccharide depolymerase produced in *Erwinia amylovora* has been cloned and expressed in *Escherichia coli*. The bacteriophage ERA103 genome was observed to consist of five *EcoRI* fragments, labeled as follows: A, 7.5 kilobases (kb); B, 5.0 kb; C, 2.7 kb; D, 2.1 kb; and E, 1.8 kb. A restriction map for ERA103 was also prepared. Each of the fragments were cloned into the positive-selection vector pOP203(*A<sup>+</sup>*) and pBR322.

*Erwinia amylovora* is the recognized primary causative agent of fireblight in rosaceous plants (2). This species has been demonstrated to produce a polysaccharide capsule that has been implicated with xylem vessel occlusion and plasmolysis of xylem parenchymal cells. These traits are associated with fireblight pathogenesis.

Bacteriophage-encoded polysaccharide depolymerases have been described for many bacterial genera (1, 7, 8). One such bacteriophage, ERA103, has been demonstrated to infect the plant pathogen *Erwinia amylovora* NCPPB595 and produce a depolymerase that degrades the polysaccharide capsule of the host. The enzyme has been purified and characterized by Vandenbergh et al. (15).

This report describes the mapping of the phage genome and the cloning of the B fragment into pBR322 (3), the positive-selection vector pOP203(*A<sup>+</sup>*) (16), and its expression in *Escherichia coli*.

The bacteriophage ERA103 was isolated from fireblight-infected plant tissue as previously described (15). High-titered stocks of bacteriophage ERA103 (10<sup>11</sup> to 10<sup>12</sup> PFU/ml) were developed by the methods of Yamamoto and Alberts (17). Polyethylene glycol-precipitated high-titer phage preparations were centrifuged in cesium chloride-ethidium bromide gradients (14). The cesium chloride-purified bacteriophage DNA was subjected for further dialysis, phenol extraction, and ethanol precipitation.

A restriction map (Fig. 1) depicting the relative positions of various restriction endonuclease recognition sites on phage ERA103 DNA was obtained by using a combination of the following procedures: (i) analysis of DNA fragments obtained after digestion with two enzymes and (ii) analysis of *Escherichia coli* plasmids containing phage ERA103 *EcoRI* fragments. DNA from phage ERA103 was cleaved by *EcoRI* into five distinct fragments labeled A (7.5 kilobases [kb]), B (5.0 kb), C (2.7 kb), D (2.1 kb), and E (1.8 kb).

Bacteriophages of *Erwinia amylovora* have been previously described and characterized by Erskine (5), Richie and Klos (11), and Vandenbergh et al. (15). The bacteriophage DNA, which was previously determined to be doubled stranded, was not cleaved by many restriction enzymes. However, the physical map of the bacteriophage ERA103 was determined, and it included the positions for cleavage sites of three restriction enzymes, *EcoRI*, *SphI*, and *BclI*.

The positions of the ERA103 *EcoRI* fragments A, B, C, D, and E on the map were also determined.

The restriction endonuclease *EcoRI* was used in cloning experiments to determine the possible location of the depolymerase gene. The bacteriophage ERA103 DNA was digested with *EcoRI* and ligated with pOP203(*A<sup>+</sup>*) or pBR322 vector cut to completion with *EcoRI*. The ligation mixture contained approximately a 1:2 ratio of vector DNA and bacteriophage DNA.

FIG. 1. Restriction map of phage ERA103 DNA. Fragments have been lettered in order of decreasing size. *Dep* represents the fragment expressing depolymerase activity.

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TABLE 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid*</th>
<th>Remarks*</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB101</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>HB101(pOP203A2+)</td>
<td>Contains positive selection vector</td>
<td>3</td>
</tr>
<tr>
<td>HB101(pBR322)</td>
<td>Contains plasmid pBR322</td>
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<tr>
<td><strong>Plasmids</strong></td>
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<tr>
<td>pOP203(A2+)</td>
<td>lac A2 tet</td>
<td>16</td>
</tr>
<tr>
<td>pBR322</td>
<td>bla tet</td>
<td>3</td>
</tr>
<tr>
<td>pSRQ51</td>
<td>Phage fragment A in pOP203(A2+) EcoRI site</td>
<td>This study</td>
</tr>
<tr>
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<td>Phage fragment B in pOP203(A2+) EcoRI site</td>
<td>This study</td>
</tr>
<tr>
<td>pSRQ53</td>
<td>Phage fragment C in pOP203(A2+) EcoRI site</td>
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<tr>
<td>pSRQ54</td>
<td>Phage fragment D in pOP203(A2+) EcoRI site</td>
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</tr>
<tr>
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<td>Phage fragment E in pOP203(A2+) EcoRI site</td>
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<td>Phage fragment B in pBR322</td>
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<tr>
<td>pSRQ58</td>
<td>SphI segment of the B fragment in pBR322 SphI site</td>
<td>This study</td>
</tr>
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</table>

* Escherichia coli strains were grown in L broth (4). Tetracycline was added to media at a concentration of (10 μg/ml), carbenicillin (50 μg/ml), or isoprpyl β-D-thiogalactoside (1.0 mM; Sigma) was added to the media as indicated in the text.

** lac lactose promoter-operator; A2, maturation protein gene of the RNA bacteriophage Qβ; tet, tetracycline resistance; blu, ampicillin resistance.

to bacteriophage DNA. Ligation was performed at 17°C for 18 h. Restriction endonucleases and T4 DNA ligase (Bethesda Research Laboratories, Gaithersburg, Md.) were used in the buffers and under the conditions recommended by the supplier. Bacterial strains used or constructed in this study are described in Table 1. Escherichia coli was transformed by the CaCl2-heat shock method (4), with cells harvested at an A600 of 0.6.

Colonies were then screened for the presence of plasmid DNA containing the five EcoRI phage fragments (14). All five EcoRI fragments were cloned into pOP203(A2+) (Table 2). The plasmid-containing strains were grown overnight in 250 ml of L broth at 37°C supplemented with either tetracycline (10 μg/ml; Sigma Chemical Co., St. Louis, Mo.) or carbenicillin (50 μg/ml; United States Biochemical Corp., Cleveland, Ohio), depending on the vector used. This culture was then diluted 1:100 into 250 ml of similar media. Cells were harvested at an A600 of 0.9. All subsequent fractionation steps were performed at 4°C. The cells then were centrifuged at 8,000 × g for 30 min. The pellet was then suspended in sterile distilled water and centrifuged at 8,000 × g for 30 min. The washed pellet was then suspended in 10 ml of 0.01 M citrate buffer (pH 6.0) containing 0.01 M 2-mercaptoethanol (CME buffer). Cell extracts were prepared by passage of washed cell suspensions through a French press at 16,000 lb/in2 three times. The resultant cell extract was then centrifuged at 27,000 × g for 30 min to remove whole cells and cell debris. Ammonium sulfate was added slowly to the supernatant to give a final concentration of 45% saturation and precipitated for 18 h. The ammonium sulfate precipitate was centrifuged at 27,000 × g for 30 min and dialyzed overnight against CME buffer. Depolymerase activity was assayed by measuring the release of galactose from the polysaccharide substrate by the method of Fairbridge et al. (6). Polysaccharide was prepared from uninfected cultures of Erwinia amylovora NCPPB595 cultivated on sheets of cellophane overlaying tryptic soy agar as described by Liu et al. (10). Enzyme assays in triplicate of cell extracts of cells containing each of the cloned fragments demonstrated that enzyme activity was associated with the EcoRI B fragment, which is 5.0 kb and is contained in pSRQ52. Depolymerase activity always was associated with the supernatants of the cell extracts and not demonstrated in the pellets.

Mapping data revealed the presence of SphI sites in the phage ERA103 DNA. The vector pBR322 was used because of the insertional inactivation of tetracycline in the SphI site of this vector. Transformants that were resistant to carbenicillin and sensitive to tetracycline were screened for plasmids and depolymerase activity. An Escherichia coli strain containing the plasmid pSRQ58 and a 1.5-kb portion of the B fragment demonstrated depolymerase activity. The subcloned SphI segment of the B fragment reflected a 43% increase in specific activity relative to the entire B fragment cloned in pBR322.

Results of the cloning experiments indicate that the expression of depolymerase in Escherichia coli is relatively low when compared with results obtained in the bacteriophage-infected Erwinia amylovora system (15).

The functional role of the phage-encoded depolymerase remains hypothetical. However, it has been suggested that the enzyme assists in the release of phage particles from infected cells (12).

Bacteriophage-encoded depolymerases have been described for many genera. However, this is the first report of a cloned depolymerase for Erwinia amylovora. Biological control has been suggested as a possible replacement for pesticide use for the control of many plant diseases (13). This enzyme may offer a potential replacement for antibiotic therapy in the control of Erwinia amylovora, the causative agent of fireblight.

LITERATURE CITED


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