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MODELLING THREE-DIMENTIONAL STRUCTURE AND VALIDATION OF POLYPHENOL OXIDASE ENZYME FROM *Camellia sinensis* (L.) O. KUNTZE

S. Ramkumar [1]*, P. Chella Perumal[2], P. Suresh kumar[3], G. Sudhakar[3], A. K. A. Mandal[4], P. Mohankumar[5], and V. K. Gopalakrishnan[1,2]

[1]Dept of Biochemistry, [2]Dept of Bioinformatics, Karpagam University, Coimbatore 641021, Tamilnadu, India.
[3]Dept of Biotechnology, Anna University, BIT campus, Trichirappalli – 620024, Tamilnadu, India.
[4]Plant Physiology & Biotechnology Division, UPASI Tea Research Foundation, UPASI Tea Research Institute, Nirar Dam BPO, Valparai 642 127, Coimbatore District, Tamilnadu, India.
[5] School of Biosciences and Technology (SBST), Plant Biotechnology division,
VIT University, Vellore-632014, Tamilnadu, India.

ABSTRACT

Polyphenol Oxidase (PPO), a major enzyme responsible for manufacture of black tea from tea leaves (*Camellia sinensis* (L) O. Kuntze). It is one of the oldest known beverages made from tender leaves of the plants. Polyphenol oxidase (PPO, EC.1.10.3.1) a key enzyme in tea processing, converts' polyphenol (catechin) to its products by undergoing enzymatic oxidation to produce black tea pigments such as theflavins (TF) and thearubigis (TR). Therefore, in this study attempt to emphasize the structural relationship of PPO enzyme based on the homology modeling analysis from United Planters' Association of Southern India (UPASI) selected clone of *Camellia sinensis* (L.). The three-dimensional structure of PPO enzyme was constructed by molecular modeling studies. Further, the model was assessed by PROCHECK, ProSA, and ERRAT plot in order to analyze the quality and consistency of computed model. The overall quality of generated model showed that, 91.1% amino acid residues were under the favored region. The ERRAT value of 68.84 % indicates that the environment profile of the model is good. Based on the results it can be concluded that, the modeled PPO enzyme can be utilized for development of good beverage and production of black tea from the selected area.

INTRODUCTION

The commercial tea comes from plants belonging to a relatively large group of cultivated species of *Camellia sinensis* (L) O. Kuntze [1]**.** It is one of the oldest known beverages made from tender leaves of the plants. Black tea is consumed throughout the world for its unique taste,

briskness and flavour. At present tea is cultivated in more than 80 countries of Asia, Africa and South America as one of the most desired plantation crops [2]. Presently, tea industry occupies significant position and plays a paramount role in the national economy. The crop shoots (apical bud and two terminal leaves) are harvested for commercial purpose for tea manufacture. The nature of plucked tea leaves decides the biochemical characteristic which in turn influences the quality of the tea [3]. Tea possess many biochemical constituents namely, phenolic components, alkaloids, vitamins, enzymes, crude fiber,

Corresponding Author

Ramkumar. S
Email: - ramkumarbiochem007@gmail.com

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proteins, lipids and carbohydrates. Enzyme like polyphenol oxidase act upon these phenolic compounds during fermentation process of black tea manufacturing [4].

Polyphenol oxidase (PPO, EC.1.10.3.1) a key enzyme in tea processing, converts polyphenol (catechin) to its products by undergoing enzymatic oxidation to produce black tea pigments such as theflavins (TF) and thearubigis (TR) [5]. PPO is also known as phenol oxidase, tyrosinase, *o*-diphenol oxidase, catechol oxidase, phenolase, and chlorogenic acid oxidase [6].PPO is a binuclear copper containing enzyme which catalyses two distinct reactions [7]. Primary reaction involves hydroxylation of monophenols to give *o*-diphenols the only specific reaction catalyzed by this enzyme (cresolase or monophenolase) and secondary reaction involves the removal of hydrogen from diphenols to give quinone by catecholase or diphenolase [8].

Molecular biology has been one of the most active research fields for the past few decades [9]. Isolation, cloning and sequencing of PPO gene is prerequisite in elucidating the molecular mechanism determining high quality, yield and disease resistance. In order to fulfill the grey area, the present study the bioinformatics tools were used to develop and analyze PPO enzyme model from United Planters' Association of Southern India (UPASI) germplasm accession.

MATERIALS AND METHODS
PPO Sequence submission
In our laboratory the PPO enzyme was purified and identified from *Camellia sinensis* (L) O. Kuntze. The isolated enzyme was sequenced; partial sequence (577 bp) of cloned cDNA was obtained due to limitations in instrumental read length, which exhibited high homology to PPO in phylogenetic tree analysis when compared to other woody plants [10]. Some functional domains were noticed within the partial sequence and registered in the GenBank and UniProt (Accession No.: JN801040 and G8IJI7) [11].

Sequence analysis and Homology modeling
Homology modeling is an efficient method for 3D structure prediction and quick experimental design for docking studies and it have been conducted based on high-resolution crystal structures of homologous proteins from the Protein Data Bank (PDB) [12]. The submitted PPO enzyme sequence was retrieved from Uniprot (Accession no. G8IJI7) and it contains 192 amino acid residues. A sequence similarity search for the protein against other sequences with available structural information was performed using the NCBI BLAST. Crystal structure of Grenache (*Vitisvinifera*) Polyphenol Oxidase (PDB ID: 2P3X with 2.20 ˚A resolution) was selected as template, having 52.63 % sequence identity with target. The homology molecular modeling was performed using SWISS-MODEL [13].

Structure validation
The constructed PPO enzyme structure was validated by saves - NIH-MBI server (http://nihserver. mbi.ucla.edu/SAVES), the inspection of phi/psi distributions of Ramachandran plot obtained through PROCHECK analysis [14]. The quality of PPO enzyme structure was further analyzed by ERRAT program [15]. The significance of consistency between template and modeled PPO enzyme was evaluated using ProSA server [16].

RESULTS AND DISCUSSION
PPO enzymes encoded by nuclear genes are localized in plastids, where they seem to be associated with the internal thylakoid membranes [17]. Thus, remaining physically separated from their phenolic substrates stored in the vacuole. Functional PPO enzymes have been purified from several higher-plant species including avocado [18], potato [19], Ferula sp. [20], broccoli[21], butter lettuce [22] and marula fruit [23]. PPO genes are highly conserved; there are numerous divergences among species. The deduced amino acid sequences of the PPO cloned genes had a relatively low degree of identity with other species which indicates that PPO sequences are highly conserved within plant species [24]. PPO genes have been cloned from several other plant species, including sugar cane, potato, grape and apricot [25].

Homology modeling is usually the method of choice when a clear relationship of homology between the sequence of target protein and at least one known structure is found [26, 27]. This approach would give reasonable results based on the assumption that the tertiary structures of two proteins will be similar if their sequences are related [28]. The structural relationship of PPO enzyme forced to construct this model. Hence, the crystal structure of Crystal structure of Grenache (*Vitisvinifera*) PPO is an appreciated template for modeling the 3D structure of PPO enzyme. The sequence alignment of PPO enzyme with *Vitisvinifera* Polyphenol Oxidase portrays that both template and target share significant similarity with each other (~53% identical), within their catalytic domains. Several homology modeling tools were used to model the protein and comparison of the results indicated that the model generated by SWISSMODEL is more acceptable than those generated by the other programs (more amino acids in the most favourable regions and less in the disallowed regions) [29]. The primary 3D model of PPO enzyme resulted from the structural modeling is shown in Fig 1.
The backbone conformation of the refined model of PPO enzyme was validated using Ramachandran plot (obtained through PROCHECK). The distribution of the phi and psi angles for the amino acid residues was also characterized. The generated model was found to be highly plausible; hence, 1.3% (only one residue) of residues alone was found to span the disallowed region in the Ramachandran plot (Fig 2). The Ramachandran plot residues of PPO

enzyme and 2P3X template were compared for validation (Table 1).

The measurement of the structural ERRAT at each amino acid residue in the developed model was given by the ERRAT plot (Fig 3). The overall quality factor of the computed model was 68.84%. Evaluation of PPO enzyme 3D model and *Vitisvinifera* Polyphenol Oxidase with ProSA server revealed a compatible *Z* score value of −3.59 and −4.99, respectively. This shows that residue energies together with pair energy, combined energy, and surface energy were all negative and had comparable surface energy affinity with template.

Table 1. Percentage residues of Target and Template predicted by Ramachandran plot statistics

Statistics	Percentage of residues in PPO enzyme (target)	Percentage of residues in (*Vitisvinifera*) Polyphenol Oxidase (template)
Residues in most favored region	91.1	87.9
Residues in additionally allowed region	6.3	9.7
Resides in generously allowed region	1.3	1.0
Residues in disallowed region	1.3	1.4

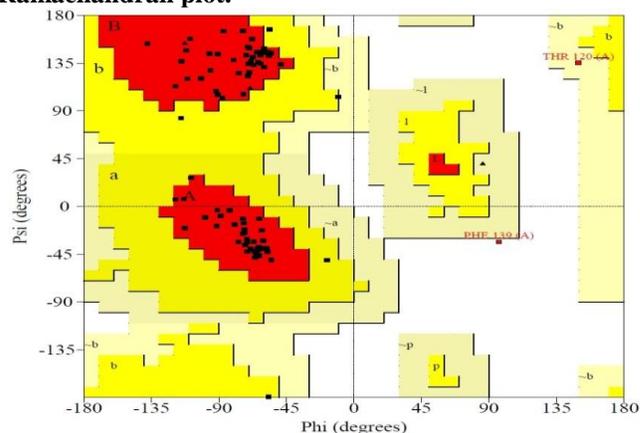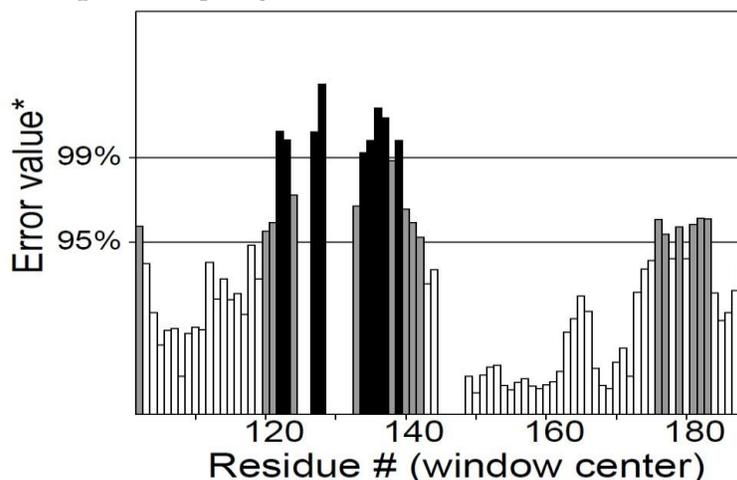| Fig 1. Three-dimensional (3D) structure of PPO enzyme based on known template protein structure by Homology modeling (Swiss Model Workspace).	Fig 2. The stereochemical spatial arrangement of amino acid residues in the modelled Three-Dimensional structure of PPO enzyme in the favoured region of the Ramachandran plot.

Fig 3. On the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value. Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolutions (2.5 to 3A) the average overall quality factor is around 91%.

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CONCLUSION

In this study the three dimensional structure of PPO enzyme was developed using bioinformatics approach of homology modeling (SWISS-MODEL) and the model was validated by PROCHECK, ERRAT plot, ProSA server and Veryfy 3D. It shows the developed model of PPO enzyme was most reliable. The stable structure of modeled PPO enzyme can be used for further Crops development process in the selected area.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this paper.

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