Nucleotide sequence of *Phaseolus vulgaris* L. alcohol dehydrogenase encoding cDNA and three-dimensional structure prediction of the deduced protein

Kassim Amelia¹,², Chin Yin Khor², Farida Habib Shah³, Subhash J. Bhore¹,²

¹Department of Molecular Biology, Melaka Institute of Biotecnology, Lot 7, Melaka International Trade Centre City, 75450 Ayer Keroh, Melaka, Malaysia, ²Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Bedong-Semeling Road, Semeling 08100, Kedah, Malaysia, ³Department of Research and Development, Novel Plants Sdn. Bhd., 27C Jln Petaling Utama 12, 7.5 Miles Old Klang Road, 46000 Petaling Jaya, Malaysia

Submitted: 14-06-2014  Revised: 10-08-2014  Published: 02-02-2015

**ABSTRACT**

**Background:** Common beans (*Phaseolus vulgaris* L.) are widely consumed as a source of proteins and natural products. However, its yield needs to be increased. In line with the agenda of Phaseomics (an international consortium), work of expressed sequence tags (ESTs) generation from bean pods was initiated. Altogether, 5972 ESTs have been isolated. Alcohol dehydrogenase (AD) encoding gene cDNA was a noticeable transcript among the generated ESTs. This AD is an important enzyme; therefore, to understand more about it this study was undertaken. **Objective:** The objective of this study was to elucidate *P. vulgaris* L. AD (*PvAD*) gene cDNA sequence and to predict the three-dimensional (3D) structure of deduced protein. **Materials and Methods:** positive and negative strands of the *PvAD* cDNA clone were sequenced using M13 forward and M13 reverse primers to elucidate the nucleotide sequence. Deduced *PvAD* cDNA and protein sequence was analyzed for their basic features using online bioinformatics tools. Sequence comparison was carried out using blastseq program, and tree-view program was used to construct a phylogenetic tree. The secondary structures and 3D structure of *PvAD* protein were predicted by using the PHYRE automatic fold recognition server. **Results:** The sequencing results analysis showed that *PvAD* cDNA is 1294 bp in length. It’s open reading frame encodes for a protein that contains 371 amino acids. Deduced protein sequence analysis showed the presence of putative substrate binding, catalytic Zn binding, and NAD binding sites. Results indicate that the predicted 3D structure of *PvAD* protein is analogous to the experimentally determined crystal structure of s-nitrosoglutathione reductase from an *Arabidopsis* species. **Conclusions:** The 1294 bp long *PvAD* cDNA encodes for 371 amino acid long protein that contains conserved domains required for biological functions of AD. The predicted deduced *PvAD* protein’s 3D structure reflects the analogy with the crystal structure of *Arabidopsis thaliana* s-nitrosoglutathione reductase. Further study is required to validate the predicted structure.

**Key words:** BAT93, common bean, homology modeling, molecular modeling, phaseomics, protein, protein structure prediction

**INTRODUCTION**

Common bean (*Phaseolus vulgaris* L.) is an important commodity in the food supply chain. It is consumed widely as it serves as a rich source of proteins, vitamins and minerals important in balanced human diet.¹,² In the Asian countries, in Latin America and Africa the production of the beans is in the greater amount to meet the demand and consumption by the increasing population. Common bean is serving as a very important source of proteins for financially weak group of people and used as a model food legume.³ We must develop new varieties of common bean that are desired by farmers and consumers. To speed up this process, an international consortium (phaseomics) was established.³ As a part of this consortium, research work of generation and characterization of expressed
sequence tags (ESTs) for bean was initiated at Melaka Institute of Biotechnology, Malaysia.[10] While processing ESTs, we found a cDNA clone for *P. vulgaris* L. alcohol dehydrogenase (*PvAD*).

Alcohol dehydrogenase (AD) encoding genes are found in all species of archaea, bacteria, fungi, plants and animals.[4] AD belongs to AD family, a group of dehydrogenases that facilitate the interconversion of alcohols and aldehydes or ketones.[4] This enzyme plays an important role in physiological processes such as alcohol and alkane metabolism, cell defense toward exogenous alcohols and aldehydes.[4] AD is studied in some flowering plants and in few legumes.[8] However, we do not know much about AD in *P. vulgaris*. Therefore, to elucidate the *PvAD* cDNA clone sequence, it was fully sequenced, and cDNA and deduced protein sequence was analyzed and annotated in this study using computational tools. The *PvAD* gene cDNA sequence, its deduced protein sequence, predicted secondary structures and three-dimensional (3D) structure is reported in this paper.

**MATERIALS AND METHODS**

Common bean (genotype BAT93) seeds were provided by Patricia Lariguet, Laboratoire de Biologie Moléculaire des Plantes Supérieures, Department of Plant Biology, University of Geneva, Geneva, Switzerland. Seed germination and maintenance of seedlings was done as reported by Bhore et al.[4]

The cDNA clone of *PvAD* was isolated and identified from the ESTs generated from 20 days old (days after anthesis) bean-pod-tissue cDNA library. This cDNA library was constructed (our unpublished data) using “CloneMiner cDNA library construction kit” obtained from Invitrogen Corporation.

*Escherichia coli* cells harboring recombinant plasmid with *PvAD* cDNA were cultivated in 10 ml LB medium supplemented with 40 µg/ml Kanamycin. Plasmid DNA was isolated and purified using Wizard® Plus SV Minipreps DNA purification system procured from Promega. Sense and antisense strand of *PvAD* cDNA clone were sequenced using M13 Forward and M13 Reverse primer.[4]

The comparative analysis of cDNA sequence was performed using online BLASTN (bl2seq) program available at NCBI. The finalized cDNA sequence was analyzed using online bioinformatics tools. The similarity search was performed using BLASTN and BLASTP programs. Bioinformatics tools available at JustBio (http://www.justbio.com/) were used to deduce the protein sequence, and to find out the general features of *PvAD* cDNA and deduced protein sequence. The tree-view program was used to construct a phylogenetic tree.

The deduced *PvAD* protein sequence was used as a BLASTP input to find the most analogous protein sequence and or structure in protein data bank (PDB).[9] However, for the prediction of secondary structures and the 3D structure of *PvAD*, Phyre2, a free web-based service for protein structure prediction was used.[10]

**RESULTS**

The *PvAD* cDNA clone isolated from 20 days old bean-pod-tissue cDNA library was sequenced for both strands. Sequence of sense (+) and antisense (−) strand was aligned and after elimination of the adaptor sequence, cDNA sequence was finalized. Our results indicate that isolated and sequenced *PvAD* cDNA is 1294 bp in length. The identity of cDNA sequence was confirmed by analyzing its nucleotide and deduced amino acid sequence. Annotated nucleotide and deduced protein sequence for *PvAD* is deposited in GenBank/DDJ/EMBL under the accession number KF569659. The basic annotated features of cDNA and deduced protein sequence are summarized in Table 1, and cDNA sequence along with its deduced amino acid sequence is depicted in Figure 1.

The comparative analysis of *PvAD* protein shows 75–80% similarity with its counterparts from other plant species. A summary is shown in Table 2.

Analysis of the deduced protein sequence suggests that *PvAD* protein is rich in Glycine (9.7%) and Valine (9.16%). However, Glutamine, Methionine, Tryptophan, and Tyrosine amino acid content was <2%. Blastp (domain enhanced lookup time accelerated basic local alignment search tool) results showed the presence

| Table 1: The basic features of *PvAD* cDNA and its deduced protein sequence |
|-----------------------------|-------|
| General features            | *PvAD* |
| cDNA sequence               |       |
| Size, bp                    | 1294  |
| Molecular weight (daltons)  | 401,353|
| 5'UTR, bp                   | 0     |
| Coding sequence             | 1116  |
| 3'UTR, bp                   | 178   |
| Stop codon                  | TGA(UGA)|
| G+C content (%)             | 44    |
| Protein sequence            |       |
| Length, amino acids         | 371   |
| Molecular weight (dalton)   | 40,402.69|
| Isoelectric point (theoretical) | 6.24 |

*PvAD=* *Phaseolus vulgaris* alcohol dehydrogenase; *UTR=* Untranslated region.
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The full length gene or its cDNA is essential for the over-expression of the gene of interest in order to increase either the production of a desired protein or natural products in the plants by using genetic engineering techniques.\textsuperscript{11} However, for the basic understanding of the gene (or its cDNA) structure, and secondary and tertiary structural features of the inferred proteins various computational tools and molecular modeling is commonly used.\textsuperscript{12-14} In this study, the main goal was to annotate \textit{PvAD} gene cDNA and its deduced protein sequence. The \textit{PvAD} cDNA clone was isolated from 20 days old-pod tissue cDNA library, an indication of its expression in bean’s 20 days old developing-pod-tissue. However, its level of expression and its expression regulation is not understood in beans (genotype BAT93) as we have not characterized its expression.

The GC content in \textit{PvAD} cDNA is 44\%. This much GC content is close to, but significantly higher than the GC content (39.4\%) reported in nuclear DNA of broad bean.\textsuperscript{15} The isolated \textit{PvAD} cDNA is truncated; hence, 5’ untranslated region is missing from its sequence [Table 1 and Figure 1]. Protein analysis results showed the presence of NAD binding site (chemical binding), catalytic...
Zn binding site (ion binding), and substrate binding site (chemical binding) those are essential for the biological functions of the AD.[16-18]

The results of phylogenetic analysis indicate that PvAD protein is closely (80%) related to *Phaseolus acutifolius*, *Lotus corniculatus*, *Lotus japonicus* and *Rosa rugosa* AD protein. On the contrary, AD from *Mangifera indica* showed less (75%) similarity with PvAD protein [Table 2 and Figure 2].

Deduced protein sequence analysis results also suggest that PvAD protein contains 12 (27%) alpha helices and 18 (29%) beta strands [Figure 3]. The predicted secondary structures and 3D structure of the PvAD protein is based on the best template, 3uko. This template is of *Arabidopsis thaliana* s-nitrosoglutathione reductase (protein), which showed the highest (56%) identity (figure not shown) with PvAD protein.[19] The reported *A. thaliana* s-nitrosoglutathione reductase structure was determined by using X-ray diffraction method (resolution: 1.40Å) (DOI: 10.2210/PDB3uko/PDB). Of 371 amino acids, 369 residues (99%) have been modeled with 100% confidence by using the single highest scoring template (3uko). There was no protein structure in PDB which shows more than 56% identity with PvAD protein; though PvAD protein’s phylogenetic analysis shows maximum (80%) similarity with AD protein from *P. acutifolius*, *L. corniculatus*, *L. japonicus* and *R. rugosa* AD the protein.[20] However, we strongly believe that the 3D structure predicted for PvAD protein in this study could be closer to its real structure based on the confidence level (key) of the prediction [Figures 3 and 4].[20] Yet, we suggest the further wet-lab experimental work is essential to

### Table 2: Comparison of PvAD cDNA nucleotide and deduced amino acid sequence with its counterparts from other plant species

| Species              | Gene bank accession number | Nucleotide (bp) | Amino acid | Similarity (%) |
|----------------------|-----------------------------|----------------|------------|----------------|
| *Arabis alpina*      | AF110429                    | 1624           | 379        | 71             |
| *Capsella rubella*   | XM_006302340                | 2860           | 379        | 74             |
| *Citrus clementina*  | XM_006434320                | 1674           | 376        | 76             |
| *Cucumis melo*       | DQ288986                    | 1502           | 379        | 74             |
| *Dianthus caryophyllus* | AY263389                  | 1522           | 380        | 77             |
| *Diospyros kaki*     | JF357957                    | 1469           | 379        | 74             |
| *Eutrema salsugineum*| XM_006390060                | 1550           | 379        | 72             |
| *Fourraea alpina*    | AF110451                    | 1789           | 379        | 71             |
| *Gossypium hirsutum* | U53701                      | 2626           | 379        | 75             |
| *Lotus corniculatus* | JN165714                    | 1143           | 380        | 81             |
| *Lotus japonicus*    | JN165714                    | 1143           | 380        | 81             |
| *Mangifera indica*   | GU233767                    | 1329           | 382        | 77             |
| *Medicago truncatula*| XM_003602081                | 1143           | 380        | 80             |
| *Nicotiana tabacum*  | AY619947                    | 1439           | 380        | 75             |
| *Phaseolus acutifolius* | ZZ3170                     | 1399           | 380        | 82             |
| *Populus trichocarpa*| XM_002302159                | 1556           | 380        | 76             |
| *Prunus persica*     | XM_007200991                | 1378           | 379        | 78             |
| *Pseudoturtius turrita* | AF110457                  | 1704           | 379        | 77             |
| *Pyrus communis*     | HO912034                    | 2290           | 380        | 76             |
| *Quercus suber*      | KF704745                    | 1146           | 381        | 75             |
| *Rosa rugosa*        | KF724973                    | 1337           | 389        | 78             |
| *Theobroma cacao*    | XM_007019324                | 1883           | 379        | 76             |
| *Zea mays*           | AF123535                    | 160480         | 379        | 72             |

Figure 2: Phylogenetic relationship of *Phaseolus vulgaris* L. alcohol dehydrogenase protein with its counterparts from other plant species.
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Figure 3: Predicted secondary structures of Phaseolus vulgaris L. alcohol dehydrogenase protein

Figure 4: Predicted three-dimensional (3D) structure of Phaseolus vulgaris L. alcohol dehydrogenase protein; (a) protein ribbon 3D structure model; red, green and yellow color shows the helices, strands and coils (loops) of protein, respectively; (b) molecular surface 3D structure of model shown in (a).

molecular modeling suggests that the deduced PvAD protein is analogous to A. thaliana s-nitrosoglutathione reductase protein. But, in order to have a full understanding of PvAD protein, further research to validate the predicted 3D structure, and to understand its expression and regulation in beans is required.

ACKNOWLEDGMENT

Authors are grateful to the Ministry of Science, Technology and Innovation (MOSTI), Malaysia for research funding [Research Grant Code Number: BSP (M)/BTK/004 (3)]; and to Patricia Lariguet, Department of Plant Biology, University of Geneva, Geneva, Switzerland for supplying seeds of bean, genotype BAT93.

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CONCLUSION

The basic features of PvAD gene cDNA and deduced protein are successfully elucidated in this study. Comparative validation of the predicted structure. Therefore, further research is necessary to understand more about PvAD protein.
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