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Extended Abstract

# Cloning, recombinant expression in Escherichia coli and DNA binding properties of Datura metel L. Myb transcription factor

**Priya Arora**

## Abstract

MYB proteins are a superfamily of transcription factors that play significant roles in developmental and defense-related processes in plants. The MYB family is characterized by the presence of a conserved DNA-binding domain called the MYB domain, which normally contains two or three imperfect repeat sequences. In the present study, we report the cloning, expression and DNA binding properties of Myb transcription factor from Datura metel L. An open reading frame of 856 bp was detected which could encode a protein of 278 amino acid residues. The cDNA encoding DmMyb was cloned in pRSETA expression vector and heterologously expressed in E.coli. The DmMyb expressed as histidine-tagged fusion protein was purified using Ni-NTA affinity chromatography. In addition, the ability of partially purified recombinant DmMYB protein to bind MBCE (Myb binding cis-elements) was evaluated with five known Myb specific cis-acting elements (MSCE 1, MSCE 2, MSCE 3, MSCE 4 and MSCE 5) using the electrophoretic mobility shift assay (EMSA). The binding assay indicated that the partially purified recombinant DmMyb interacts with three Myb-specific cis-acting elements (MBCE 3, MBCE 4 and MBCE 5). Further studies are required for providing deep insights into transcriptional regulation of Datura metel genes/gene networks which is associated with the DmMyb transcription factor.

Complete PCR-derived DNA fragments containing the structural genes for DNA polymerases of the archaeons Pyrococcus furiosus and Pyrococcus woesei were cloned into an expression vector. The clones expressing thermostable His-tagged DNA polymerases were selected. The cloned fragments were sequenced. The DNA sequences were verified to be authentic by sequencing several clones. The nucleotide (nt) sequence revealed that DNA polymerase of P. woesei (Pwo DNA polymerase) consists of 775 amino acids and has a molecular weight of 90,566. It shows 100% nucleotide identity to the nucleotide sequence of DNA polymerase from P. furiosus (Pfu DNA polymerase). The results confirm that nucleotide sequences of both archaeons (P. furiosus and P. woesei) are highly similar. The recombinant DNA polymerases (His-tagged Pfu and His-tagged Pwo) contained a polyhistidine tag at the N-terminus (43 additional amino acids) that allowed single-step isolation by Ni-affinity chromatography. We found that recombinant plasmids are toxic or unstable in the expressing strain BL21(DE3), even in the absence of the inducing agent, IPTG. However, the plasmids were stable in BL21(DE3) containing the pLysS plasmid, which suppresses expression prior to induction, and His-tagged proteins were expressed upon IPTG addition. The proteins were purified by heat treatment (to denature E. coli proteins), followed by metal-affinity chromatography on Ni2+-Sepharose columns. The enzymes were characterized and displayed high DNA polymerase activity and thermostability. This bacterial expression system appears to be the method of choice for production of Pfu or Pwo DNA polymerases.

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Priya Arora
Indian Institute of Science, India E-mail: meetpriyaarora@gmail.com