DNA sequence and shuttle vector construction of plasmid pGL3 from Plectonema boryanum PCC 6306

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The cyanobacteria are an ancient group of Gram-negative prokaryotes. In addition to photosynthesis, the cyanobacteria possess many other interesting features: a) nitrogen-fixation, b) cellular differentiation, c) regulated genomic rearrangements (1, 2) and, d) introns (3). Studies of these features in the filamentous cyanobacteria are hampered by a lack of shuttle vectors. Currently, there is only one series of vectors available, based on the Nostoc sp. PCC 7524 plasmid pDU1 (4).

Many of the filamentous cyanobacteria possess plasmids but all remain cryptic. Characterization of the plasmids has been limited to restriction mapping and cross hybridizations. An exception to this is the isolation of the replication region of pDU1 (5) and its sequence (6).

In this study the small plasmid pGL3 from Plectonema boryanum (2) has been sequenced and a shuttle vector which replicates in Plectonema and Anabaena PCC 7120 constructed. Plasmid pGL3 possesses one large ORF which is putatively involved in the replication of the plasmid. Comparative searches (TFASTA and FASTA (7)) of pGL3 with GenBank (version 66) and pDU1 (6) revealed no similarities.

Plasmid pGL3 is 1,504 bp in length and contains one large open reading frame (482 - 1489) coding for a putative protein of 37661 Da. The start site for numbering begins at the unique HpaI site. The G+C content of pGL3 is 46.9 percent. This is within the expected range for the Plectonema genus which has a chromosomal G+C content of 42 - 67 percent (8). The ORF is followed by a region of dyad symmetry (43 - 58) which may be a rho dependent terminator. Three inverted repeats (IR1, IR2 and IR3) lie outside of the ORF at positions 79 - 112, 237 - 259 and 567 - 603, respectively.

A 4.6 kb shuttle vector (pPBH201) was constructed by restriction of pGL3 at the unique HpaI site and ligation into the Small site in the MCS of pBluescript II SK+ (Stratagene). The entire pGL3 plasmid, flanked by the MCS, was excised from pBluescript II SK+ with BssHII, treated with S1 nuclease, and cloned into the EcoRV site of pBOC. pBOC is a three kbp plasmid derived in this study by the complete digestion of pRL1 (4) with EcoRV, and contains the chloramphenicol resistance gene, a basis of mobility (bom) site and the ColE1 ori.

Cyanobacterial filaments were reduced by sonication to one to four cells in preparation for conjugation. Conjugations of shuttle vector pPBH201 into Plectonema and Anabaena were performed according to Thiell and Wolk (9). Selection was on BG-11 medium (10) petri plates (25 μg/ml Cm). Shuttle vector plasmid isolations (11) were performed on cells grown in liquid BG-11 medium (25 μg/ml Cm). The shuttle vector was stably maintained in both cyanobacteria.

The small size of pPBH201, its two multiple cloning sites, and its ability to replicate in both Plectonema and Anabaena make it very useful for molecular genetic analysis. Also, the fact that it has no similarity to the pDU1 replication region suggests that it can coexist with pDU1 based shuttle vectors.

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REFERENCES


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