NOTE

Construction of an Escherichia-Pseudomonas Shuttle Vector Containing an Aminoglycoside Phosphotransferase Gene and a lacZ' Gene for α-Complementation

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A new 4.87 kb Escherichia-Pseudomonas shuttle vector has been constructed by inserting a 1.27 kb DNA fragment with a replication origin of a Pseudomonas plasmid pRO1614 into the 3.6 kb E. coli plasmid pBGS18. This vector, designated pJH1, contains an aminoglycoside phosphotransferase gene (aph) from Tn903, a lacZ' gene for α-complementation and a versatile multiple cloning site possessing unique restriction sites for EcoRI, SaeI, KpnI, Smal, BamHI, XbaI, SalI, BspMI, PstI, SpI, and HindIII. When pJH1 was transformed into E. coli DH5α and into P. putida HK-6, it was episomally and stably maintained in both strains. In addition, the enhanced green fluorescent protein (EGFP) gene which was transcriptionally cloned into pJH1 rendered E. coli cells fluorescence when its transformants were illuminated at 488 nm.

Keywords: Pseudomonas, shuttle vector, kanamycin-resistant gene, cloning
B.G. Spratt at Imperial College London. pBGS18, which was employed as a back-born plasmid in this study, has a kanamycin-resistant gene (aphI) from a transposon Tn903, which appeared to induce well in Pseudomonas species. Tn903 has been used for insertional mutagenesis studies of Pseudomonas (Itoh and Haas, 1985; Lam et al., 1987). Thus, the newly constructed Escherichia-Pseudomonas shuttle vector designated pJH1 possesses not only all convenient features of pUC18 including the multiple cloning site (MCS) and a lacZ' gene for the blue/white colony test through α-complementation, but also a kanamycin-resistant gene, a pRO1614 Pseudomonas origin and ColE1 Escherichia origin (Fig. 1).

The unique restriction sites of the MCS of pJH1 are EcoRI, SalI, KpnI, SmaI, BamHI, XbaI, Sall, BspMI, PstI, SpII, and HindIII, which are equivalent to those of a pUC18 plasmid. Kanamycin-resistance will especially enhance the worth of this vector and facilitate molecular genetic research of P. putida because the P. putida strains that are currently in use in research are already resistant to various antibiotics in many cases. This means that previously-developed vectors are seldom available for selection.

In order to verify that pJH1 works as a cloning vector for P. putida, both electroperoration and traditional transformation techniques were used to introduce it into P. putida HK-6, a bacterium that degrades explosives such as TNT and RDX (Chang et al., 2004). The electroperoration was carried out using Gene Pulser (Bio-Rad, USA), with a 2.5 kV/cm field strength, a 25 μF capacitor, a 200 Ω resistor and a time constant of about 5 ms. The method of Mercer and Loutit (1979) for traditional transformation using 0.15 M MgCl2 and 37°C heat shock was originally designed for P. aeruginosa (Mercer and Loutit, 1979), however it has also proved to work well for P. putida. After transformation, either LB or Pseudomonas selection agar medium containing 20 µg/ml of kanamycin was used to select transformants. Transformation frequency measured by Mercer and Loutit’s method was about 2.5 × 10⁶ P. putida HK-6 cells/µg of pJH1. Electroporation resulted in about 100-fold higher transformation frequency than was achieved using the traditional method.

We next examined whether pJH1 was maintained episomally in the P. putida HK-6 strain. Episomal maintenance means that the vector was not integrated into the chromosome and therefore kanamycin-resistance is expressed from cytoplasmic plasmid and not from the chromosome. pJH1 was purified from P. putida HK-6 and successfully re-transformed into E. coli cells. In addition, pJH1 plasmid purified from P. putida HK-6 was observed through 1.0% (w/v) agarose gel electrophoresis (Fig. 2). These results suggested that pJH1 was indeed episomally maintained in the cytoplasm of P. putida HK-6 cells.

Green fluorescent protein (GFP) has been used as a marker of gene expression. To examine the additional use of pJH1 as a potential expression vector, an enhanced GFP gene (EGFP) from the pYEGFP plasmid (Cormack et al., 1997) was amplified and inserted between the PstI and HindIII sites of pJH1. The inserted gene was transformed into E. coli DH5α and P. putida HK-6. In this configuration, the EGFP gene is under control of lac promoter which originated from pBGS18. This means that it is highly inducible by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG). After induction with 0.1 mM IPTG, both E. coli DH5α and P. putida HK-6 cells were observed...
under a confocal microscope. E. coli cells harboring pJH1 with an EGFP gene were fluorescent when illuminated at 488 nm while _P. putida_ cells were not (Fig. 3). At the present, the reason for this lack of fluorescence in _P. putida_ cells remains unknown. pJH1 containing the EGFP gene seemed to be maintained well in _P. putida_ HK-6 (Fig. 2, lane 5). It may be due to lack of transcription of the lac promoter or instability of the EGFP in _P. putida_ HK-6 cells. Some of _E. coli_ cells were not fluorescent as well (Fig. 3). Although exact reason for this remains unknown, it could also be due to instability of the expressed EGFP or formation of inactive proteins such as inclusion bodies.

There have been several reports regarding the construction of the _Escherichia-Pseudomonas_ shuttle vectors. This, however, is the first report to construct a shuttle vector with the pRO1600 replication origin possessing the kanamycin resistant gene (aph) of _Tn903_. A series of pUCPs were constructed by West _et al._ (1994). pUCP18 and pUCP26 carried a β-lactamase gene and a tetracycline-resistant gene, respectively. pUCP24 is the most similar to pJH1. It had a 832 bp gentamycin acetyltransferase gene (aacC1) instead of a 1.3 kb kanamycin phosphoransferase gene (aph). The aacC1 gene can also confer kanamycin-resistance.

In conclusion, a newly-constructed 4.87 kb shuttle vector with a replication origin of a plasmid pRO1614 was transformed into both _E. coli_ and _P. putida_ HK-6 and episomally maintained well. Kanamycin-resistance makes pJH1 of greater value since many _Pseudomonas_ strains naturally possess ampicillin-resistance. This vector will facilitate molecular genetic studies of the biodegradation of _P. putida_ HK-6. It will be especially useful when a genomic library is constructed. If pJH1 is manipulated by inserting an inducible or controllable promoter for _Pseudomonas_, it will be of great importance in _Pseudomonas_ research.

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References


