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

**Ligang Ma**¹,², **Yongguang Han**¹, **Le Zhao**¹,², **Weisheng Feng**¹,², **Haixue Kuang**³, **Xiaoke Zheng**¹,²,*

¹School of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou, China
²Collaborative Innovation Center for Respiratory Disease Diagnosis and Treatment & Chinese Medicine Development of Henan Province, Zhengzhou, China
³College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin, China

*Corresponding author e-mail: zhengxk.2006@163.com

**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 mL using Takara PrimeScript first strand cDNA synthesis kit. The entire LaDXS cDNA was amplified by PCR with primers (LaDXS-F: TTAGTGATTCTAAAGTGCAA, LaDXS-R: GATTAATTTAGCCGCAACGA), and cloned into the pMD19-T cloning vector to generate the plasmid pMD19-LaDXS. The vector was transformed into *E.coli* Trans5α 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(CCGAATTCTAGGCTCTTGCA) and HindIII-R (CCCAAGCTTTCAAAATAGAGCTTC) 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* Trans5α 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 μg.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* (SIDXS, 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.

![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.](image-url)
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
This work was financially supported by the National Key Basic Research Development Program of China (973 Program) (2013CB531802), Collaborative Innovation Center for Respiratory Disease Diagnosis and Treatment & Chinese Medicine Development of Henan Province, and Doctoral Research Fund of Henan University of Traditional Chinese Medicine (Grant No. BSJJ2011-18).

References
[1] Wang S, Shi P, Qu L, et al. Bioactive constituents obtained from the seeds of *Lepidium apetalum* Willd. Molecules. 2017, 22 (4): 540.
[2] Yuan P, Zheng X, Li M, et al. Two Sulfur Glycoside Compounds Isolated from *Lepidium apetalum* Willd. Protect NRK52e Cells against Hypertonic-Induced Adhesion and Inflammation by Suppressing the MAPK Signaling Pathway and RAAS. Molecules, 2017, 22 (11): 1956.
[3] Han L, Shi P, Dong Y, et al. New Rare Sinapoyl Acetylated Flavonoid Glycosides Obtained from the Seeds of *Lepidium apetalum* Willd. Molecules, 2015, 20 (8): 13982-96.
[4] Shi P, Chao L, Wang T, et al. New bioactive flavonoid glycosides isolated from the seeds of *Lepidium apetalum* Willd. Fitoterapia, 2015, 103: 197-205.
[5] Kim S J, Kim H Y, Lee Y J, et al. Ethanol Extract of *Lepidium apetalum* Seed Elicits Contractile Response and Attenuates Atrial Natriuretic Peptide Secretion in Beating Rabbit Atria. Evid Based Complement Alternat Med, 2013, 2013: 404713.
[6] Zhao L, Chang W-C, Xiao Y, et al. Methylerthritol Phosphate Pathway of Isoprenoid Biosynthesis. Annu Rev Biochem, 2013, 82 (1): 497-530.
[7] Zhou W, Huang F F, Li S, et al. Molecular cloning and characterization of two 1-deoxy-D-xylulose-5-phosphate synthase genes involved in tanshinone biosynthesis in *Salvia miltiorrhiza*. Mol Breed, 2016, 36 (9).
[8] Fan H H, Wu Q J, Wang X, et al. Molecular cloning and expression of 1-deoxy-D-xylulose-5-phosphate synthase and 1-deoxy-D-xylulose-5-phosphate reductoisomerase in *Dendrobium officinale*. Plant Cell Tissue and Organ Culture, 2016, 125 (2): 381-385.
[9] Lv H, Zhang X, Liao B, et al. Cloning and analysis of 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase genes HsHDR1 and HsHDR2 in *Huperzia serrate*. Acta Pharm Sin B, 2015, 5 (6): 583-9.
[10] Tong Y, Su P, Zhao Y, et al. Molecular Cloning and Characterization of DXS and DXR Genes in the Terpenoid Biosynthetic Pathway of *Tripterygium wilfordii*. Int J Mol Sci, 2015, 16 (10): 25516-35.
[11] Xu Y, Liu J, Liang L, et al. Molecular cloning and characterization of three cDNAs encoding 1-deoxy-d-xylulose-5-phosphate synthase in *Aquilaria sinensis* (Lour.) Gilg. Plant Physiol Biochem, 2014, 82: 133-41.
[12] Mendoza-Poudereux I, Munoz-Bertomeu J, Arrillaga I, et al. Deoxyxylulose 5-phosphate reductoisomerase is not a rate-determining enzyme for essential oil production in spike lavender. J Plant Physiol, 2014, 171 (17): 1564-70.
[13] Wang J, Shen Y M, Li B, et al. Characterization of a functionally active recombinant 1-deoxy-D-xylulose-5-phosphate synthase from *Babesia bovis*. J Vet Med Sci, 2014, 76 (7): 1021-7.
[14] Sun R, Liu S, Gao J-L, et al. Cloning and expression analysis of 1-deoxy-D-xylulose-5-phosphate synthase gene from the medicinal plant *Conyza blintii* H.Lév. Turkish Journal of Biology, 2014, 38 (5): 664-670.