Characterization of Temperate Bacteriophages of *Bacillus subtilis* by the Restriction Endonuclease EcoRI: Evidence for Three Different Temperate Bacteriophages

G. A. WILSON, M. T. WILLIAMS, H. W. BANEY, AND F. E. YOUNG

Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Received for publication 29 April 1974

Temperate bacteriophages of *Bacillus subtilis* were characterized according to host range and digestion of the bacteriophage genome by endonuclease EcoRI. The three bacteriophages, φ3T, SPO2, and φ105, were all heteroimmune, and the DNA digestions showed dissimilar patterns by agarose-ethidium bromide gel electrophoresis.

The discovery of a restriction endonuclease in *Haemophilus influenzae* that was capable of cleaving DNA at sites determined by a unique sequence of nucleotides (10, 20) provided a new dimension in molecular genetics. Now for the first time fragments of DNA could be generated with specific ends, recombined, and studied in vitro and in vivo. The development of this new, powerful tool for manipulating genetic information prompted a search for similar enzymes with different specificities. Such enzymes have been isolated from *Haemophilus parainfluenzae* (7, 19), *Haemophilus aegyptius* (12), and *Escherichia coli* (8). By preparing a limit digest of DNA with these enzymes, it is possible to obtain a series of unique fragments that retain biological activity. For instance, Edgell and Hutchison (6) subjected DNA from bacteriophage φX174 to this digestion and determined genetically which markers resided on the fragments. Genomes that have proven to be difficult to map by conventional means have recently been successfully investigated by fragmentation with restriction endonucleases. Morrow and Berg (13) and Mulder and Delius (14) combined the techniques of endonuclease digestion and electron microscopy to map the restriction site on simian virus 40 DNA. With the introduction of agarose-ethidium bromide gel electrophoresis (19), it became possible to rapidly visualize the products of digestion and to separate them according to size. Thus, a “fingerprint” of a genome can be obtained for taxonomic studies. It is important to emphasize the limitations of this methodology. The fingerprint will only indicate whether the genomes are broken into similar or dissimilar sizes. To determine rigorously if the fragments of the same size do indeed represent a similar region of the genome, it would be necessary to hybridize the two fragments to show base sequence homology. Regardless of this limitation, it is doubtful if genomes that show markedly dissimilar patterns can be closely related. Therefore, we decided to apply this technique to examine the degree of relatedness between the temperate bacteriophages of *B. subtilis*.

EcoRI was isolated from *E. coli* RY13, using a procedure communicated to us by H. O. Smith and P. Geshelin. Essentially, the enzyme was purified in the following manner. Cells (124 g [wet weight]) were harvested from LB broth during the late logarithmic stage of growth and sonically treated for 30 min, and the cell debris was removed by low-speed centrifugation followed by high-speed centrifugation (100,000 × g). Nucleic acids were removed by precipitation with streptomycin sulfate. The enzyme was precipitated from the supernate with ammonium sulfate (50% saturation). After dialysis, the precipitated fraction was purified by column chromatography on Whatman P11 phosphocellulose and Whatman DE-52 DEAE-cellulose. The final enzyme preparation was stored at 4°C in 50% glycerol containing 0.015% Triton X-100. The enzyme appears to be free of exonuclease activity and cleaves DNA from bacteriophage λp1ac into seven fragments and polynucle DNA once (11, 13, 14). This evidence confirms that the enzyme is the EcoRI restriction endonuclease.

Bacteriophages were treated with pancreatic DNase and RNase and further purified by equilibrium centrifugation in CsCl. DNA was isolated from these bacteriophage preparations by treatment with 1% sodium dodecyl sulfate followed by three extractions with buffer-saturated (0.1 M Tris-hydrochloride, pH 8.0)
phenol. An equal volume of saturated NaCl was added before precipitation of the DNA with two volumes of ethanol. The preparations of DNA were added to an agarose-ethidium bromide gel and subjected to electrophoresis. The DNA from bacteriophages $\phi 105$ and SPO2 yielded a single band indicating homogeneity of the preparations (Fig. 1). Both genomes have a molecular weight of $26.3 (\pm 0.3) \times 10^6$ (4).

When a digest of the DNA with EcoRI endonuclease was fractionated by agarose-ethidium bromide gel electrophoresis, the patterns were quite different (Fig. 1). EcoRI cleaved $\phi 105$ DNA into 11 fragments (8 major bands and 3

![Diagram](http://jvi.asm.org/)
minor low-molecular-weight bands) and SPO2 DNA into 7 fragments (the two minor bands in the middle are often difficult to observe), as indicated diagrammatically in Fig. 1. Two of the fragments have approximately the same molecular size; however, further experiments will be required to establish whether these fragments are identical.

During the course of these studies, we received another temperate bacteriophage, φ3T. This phage was originally isolated and designated φ3 by R. G. Tucker. To avoid confusion with the bacteriophage φ3 isolated by B. E. Reilly (Ph.D. thesis, Western Reserve University, Cleveland, Ohio, 1965), we have changed the designation of the bacteriophage isolated by Tucker to φ3T. Bacteriophage φ3T has a guanine plus cytosine content of 35.9% (21). This guanine plus cytosine content is markedly different than the 43% obtained for bacteriophages φ105 and SPO2 (1, 16). R. E. Yasbin, in this laboratory, constructed lysogenic strains in our standard isogenic strain BR151 to determine if this temperate bacteriophage interfered with transformation as did bacteriophages SPO2 and φ105 (23). The efficiency of plaque formation on these lysogenic strains by the three bacteriophages indicated that φ3T was heteroimmune to φ105 and SPO2 (Table 1). The decreased efficiency of plating of bacteriophage φ3T on B. subtilis lysogenic for φ105 and SPO2 may be related to bacteriophage interference (15, 22). The bacteriophage φ3T forms a stable association with the host, is immune to superinfection, and can be induced with mitomycin C (M. T. Williams, unpublished data) or hydrogen peroxide (21), thus indicating that the bacteriophage is temperate.

DNA from DNase-treated CsCl purified φ3T was digested with EcoRI endonuclease and chromatographed on agarose-ethidium bromide gel electrophoresis. There are many more EcoRI cleavage sites for bacteriophage φ3T than were found for bacteriophages φ105 or SPO2 (Fig. 1). It is difficult to resolve all of the low-molecular-weight fragments; however, it appears that 24 fragments are generated by EcoRI digestion.

The degree of relatedness between the temperate bacteriophages has been the subject of a number of investigations. Two of the bacteriophages, φ105 and SPO2, have DNA with the same molecular weight and are morphologically similar (2, 3, 16). Bacteriophages SPO2 and φ105 are certainly different in that they are heteroimmune (2), integrate at different locations in the host chromosome (9, 17), and only share approximately 14% base sequence homology as determined by electron microscopy of heteroduplex DNA molecules (4). In addition, Chow and Davidson (5) demonstrated that the SPO2 prophage is a circular permutation of the mature phage, whereas φ105 prophage and mature phage are co-linear. None of the mutants of bacteriophage φ105 can be complemented by bacteriophage SPO2 in the genetic studies performed to date (18). This communication indicates yet another difference between the two phages. By digestion with restriction enzyme EcoRI, dissimilar fragments of the genome are produced, thus indicating that recognition palindromes for the enzyme are located at different sites on the genome.

The bacteriophage φ3T has not been compared to the other temperate bacteriophages; however, one would expect this phage to be quite different because of its abnormally low content of guanine and cytosine (35.9%). Digestion of the φ3T genome by EcoRI emphasizes the unrelatedness of this temperate bacteriophage. The “fingerprinting” of the genome by

<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th>BR151</th>
<th>BR151 (φ3T)</th>
<th>BR151 (φ105)</th>
<th>BR151 (SPO2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>φ3T</td>
<td>5.14 × 10⁸ (1.00)</td>
<td>0</td>
<td>3.33 × 10⁸ (0.65)</td>
<td>4.33 × 10⁸ (0.84)</td>
</tr>
<tr>
<td>φ105</td>
<td>3.60 × 10⁸ (1.00)</td>
<td>2.90 × 10⁸ (0.81)</td>
<td>0</td>
<td>3.23 × 10⁸ (0.90)</td>
</tr>
<tr>
<td>SPO2</td>
<td>5.92 × 10⁸ (1.00)</td>
<td>8.45 × 10⁸ (0.14)</td>
<td>7.69 × 10⁸ (1.30)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Bacteriophages were titered on different hosts by the semisolid overlay method described previously (23). Parentheses after the strain indicate that the host is lysogenic for the bacteriophage indicated. The efficiency of plating is given relative to strain BR151 in parentheses beneath the bacteriophage titer.
restriction endonucleases is, therefore, a rapid diagnostic tool to test relatedness between genomes in the absence of characterized mutants.

We wish to thank H. O. Smith and the members of his laboratory who generously provided suggestions for the isolation and assay for EcoRI. We are indebted to Carel Mulder who ran preliminary assays of a number of restriction enzymes on our DNA samples and offered suggestions on the choice and purification of the enzyme and communicated the electrophoresis methodology to us prior to its publication (19). C. Hutchison III provided us with E. coli strain RY-13. This research was supported by grant VC-27-J from the American Cancer Society and Public Health Service grant IT05 GM-02263 from the National Institute of General Medical Science to M.T.W.

LITERATURE CITED