Cloning and expression of the *Nsp*V restriction-modification genes of *Nostoc* sp. strain PCC7524

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Received June 16, 1993; Accepted June 23, 1993 DDBJ accession no. D14719

The *Nsp*V restriction-modification system of the filamentous cyanobacterium *Nostoc* sp. strain PCC7524 consists of *Nsp*V restriction endonuclease (R.*Nsp*V) and *Nsp*V modification enzyme (M.*Nsp*V). R.*Nsp*V recognizes and cleaves double-stranded DNA at the sequence 5'-TTCGAA-3', and M.*Nsp*V recognizes and protects this sequence by methylation against R.*Nsp*V (1). We cloned the genes in *Escherichia coli* and analyzed their structure.

A genomic library was constructed with pACYC184 [Cm^r, Tc^r] and *E.coli* MC1061 as the host-vector system. The library was screened for genes of the *Nsp*V system by the so-called Hungarian trick (2). One clone with both *Nsp*V restriction and modification activities was selected.

The fragment containing the genes of the *Nsp*V system was 2713 bp long, and it included two ORFs, 663 and 1323 bp long, with the same orientation. The results of deletion assays showed that the upstream ORF encoded R.*Nsp*V and that the downstream ORF encoded M.*Nsp*V (data not shown). The putative amino acid sequence of the *Nsp*V restriction enzyme was compared with the sequences of other restriction enzymes, but homology was not found.

An expression plasmid, pNSPV, was constructed with plasmid pKH1 (3; Figure 1). *E.coli* MC1061 cells carrying pNSPV and a regulatory plasmid, pNT203, for the P_L promotor (4) overproduced R.*Nsp*V. The R.*Nsp*V activity from this recombinant was 350-fold that of *Nostoc* sp. strain PCC7524. The N-terminal sequence of R.*Nsp*V purified from the recombinant was identical with that deduced from the nucleotide sequence, except that it lacked the first methionine residue.

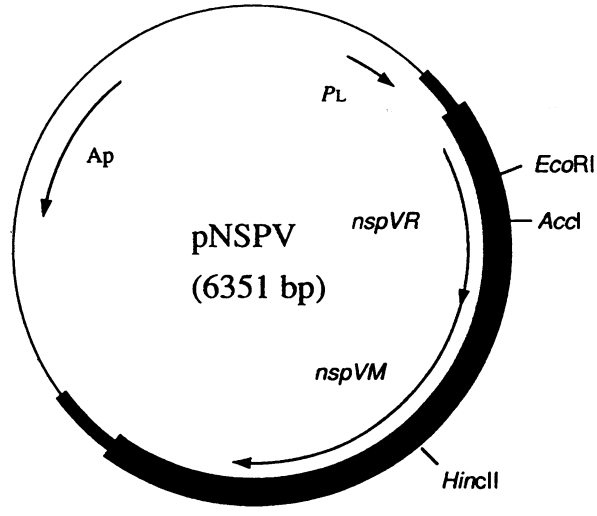

Figure 1. Map of the expression plasmid pNSPV. The genes encoding R.*Nsp*V and M.*Nsp*V, *nspVR* and *nspVM*, are expressed under the control of a P_L promotor. Thin line, DNA derived from pKH1; medium-bold line, DNA from pACYC184; bold line, genome DNA from *Nostoc* sp. strain PCC7524. Ap, ampicillin resistance gene; P_L, P_L promotor.

ACKNOWLEDGEMENTS

We thank Yoshiko Nomura, Harumi Shimato, and Minoru Ueda for discussions and advice.

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