NOTES

Determination of the Packaging Capacity of Bacteriophage VWB

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VWB is a temperate bacteriophage whose chromosome has cohesive ends. VWB can stably package modified chromosomes that contain insertions of up to about 4 kilobases of foreign DNA. Phage particles containing extra DNA differ from the wild type in their increased sensitivity to chelating agents. Because of these properties, VWB is a promising cloning vector for Streptomyces venezuelae.

Bacteriophage-based cloning vectors have already demonstrated their usefulness in cloning work and genetic analysis with different genera, including streptomyces. With respect to phage-derived cloning vehicles for streptomyces, several phages or their in vitro-manipulated derivatives have been described as potentially interesting (for a review, see reference 6). However, only φC31 derivatives have, so far, been applied extensively to both gene cloning (5) and mutational inactivation (18). A major advantage of φC31 derivatives is that up to 9.5 kilobases (kb) of foreign DNA can be cloned and packaged in the φC31 phage heads (9), while in other phage derivatives the amount of supplementary DNA is limited to 2.0 kb at the most.

We were investigating gene expression in Streptomyces venezuelae and intended to use phage-based vector systems. Although φC31 has a broad host range, it does not infect S. venezuelae. Therefore, we had to look for other phages.

In a previous article we described the isolation of bacteriophage VWB (2). This temperate phage with a genome size of 47.3 kb (1) was isolated from soil seeded with S. venezuelae. During the molecular analysis of this phage and the attempt to obtain deletion mutants, we observed that VWB was unusually resistant to chelating agents. This was rather surprising, since the phage DNA has cohesive ends (1) and there was no indication from the restriction mapping of a headful mechanism of packaging. The resistance to chelating agents suggested that the VWB phage head has substantially less DNA than one headful (17). This suggestion is confirmed by the results presented here. This report describes investigations of the amount of DNA that could be packaged in addition to the wild-type genome in the VWB phage heads and the influence of this amount of DNA on the sensitivity of the phage head to chelating agents. The results also indicate that because of the unusually high amount of DNA that can be packaged supplementary to the VWB wild-type genome, this phage is an interesting candidate to be developed as a phage-based cloning vector for S. venezuelae.

VWB DNA was obtained from phage particles multiplied in S. venezuelae ETH 14630 (ATCC 40755) as described elsewhere (2). Plasmids pIJ486 (20), pIJ699 (15), and pIJ702 (12) were propagated in Streptomyces lividans TK24 (10), and pGVaph2, a derivative plasmid of pGV451 (19), was amplified in Escherichia coli JM83 [ara Δ(lac proAB) rpsL φ80 lacI4 ΔlacZM15]. Plasmid DNA was extracted from cells by alkaline lysis (3, 13) and purified by CsCl-ethidium bromide centrifugation. DNA was stored in TE buffer (10 mM Tris hydrochloride, 1 mM EDTA; pH 7.5). Prior to being cloned in VWB or its derivatives, phage DNA was ligated with T4 DNA ligase (Boehringer GmbH, Mannheim, Federal Republic of Germany). Concatemer formation during ligation was avoided by using diluted VWB DNA solutions (ca. 0.05 µg of DNA per µl). Other ligations were also carried out with T4 DNA ligase.

For cloning in VWB, the ligated VWB DNA was partially digested with BglII because VWB DNA has, so far as is known, a unique restriction site only for EcoRI (1). However, because this site is located in an essential region (unpublished data), it is unsuitable for cloning purposes. BglII partial digestions were carried out by treatment with 0.1 U of enzyme for 1 to 5 min. Reactions were stopped by heat inactivation (65°C, 10 min). BglII and other restriction enzymes were obtained from Boehringer or Bethesda Research Laboratories, Inc. (Bethesda, Md.) and were applied as recommended by the manufacturers. Results of digestion were controlled by horizontal agarose gel electrophoresis with 40 mM Tris hydrochloride (pH 7.8)–10 mM sodium acetate–1 mM EDTA as buffer (16). When appropriate, restriction fragments to be cloned were isolated from the gel after electrophoresis and purified by the GeneClean process (BIO 101, La Jolla, Calif.).

Several derivatives of VWB were constructed (Table 1). To facilitate the selection of transfectants, the cloned fragments contained one or two resistance markers, including those for thiostrepton (tsr), neomycin (aph), or viomycin (vph). VWB4 correlated with VWB and the polylinker (76 base pairs) of pIJ486 which contains in the BamHI site the 1.08-kb Bell fragment of pIJ702 with a tsr gene (Fig. 1). As a consequence, VWB4 possesses a unique XbaI cloning site (situated in the 76-base-pair polylinker) and the tsr selection marker. To increase the number of unique cloning sites in this vector, VWB05 was developed. This construction is similar to VWB4, except that it contains three unique cloning sites, i.e., XbaI, DraI, and XhoI. These sites were added to VWB04 by cloning the synthetic oligonucleotide

\[
5'\text{CTAGAGTTTAAACTCGAG3'}
\]

TCAAAATGAGCTCGATC

in the XbaI site. VWB10 was obtained by insertion of
TABLE 1. Properties of VWB and its derivatives

<table>
<thead>
<tr>
<th>Phage</th>
<th>Selection marker(s)</th>
<th>Size (kb)</th>
<th>% of size of wild-type isolate</th>
<th>Sensitivity to EDTA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWB</td>
<td>None</td>
<td>47.3</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>VWB04</td>
<td>tsr</td>
<td>48.5</td>
<td>102.5</td>
<td>-</td>
</tr>
<tr>
<td>VWB10</td>
<td>tsr, aph</td>
<td>50.9</td>
<td>107.6</td>
<td>±</td>
</tr>
<tr>
<td>VWB20</td>
<td>tsr, vph</td>
<td>52.5</td>
<td>111.0</td>
<td>+</td>
</tr>
</tbody>
</table>

* Symbols: -, not sensitive; +, sensitive; ±, somewhat sensitive.

pGVaph2 in VWB04. Therefore, circularized VWB04 and the plasmid pGVaph2 were linearized with XbaI and subsequently ligated. Since pGVaph2 comprises the aph gene, VWB10 lysogens should be both thiostrepton and neomycin resistant. For the construction of VWB20, a 4.05-kb XbaI digested with K- BamHI. XbaI BglII polylinker (76bp) BamHI 1.085kb fragment ligated to plJ486 digested with BamHI 1.085kb fragment ligated to plJ486 digested and collected from gel with BclI

FIG. 1. Construction of VWB04. To clone the polylinker with the inserted tsr gene in VWB, phage VWB DNA was circularized at the cohesive ends and subsequently digested with BglII in such a manner that most of the molecules were digested at only a single site ( ). Nondigested BglII sites located on VWB DNA are also indicated ( ). bp, Base pairs.
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VWB20. VWB (lane 2) and VWB04 (lane 3) are compared by BglII digestion showing the 1.16-kb insert of VWB04 ( ). After XbaI digestion, the 2.4- and 4.05-kb XbaI fragments ( ) inserted in the unique XbaI site of VWB04 (lane 5) could be reexcised from VWB10 (lane 6) and VWB20 (lane 7). VWB (lane 4) does not contain XbaI sites. Lane 1 is a standard containing bacteriophage lambda DNA digested with EcoRI and HindIII. Sizes (at the left) are in kilobases.

replica plated on selective medium to detect the transfectants.

In the cases of VWB04 (48.5 kb) and VWB05 (48.5 kb), the lysogens were detected as thiostrepton-resistant clones. Phages released from the lysogens after reinfection gave rise to thiostrepton-resistant colonies. VWB10 (50.9 kb), containing the complete pGVaph2 plasmid comprising the aph gene of pIJ211 (14), produced after transfection lysogens that were both thiostrepton and neomycin resistant, and phages released from these lysogens produced upon reinfection thiostrepton- and neomycin-resistant lysogens. Restriction enzyme analysis (Fig. 2) of the DNA extracted from single plaque isolates and obtained after several subcultures indicated that the 1.16-kb fragment (for VWB04 and VWB05) and the 2.4-kb fragment (for VWB10) were stably integrated in the phage genome. These chromosome lengths could, in consequence, be packaged without any problem, even though the genome sizes of VWB04 (like VWB05) and VWB10 were 102.5 and 107.6%, respectively, of that of the VWB wild-type isolate. Restriction enzyme analysis using double digests (data not shown) indicated that the cloned fragments were inserted only once in each VWB molecule.

The cloning of the 4.05-kb fragment of pIJ699 in VWB04, which resulted in VWB20, demonstrated that even larger DNA fragments could be packaged in VWB heads. The addition of the 4.05-kb fragment increased the amount of DNA to be packaged to 111% of that in the wild-type isolate, which is higher than the amount of DNA packaged by any other known phage (7, 8, 11). It appeared, however, that 52.5 kb (i.e., 5.2 kb of additional DNA) is probably almost the upper limit of packaging in VWB phage heads, since from the transfectants obtained, only about 20% of the isolates contained the whole 4.05-kb fragment (Fig. 2). Other isolates had deletions of 1.0 to 2.0 kb of the inserted fragment. No deletions in the phage DNA itself were observed, indicating that deletions specifically occurred in the inserted DNA. Similar specificity of deletion in the inserted DNA was observed when an EcoRI-PvuII fragment of pBR322 (4) was cloned in VWB10, increasing the amount of DNA inserted in VWB to 5.88 kb (results not shown). Once again, a majority of phages released from the transfectants contained deletions specifically in the inserted DNA and not in the phage DNA.

As mentioned above, VWB is resistant to the chelating agents PP, and EDTA. To investigate the resistance of phage particles with modified VWB chromosomes to these compounds, phages were suspended at 10^6 to 10^7 PFU/ml in phosphate-buffered saline (in grams per liter: NaCl, 8.5; KH2PO4, 0.3; Na2HPO4·2H2O, 0.75 [pH 7.8]) and increasing concentrations of sodium PP, (0 to 30 mM) or EDTA (0 to 40 mM). Suspensions were incubated in sodium PP, and EDTA solutions at 37°C for 60 min and at 45°C for 10 min, respectively. Treatment was stopped by a 10-fold dilution of the suspension with phosphate-buffered saline. The survival rate of the phages was then estimated by plating serial dilutions and comparing the phage titers of treated and untreated samples. The results (Fig. 3) showed that VWB04 remained resistant, VWB10 became somewhat sensitive, and VWB20 was very sensitive and comparable to other sensitive phages, e.g., CPT (2). Furthermore, VWB20 phages with deleted DNA sequences of 1 to 2 kb (ΔVWB20) became resistant again.

From the results of the investigations involving both the estimation of the packaging capacity of VWB phages and the comparison of the sensitivity of VWB phage particles containing different amounts of DNA, it appears that VWB contains about 3 to 4 kb less DNA than a headful. Because VWB has the capacity to package stably at least 3.5 to 4 kb in addition to the phage genome, this phage has much promise for development into a useful cloning vector for S. venezuelae. A first step in this direction was the construction of VWB05.

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LITERATURE CITED