

Cloning and prokaryotic expression of 1-deoxy-D-xylulose-5-phosphate synthase (DXS) from *Lepidium apetalum*

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Abstract. 1-Deoxy-D-xylulose-5-phosphate synthase (DXS) is the first enzyme in plastidial methylerythritol phosphate pathway for isoprenoid biosynthesis. With primers designed according to seedling transcriptome data, we cloned a full-length cDNA fragment of *DXS* gene from *Lepidium apetalum* by PCR amplification, and designate it as *LaDXS* (GenBank accession no. KU314760). The gene contains a 2148-bp ORF and encodes a protein with 715 amino acids. Recombinant *LaDXS* protein was successful expressed in *E.coli* BL21 (DE3). Cloning and prokaryotic expression of *LaDXS* is important for further studies of isoprenoid biosynthesis in *L. apetalum*.

1. Introduction

Lepidium apetalum Willd is a commonly used Chinese medicine in the treatment of cough and asthma [1-4]. Various isoprenoids have been isolated from seeds of *L. apetalum*, but biosynthetic pathways of isoprenoids in *L. apetalum* are not yet clear [5].

All known isoprenoids are derived from two common precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) [6]. IPP and DMAPP are generated through two pathways: cytosolic MVA pathway and plastidial MEP pathway. The MEP pathway is principally involved in the synthesis of monoterpenes, diterpenes and carotenoids [6]. The first step of MEP pathway is conversion of pyruvate and D-glyceraldehyde-3-phosphate (D-GAP) to 1-deoxy-D-xylulose-5-phosphate. This reaction is catalyzed by 1-deoxy-D-xylulose-5-phosphate synthase (DXS) [7-14].

In the present study, we cloned full-length cDNA fragment of *DXS* gene from *L. apetalum*, and expressed the gene in *E.coli* BL 21 (DE3).

2. Materials and methods

2.1. Plant Materials

Seeds of *L. apetalum* were planted in green house with temperature 23/20°C (day/night). After 60 days, fresh leaves were collected and stored at -80°C before RNA isolation.



2.2. cDNA Cloning

Total RNA was extracted from leaves of *L. apetalum* using a Trizol method. The RNA quantity was determined by using a NanoDrop 2000 Spectrophotometer. The RNA integrity was analyzed on a 1% agarose gel. One microgram of total RNA was reverse transcribed in a final volume of 20 μ L using Takara PrimeScript first strand cDNA synthesis kit. The entire LaDXS cDNA was amplified by PCR with primers (LaDXS-F: TTAGTGGATTCTAAAGTGCAA, LaDXS-R: GATTAATTTAGCCGCAAACGA), and cloned into the pMD19-T cloning vector to generate the plasmid pMD19-LaDXS. The vector was transformed into *E.coli* Trans5 α cells and cultured at 37°C. The plasmids from transformed colonies were sequenced and assembled to verify the correct LaDXS insertion.

2.3. Expression of Recombinant Protein

The fragment containing LaDXS ORF and the correct restriction enzyme sites was amplified using EcoRI-F(CGGAATTCATGGCTTCGTCTGCA) and HindIII-R(CCCAAGCTTTCAAATAGAGCTTC) as primers. The resulting PCR fragment was double-digested with EcoRI and HindIII, and inserted into the pET-32a vector that had been digested with the same restriction enzymes, yielding prokaryotic expression plasmids pET32-LaDXS. The recombinant plasmid was then transformed into *E.coli* Trans5 α cells. The plasmids from transformed colonies were sequenced and assembled to verify the correct LaDXS insertion.

The *E. coli* BL21 (DE3) cells containing the pET32a-LaDXS plasmid grew in LB liquid medium (containing ampicillin 100 μ g.ml⁻¹) at 37°C overnight, then 5ml cultured cells were inoculated in 500 ml of LB liquid medium (volume ratio 1:100) at 37°C, 200 rpm for 2~3 h. When the OD₆₀₀ reached 0.6, isopropyl β -D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM to initiate overexpression for 6 more hours at 28°C. The cells were collected through centrifuging at 6000 g, 4°C for 10 min. Protein expression was analyzed through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 15% polyacrylamide separation gel) with Mini-PROTEAN 3 Cell (Bio-Rad), then detected by Coomassie Brilliant Blue R-250 staining.

3. Results

3.1. Cloning and Sequence Analysis of La DXS

The full-length cDNA of *LaDXS* was obtained and analyzed. The gene contains a 2148-bp ORF and encodes a protein with 715 amino acids. The deduced amino acid sequence of *LaDXS* exhibited a high degree of homology with the DXS sequences from other plant species, e.g., *Arabidopsis thaliana* (AtCLA1, NP_193291.1, 97% identity), *Brassica rapa* (BrDXS1, AHN09416.1, 93% identity), *Siraitia grosvenorii* (SgDXS, AEM42997.1, 84% identity), *Capsicum annuum* (CaDXS, O78328.1, 84% identity), *Catharanthus roseus* (CrDXS1, AGL40532.1, 83% identity), and *Solanum lycopersicum* (SlDXS, NP_001234672.1, 84% identity) (Figure 1).

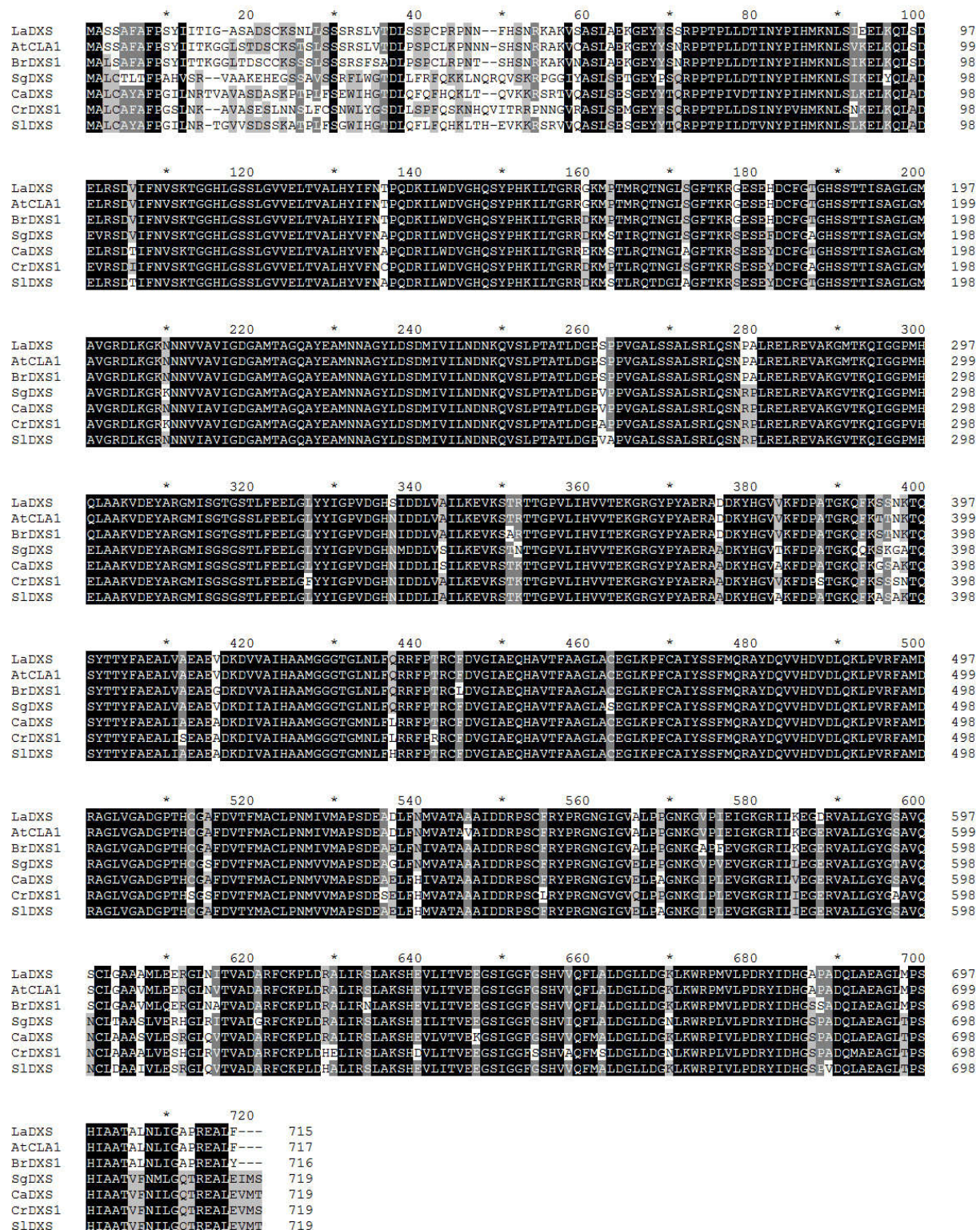


Figure 1. Multiple alignments of LaDXS with other plant DXSs.

The evolutionary position of LaDXS was shown in a phylogenetic tree of the DXSs (Figure 2). LaDXS was more identical to DXS from *Arabidopsis thaliana* and *Brassica napus*.

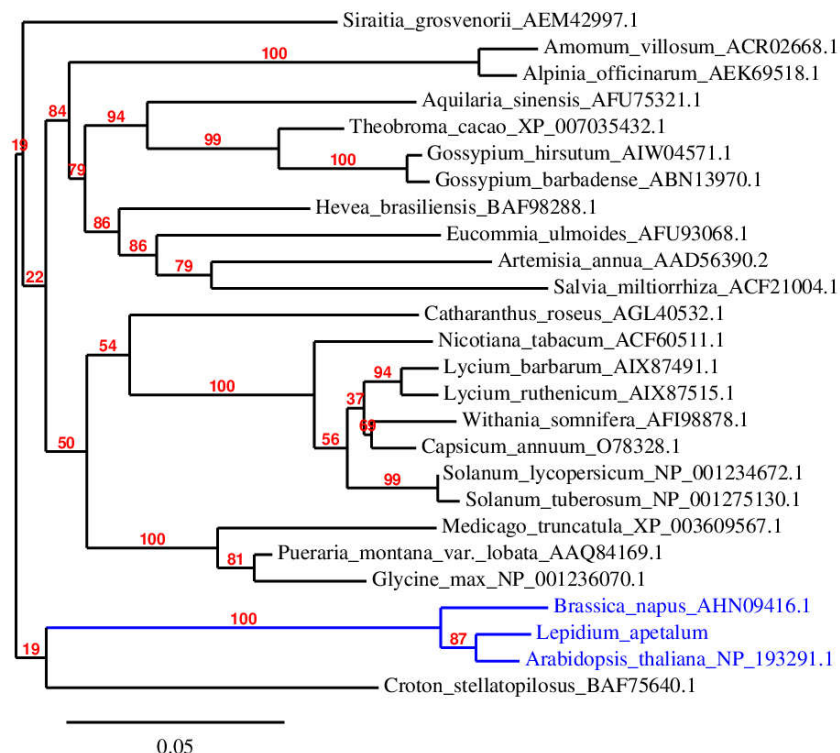


Figure 2. Phylogenetic analysis of the amino acid sequences of DXSs.

3.2. Expression of LaDXS Recombinant Protein

The entire reading frame of LaDXS was cloned into the pET-32a vector and expressed in *E. coli* BL21 (DE3) cells. After induction by IPTG, the recombinant protein was expressed. The molecular mass of LaDXS (Fig 3) fused with Trx-tag, His-tag and S-tag on N-terminal is approximately 94.5 kDa, as determined by SDS-PAGE.

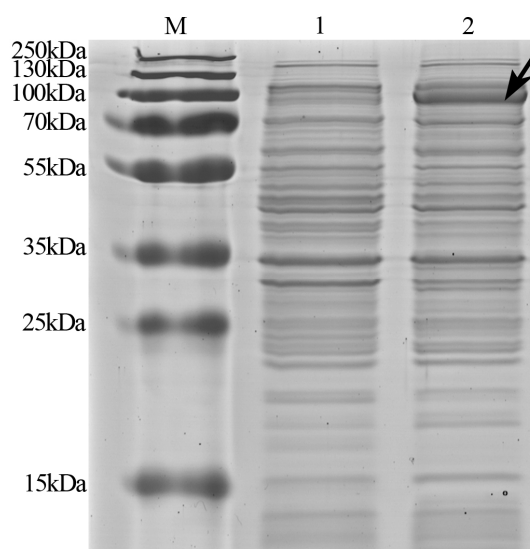


Figure 3. SDS-PAGE analysis of recombinant LaDXS protein expressed in *E. coli*. Lane M, protein molecular weight marker; Lane 1, the protein of the pET32a-LaDXS without the induction; Lane 2, the protein of the pET32a-LaDXS with the induction.

4. Conclusion

The DXS gene in *Lepidium apetalum* was first cloned; bioinformatics and phylogenetic analysis clearly suggested that LaDXS shared high sequence similarity with DXS genes of other plants. Prokaryotic expression demonstrated that LaDXS was expressed highly in *E.coli* BL21 (DE3) with 0.5 mM IPTG at 28°C.

Acknowledgments

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