Construction of a Hybrid Plasmid Capable of Replication in the Bacterium *Escherichia coli* and the Cyanobacterium *Anacystis nidulans*

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A hybrid plasmid was constructed between the 5.3-megadalton plasmid (pUH24) of *Anacystis nidulans* R2 and the *Escherichia coli* plasmid pBR322. This was accomplished by adding a transposon to pBR322 and transforming this DNA into *A. nidulans*. One resultant hybrid, pLS103, had a molecular weight of \(6.8 \times 10^8\), replicated in both organisms, had unique sites for two restriction endonucleases, conferred ampicillin resistance on both organisms, and could be used as a cloning vector in *A. nidulans*.

Interest in photosynthesis and photosynthetic organisms has increased greatly in recent years because of the combined impact of problems in energy and agriculture. Concomitantly, the development of molecular cloning techniques in bacteria has made it possible for individual genes to be cloned in a phage or plasmid vector and for the corresponding DNA fragments to be isolated and studied (2). Not surprisingly, the two lines of research are beginning to converge, and the cloning of genes from plants and algae has been reported with increasing frequency (1, 6, 9, 10). In this context, unicellular cyanobacteria would provide an excellent system for the cloning of genes coding for photosynthetic functions. These organisms perform an aerobic photosynthesis nearly identical to that of green plants; they can be manipulated like bacteria, and they possess only one major chromosome coding for photosynthetic proteins (5). Suitable mutations can now be isolated in a wide variety of functions, and we have recently obtained numerous temperature-sensitive photosynthetic mutants in unicellular cyanobacteria (8, 13, 14).

The suitability of cyanobacteria for molecular cloning has improved in the past 2 years for three reasons. First, plasmids have been observed in many strains (9, 16, 18), although the plasmids are presently genetically cryptic. Second, a unicellular strain with a high frequency of transformation is now available (15, 19). Finally, van den Hondel et al. (19) used a transposon to produce a plasmid that contains a selectable genetic marker. This was accomplished by transforming a bacterial plasmid that contained a transposon with a gene conferring ampicillin resistance into the transformable strain, *Anacystis nidulans* R2. The transposon, Tn901, was able to transpose onto the cyanobacterial plasmid of 5.3 megadaltons (Mdal) (pUH24); this transposition generated an 8.3-Mdal plasmid that conferred ampicillin resistance on *A. nidulans*. The purpose of the present work was to take this process one step further and produce a hybrid plasmid capable of replication in both *A. nidulans* and *Escherichia coli*. This was accomplished by adding a chloramphenicol transposon to the *E. coli* plasmid pBR322 and transforming the pBR322::Cm into *A. nidulans* R2. Cointegrate formation between this plasmid and the indigenous 5.3-Mdal species was detected by selecting for *A. nidulans* colonies resistant to ampicillin. This yielded hybrid plasmids containing the origins of replication of pUH24 and pBR322 as well as the ampicillin gene of pBR322. We report on the biological and physical properties of one such hybrid plasmid, pLS103. This plasmid is 6.8 Mdal, has unique sites for two restriction enzymes, replicates in either organism, and confers ampicillin resistance on both organisms. Thus, we now possess the capability of cloning photosynthetic genes directly in photosynthetic mutants; further analyses of the cloned genes will be facilitated by growth of the cloned vector in *E. coli*.

The cyanobacterial strain *A. nidulans* R2 (isolated in Russia by Grigor'eva and Shestakov [7]) was obtained from G. van Arkel. The cells were grown axenically in liquid culture as previously described (8, 13). The *E. coli* K-12 strains used were HB101 (hsdR\(^+\) hsdM\(^-\) recA supE44 lacZ4 leu thi), obtained from H. Boyer, Stanford University) and GDB4::Cm (obtained from C. van Sluis, University of Leiden). Cultures of *E. coli* were routinely grown in LC medium containing 10 g of tryptone (Difco Laboratories)—5 g of...
yeast extract (Difco)–8 g of NaCl–5 ml of 0.1 M Tris per liter supplemented with 12.5 mg of thymine and 1 g of MgSO₄.

Plasmid pBR322 (3) was kindly provided by E. van Leeram, University of Leiden, whereas the 8.3-Mdal plasmid (pCH1 of van den Hondelel al. [19]) was a gift of C. van den Hondelel and G. van Arkel, University of Utrecht. For selection of antibiotic-resistant strains of E. coli, 20 μg of tetracycline, 25 μg of chloramphenicol, and 40 μg of ampicillin per ml were used. For selection of ampicillin-resistant cyanobacterial strains, 0.5 μg of ampicillin per ml was used on plates, and 1.0 μg/ml was used in liquid culture. Plasmid DNA was isolated from bacteria by a cleared lysate procedure similar to that of Meagher et al. (12) and from A. nidulans by the procedure of van den Hondelel et al. (18). Electrophoresis of plasmid DNA or DNA fragments on agarose gels (Seakem) was performed on 0.7 to 1.0% gels. Horizontal submerged gels were run by using EB buffer (80 mM Tris, 2 mM sodium acetate, 2 mM EDTA, pH 8.3). The determination of covalently closed circular DNA on gels was made by the heat treatment procedure described previously (18).

The hybrid plasmid was constructed in a two-stage procedure. The initial step consisted of insertion of a chloramphenicol transposon into pBR322. The donor strain, E. coli GD84, contained a transposable Cm element in the chromosome; however, this transposon was not Tn9 as anticipated. The transposon had a different restriction pattern than that of Tn9 (one extra EcoRI site and different cleavage sites for SalI and PsiI), transposed with much higher frequency, and appeared to be a previously uncharacterized transposon (P. van de Putte, unpublished data). Of the 12 pBR322::Cm molecules produced, we chose one that was tetracycline sensitive and had the transposon inserted between the BamHI and SalI sites in the tetracycline gene (unpublished data).

The pBR322::Cm DNA was next transformed into A. nidulans R2 by using 20 μg of plasmid DNA per 10⁸ cells (Fig. 1a). Selection was performed on 0.5 μg of ampicillin per ml, and six ampicillin-resistant colonies were obtained after 6 days (all controls yielded no ampicillin-resistant colonies). Plasmid DNA was isolated from each strain, designated pLS101–pLS106, and analyzed by agarose gel electrophoresis. The plasmids ranged in size from 6.0 Mdal for pLS104 to 14.0 Mdal for pLS105. None of the strains contained the 5.3-Mdal plasmid, but all still had the 33-Mdal species. Since the strain containing pLS103 grew best on ampicillin and since this plasmid had a relatively small size (6.8 Mdal), it was chosen for more detailed studies.

The restriction map of pLS103 is shown in FIG. 1. (a) Diagrammatic representation of the construction of pLS103 in vivo. The cyanobacterium A. nidulans was transformed with pBR322::Cm, and Ap' cells were selected. Cointegrate formation between pBR322::Cm and the indigenous plasmid pUH24 (5.3 Mdal) resulted in the formation of the hybrid pLS103. The boldface line in the hybrid represents the position of pBR322. (b) Circular restriction map of pLS103. Locations of the restriction cleavage sites were determined by digesting the plasmid with one or more restriction enzymes and analyzing the size and number of the fragments produced (see Fig. 2). The positions of certain sites were normalized to 6.8 Mdal, although the total size of the fragments deviated somewhat from this value (see Fig. 2). The following restriction sites in pUH24 were missing in pLS103: two BglII, two HindIII, three KpnI, three PstI, and one XhoI.
molecules were deleted after transposition (17, 19). The deletion of a number of restriction sites can be seen from the data in Fig. 1. For example, two BglII sites, two HindIII sites, three KpnI sites, and the unique XhoI site have been deleted. Interestingly, most of the deleted pieces are from two large segments of pUH24 (19). The pLS103 hybrid retains the unique Sall site from pBR322, the HindIII site from pBR322, and one BamHI site from each original molecule. The hybrid has three EcoRI sites, implying that there may be vestigial fragments of the transposon remaining. The map indicates that the site of transposition was between the two PvuII sites on pUH24.

As shown in Table 1, pLS103 could be transformed into A. nidulans R2 and E. coli HB101 and replicate stably in both organisms. This DNA could confer ampicillin resistance on both strains with an efficiency comparable to that of standard plasmid DNA controls. This implies that pLS103 contains the replication origin of both pBR322 and pUH24. In addition, pLS103 could be amplified in E. coli (but not in A. nidulans) with chloramphenicol and spectinomycin. Since the transposon had been inserted into the tetracycline gene and the transposon had been deleted, the plasmid only conferred ampicillin resistance on the two strains.

After the first cycle of transformation, one colony each of ampicillin-resistant E. coli and A. nidulans was picked and grown in liquid medium, and the plasmids from each strain were isolated. This DNA was again transformed into the two strains (Table 1); transformation frequencies were quite similar to those obtained with the original pLS103 isolated from A. nidulans. Thus, biological activity was not modified by growth in heterologous bacteria. The restriction patterns of the plasmid DNA from any of these sources was identical, implying that the DNA is not changed during growth in different organisms. Since plasmid DNA can be amplified in E. coli and there is no contamination with the large cyanobacterial plasmid, the bacterial source of the plasmid is preferable.

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TABLE 1. Transformation of hybrid plasmid DNA, pLS103, into A. nidulans and E. coli

<table>
<thead>
<tr>
<th>Plasmid donor DNA</th>
<th>Recipient</th>
<th>No. (×10^3) of Ap&lt;sup&gt;+&lt;/sup&gt; transformants/ml per μg of DNA</th>
<th>Recipient</th>
<th>No. (×10^3) of Ap&lt;sup&gt;+&lt;/sup&gt; transformants/ml per μg of DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2(pLS103)&lt;sub&gt;1&lt;/sub&gt; (6.8 Mdal)</td>
<td>A. nidulans R2</td>
<td>135; R2(pLS103)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>R2</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HB101</td>
<td>715</td>
</tr>
<tr>
<td></td>
<td>E. coli HB101</td>
<td>725; HB101(pLS103)&lt;sub&gt;1&lt;/sub&gt;</td>
<td>R2</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HB101</td>
<td>675</td>
</tr>
<tr>
<td>pCH1</td>
<td>A. nidulans R2</td>
<td>225</td>
<td>R2</td>
<td>270</td>
</tr>
<tr>
<td>pBR322</td>
<td>E. coli HB101</td>
<td>1,650</td>
<td>HB101</td>
<td>1,530</td>
</tr>
</tbody>
</table>

<sup>a</sup> Transformation of A. nidulans was performed by a method similar to that described by van den Hondel et al. (19); 0.5 ml of exponentially growing cells (10<sup>8</sup> cells per ml) was incubated with 1 μg of cyanobacterial plasmid DNA or hybrid plasmid DNA. Cells were always illuminated with white light at an intensity of 3000 lx. Aliquots of 0.1 ml were plated on agar that did not contain antibiotic and incubated for 20 h in the light. Then 0.5 ml of ampicillin was added underneath the agar to give a final concentration of 0.5 μg/ml. Resistant colonies could be scored after 6 days, and appropriate colonies were then grown in medium containing 1 μg of ampicillin per ml. All results are the average of two experiments performed in triplicate. Transformation of E. coli was performed by using the Ca<sup>2+</sup> treatment of Cohen et al. (4). R2(pLS103)<sub>1</sub> and HB101(pLS103)<sub>1</sub> indicate that plasmid DNA from a specific colony of the original transformation was isolated and used for the second round of transformations. No transformants were ever obtained when pBR322 was incubated with A. nidulans or when pCH1 or pUH24 was incubated with E. coli.


