Proceedings of the Indiana Academy of Science
(1988) Volume 98 p. 367-371.

A COMPARISON OF TRANSFORMATION EFFICIENCY OF HYBRID CLONING VECTORS pPGV5 AND pTNTV IN THE CYANOBACTERIA *ANACYSTIS NIDULANS* R2 AND *A. NIDULANS* R2SPc

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ABSTRACT: In this study, a comparison was made of the transformation efficiencies of two hybrid vectors into the cyanobacteria *Anacystis nidulans* R2 and *A. nidulans* R2SPc. The two vectors were hybrid cloning vectors capable of replication in both strains of *A. nidulans* as well as in *Escherichia coli*. In addition, these vectors contained the powerful bacteriophage lambda promoter sequences for enhanced expression of cloned genes. Plasmid pTNTV was constructed from pPGV5 to create a unique restriction site to facilitate the cloning of DNA fragments into the vector and to create a smaller vector so that the vector with an inserted DNA fragment would be of a reasonable size for transformation. The transformation frequencies obtained using pTNTV were 1.5 x 10^7 transformants/μg DNA for *A. nidulans* R2 and 6.6 x 10^6 transformants/μg DNA for *A. nidulans* R2SPc. Thus, the modified vector pTNTV retains the high transformation efficiency of the parent vector pPGV5 but will be a more useful cloning vector as a result of its smaller size and unique cloning site.

INTRODUCTION

Cyanobacteria are prokaryotes that perform oxygenic photosynthesis much like that of eukaryotic plants. They also contain important bacterial properties that allow for easy genetic manipulation. Some of these properties are the existence of indigenous plasmids useful for constructing hybrid cloning vectors, short reproduction time, a small genome, and most importantly, the capability to be transformed.

Transformation is a process that involves genetic modification induced by the assimilation of DNA. In this study, transformations were performed using hybrid cloning vectors that were constructed from plasmids capable of autonomous replication in the cyanobacteria, *Anacystis nidulans* R2 and *A. nidulans* R2SPc. The use of plasmid vectors in transformation is a useful technique in recombinant DNA technology in that it enables researchers to easily alter the genetics of an organism, to enhance the expression of particular genes by increasing the number of gene copies in an organism, to introduce foreign or mutated genes, and to simply recover the introduced DNA by plasmid isolation.

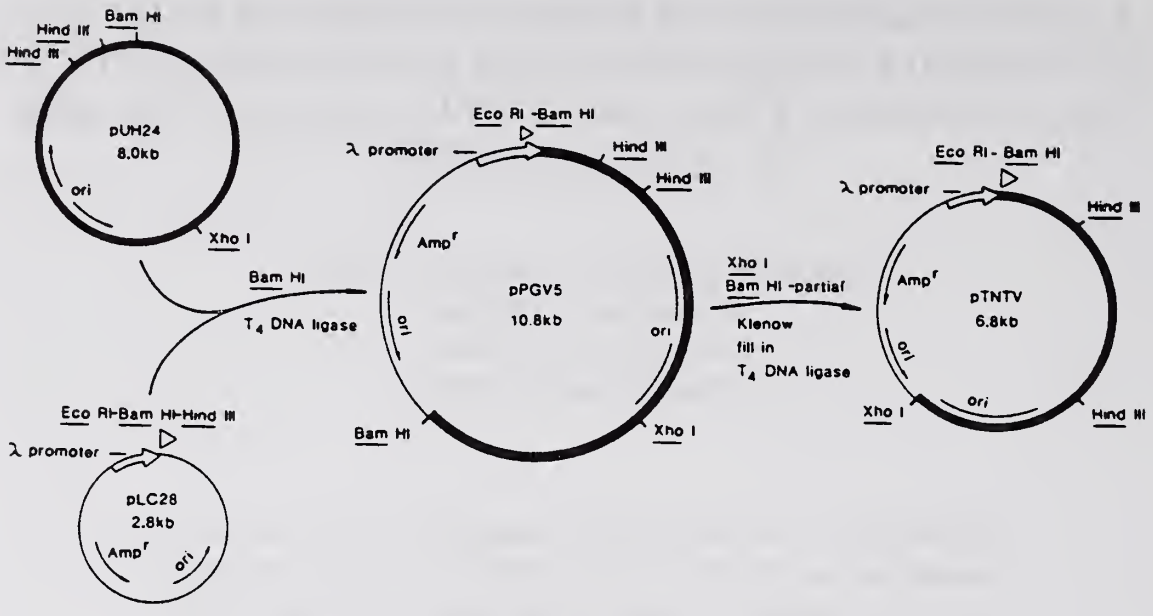

FIGURE 1. Construction of the plasmids pPGV5 and pTNTV.

The two species of cyanobacteria transformed were *A.nidulans* R2 and *A. nidulans* R2SPc. *A. niulans* R2 has been well characterized biochemically and biophysically (Olsen, *et al.*, 1979; Pakrasi, *et al.*, 1985). It contains two plasmids, a 50 kb plasmid known as pUH25, and an 8 kb plasmid known as pUH24 or pANS (van den Hondel, *et al.*, 1971). *A. nidulans* R2SPc is different from *A. nidulans* R2 in that it has been cured of the smaller 8 kb plasmid, pANS (Kuhlemeier, *et al.*, 1981), which helps reduce homologous recombination between genes cloned on the plasmid and a chromosomal gene.

The transformation efficiencies of two different hybrid cloning vectors, pPGV5 and pTNTV were compared. The vector pPGV5 was constructed by digesting both pANS of *A. nidulans* R2 and the *Escherichia coli* plasmid pLC28 with *Bam* HI restriction endonuclease. These two plasmid fragments were then ligated together to form a 10.8 kb hybrid vector (Vann, *et al.*, 1986). This hybrid cloning vector contains the powerful leftward promoter of bacteriophage lambda, allowing for enhanced expression of cloned genes downstream of the promoter (Remant, Stanssens, and Fiers, 1981). It also contains an ampillicin resistance gene and two *Bam* HI restriction endonuclease cloning sites (Figure 1).

The hybird cloning vector pTNTV was modified to make pPGV5 in order to create a smaller vector that contained a unique *Bam* HI restriction site (Figure 1). The *Bam* HI cloning site most distant from the lambda promoter and nonessential cyanobacterial sequences was removed (Nahreini, *et al.*, in press). it was hoped that this vector would transform as efficiently as pPGV5. It is important for a vector to be stable in cells and transform as efficiently as possible so that a higher number of transformants can be obtained for easier manipulation and study. This study was concerned with comparing the tranformation frequency obtained using pTNTV versus pPGV5 in the two cyanobacterial strains.

MATERIALS AND METHODS

Four experiments were performed using the same transformation procedure. First, cell cultures are grown for two days at 30°C on a shaker in BG-11 media

TABLE 1. Transformation frequencies of *Anacystis nidulans* R2 and *A. nidulans* R2SPc. All of the transformation frequencies were calculated in transformants/μg.

Strains	R2	R2SPc	New R2
Experiment 1			
pTNTV	5.6×10^4		
pGV5	1.2×10^2		
Experiment 2			
pTNTV	4.6×10^4	6.6×10^6	
pGV5	4.8×10^4	9.9×10^5	
Experiment 3			
pTNTV	6.2×10^4		
pGV5	6.5×10^4		
Experiment 4			
pTNTV	6.1×10^6		1.5×10^7
pGV5	1.4×10^6		4.6×10^7

(Allen, *et al.*, 1968). Thirty milliliters of these cells were then centrifuged at 5-7 Krpm for ten minutes at 4°C. The pellets were resuspended in 1/2 volume 10 mM NaCl and centrifuged again. The pellets were resuspended again in BG-11 media to a concentration 5×10^8 cells/ml. Fifty nanograms of plasmid DNA was added to 400 μl aliquots of cells. Aliquots of cells containing no added DNA served as controls. All of the cells were covered (to inhibit photosynthesis, which has been found to increase transformation efficiency) and shaken at 30°C overnight.

Dilutions were made and plating was performed on the following day. Aliquots of cells not receiving DNA were diluted to 1×10^{-2}, 10^{-4}, 10^{-5}, and 10^{-6} cells/ml. Cells inoculated with DNA were diluted in the following manner: 1/4x, 1/16x, 1/64x, 1/128x, 1/256x, 1/512x. One hundred microliters of each dilution was plated on BG-11 agar plates. These plates were placed under lights for 4-6 hours to allow time for the antibiotic resistance gene to be expressed. After 4-6 hours, 400 microliters of the selective agent (ampicillin) was added under the agar of the plate, to a final concentration of 0.5 μg/ml. The plates were returned to the light at 30°C for 5-7 days. Colonies were then counted, and the transformation frequencies determined.

The same amount of DNA, 50 ng, was used in transformations by pPGV5 and pTNTV. DNA concentrations of stock solutions were obtained using a UV spectrophotometer set at a wavelength of 260 nm.

RESULTS AND DISCUSSION

The transformation efficiencies obtained in four experiments are illustrated in Table 1. In the first experiment, only *A. nidulans* R2 was transformed. The hybrid cloning vector pPGV5 tranformed this strain at a frequency of 1.2×10^5 transformants/μg, slightly higher than the efficiency of pTNTV which was 5.6×10^4 transformants/μg. This difference was not considered significant, since variations of this magnitude are common.

In the second and third experiments, similiar results were obtained using both plasmid vectors and *A. nidulans* R2 and *A. nidulans* R2SPc. These results suggested that there was no significant difference in the transformation efficiencies, when comparing the two vectors. However, contrary to results obtained in previous studies (Vann, *et al.,* 1986), *A. nidulans* R2SPc transformed at a much higher level than did *A. nidulans* R2, and the latter strain showed much lower transformation efficiency than previously seen.

Prior to performing another experiment, two parameters were altered in order to obtain transformation efficiencies which were similar to those seen in the earlier studies. In the first three experiments, the cyanobacterial cultures had been contaminated with bacterial organisms. It is difficult to maintain axenic cultures of cyanobacteria, and earlier transformation experiments had suggested that slight contamination did not significantly interfere with transformation efficiency (Vann, unpublished observations). However, we decided to perform the transformations with axenic cultures. Previously, cultures had been made axenic by selecting several isolated single colonies from a plate (in order to be sure to select transformable colonies) and growing them up to a dense colony. However, in this case, a recently described technique (Thiel, 1988) was used to make the cultures axenic prior to the fourth experiment. Thiel showed that cyanobacteria (but not bacteria) were protected from arsenate toxicity (10 mM) in the presence high levels of phosphate (100 mM). The cyanobacteria were resistant to this treatment, which killed the contaminating microorganisms.

The last transformations were also performed using a culture called new *A. nidulans* R2, which was kindly donated by Louis Sherman (University of Missouri). The *A. nidulans* R2 used in early experiments had also been obtained from L. Sherman and had previously shown high levels of transformation. It is believed that organic substances which briefly contaminated our water supply during the summer of 1988 may have been responsible for the detrimental effects to the cyanobacteria, resulting in the observed decrease in transformation competency.

The use of the new culture and the removal of contaminants increased transformation efficiencies by two orders of magnitude. Thus, the fourth experiment yielded transformation efficiencies comparable to those seen in other published studies.

Three important conclusions were reached from the data obtained in these experiments. First, both *A. nidulans* R2 and *A. nidulans* R2SPc were transformed by both vectors at a reasonable level as compared to previously reported levels. Second, in the first experiments, contaminating microorganisms reduced transformation efficiencies, but pretreating the cells with 100 mM phosphate and 10 mM arsenate resulted in high efficiencies in experiment four. Third, the transformation efficiencies of pTNTV and pPGV5 are not significantly different in either cyanobacterial species. Thus, pTNTV is capable of high levels of transformation making it a good candidate for gene expression studies.

ACKNOWLEDGMENTS

This research was funded by a grant from the Indiana Academy of Science and a Research Corporation Cottrell grant.

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