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Overexpression and Purification of the Oat (Avena Fatua) Protein AFN1 and Construction of a pMAL/AFN1 Expression Plasmid

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ABSTRACT

Abscisic acid (ABA) is an important phytohormone with regulatory roles in many physiological processes. ABA expression is induced by environmental stresses such as drought and it is known to be an inhibitor of seed germination. A wild oat (*Avena fatua)* called AFN1 has been hypothesized to initiate the early stages of germination as its mRNA accumulates in nondormant seed embryos during imbibition. The polypeptide sequence of AFN1 suggests that it is an ABA glucosyl transferase. Glucosylation by AFN1 and thereby inactivation of ABA could lead to seed germination. In order to understand the role of AFN1 in germination, an ample quantity of AFN1 polypeptide is needed to test for enzymatic ABA glucosylase activity. My work has been to overexpress recombinant AFN1containing a $(His)_6$ tag using a pRSETC *E.coli* expression system followed by purification of the AFN1 protein by means of a nickel-affinity column that bind to the $(His)_6$ tag. Due to the insufficient yield of AFN1 fusion protein obtained with this procedure, another method using a pMAL-c2x vector is now being employed. The pMAL expression system provides a method for expressing and purifying protein by tagging proteins with maltose-binding protein (MBP). It is anticipated that MBP tag will be advantageous as it can make the fusion protein more soluble and thereby yield a larger quantity of protein. Currently, work is underway on the construction of pMAL/AFN1 plasmid.

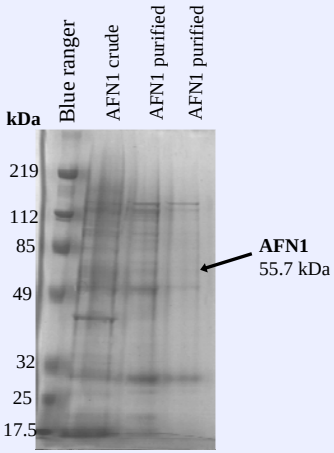

Figure 1. Crude protein was obtained from the E. *coli* cells by sonication. The AFN1 fusion protein, carrying a $(His)_6$ tag, was purified using a nickel affinity column. Crude and purified protein samples were analyzed on polyacrylamide gels. An AFN1 fusion protein may have been produced in E. *coli* cultures but it cannot be visualized. Blue ranger is the prestained molecular weight marker.

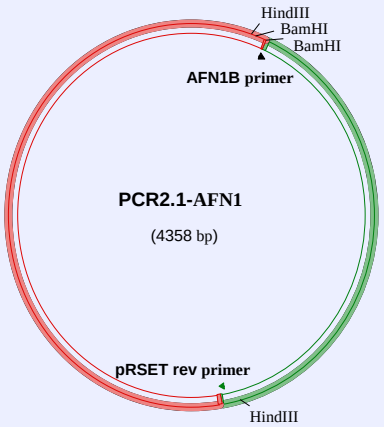

Figure 2. The Construct of pCR2.1/AFN1 plasmid. The AFN1 insert from this plasmid can be excised and inserted into the pMAL-c2x vector.

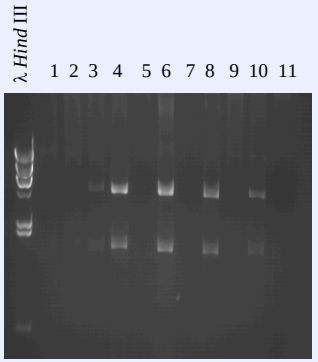

Figure 3. Digest of plasmid prep pCR2.1/AFN1 with *Bam* H1 and *Hind* III restriction enzymes show that cultures numbered 3, 4, 6, 8, and 10 contain AFN1 insert.

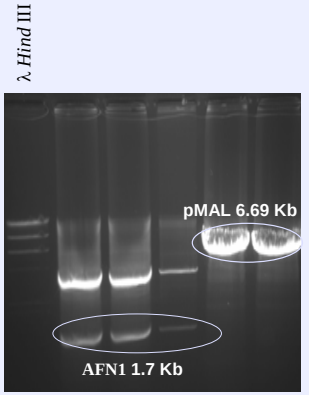

Figure 4. A large scale digest of pCR2.1/AFN1 plasmid and dephosphorylated pMAL-c2x vector.

RESULTS AND DISCUSSION

Expression of AFN1 and Protein Purification using Nickel-affinity column:

Crude and purified AFN1 expression extracts were examined on a polyacrylamide gel (Fig. 1). The lane corresponding to AFN1 crude extract contain many other E. *coli* protein and AFN1 fusion proteins could not be viewed. Purification did not separate out all of E. *coli* proteins from the fusion proteins. Similarly, past results have yielded little or no expression of AFN1 and have suggested that pRSETC/AFN1 plasmid may not be compatible with the E. *coli* expression system for induction of AFN1 expression. Therefore, another method using pMAL-c2x vector is now being employed.

Construction of pMAL/AFN1 plasmid:

Digest of plasmid preps with *Eco* RI and *Hind* III enzymes confirmed successful construction of intermediate pCR2.1/AFN1 plasmid (Fig. 2, 3). After a medium scale plasmid isolation was performed on the dense TB culture followed by phenol/chloroform extractions, a large scale digest on the plasmid and pMAL-c2x vector was performed (Fig. 4). Gel purification of AFN1 and pMAL-c2x bands were performed from the agarose gel to be used for ligation of AFN1 insert into pMAL-c2x vector.

FUTURE PLANS

Once the pMAL/AFN1 plasmid is constructed, the pMAL expression system can be employed for expressing and purifying AFN1 by tagging proteins with maltose-binding protein (MBP).

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