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Research Article

Comparative Analysis of Amino acid Sequence Diversity and Physiochemical Properties of Peroxidase Superfamily

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Abstract

Peroxidases superfamily is amongst the most widespread enzymes among living organisms, which maintains vital role(s) in many biological processes. Found in variety of species ranging from microbe to animals their main functions differ from organism to organism. Peroxidases mainly catalyze the oxidation of diverse substrates using hydrogen peroxide (H_2O_2). Peroxidases are a big family of enzymes which comprises of animal peroxidase and plant peroxidase superfamily. Plant peroxidases are further divided into three classes on basis of their origin and functions. In the present study an attempt has been made to relate three classes of plant peroxidase superfamily on the basis of enzyme's amino acid sequences and physiochemical properties by using bioinformatics tools (Assistat version-7.7, Clustal W, etc). Further phylogenetic relation was set up among them after doing multiple sequence alignment. Significant difference in the total number of amino acid residues among the classes was sufficient to determine factors like stability and nature (charge) of the enzyme. It was observed that class III peroxidase appeared to be the most stable class in plant peroxidase superfamily while class I being the least stable one. The study provides more insight about how closely the peroxidase classes are related to each other structurally. Distribution of amino acids also helps in stating the intracellular nature of class I plant peroxidase which differs from rest two classes of the plant peroxidase family which are extracellular in nature.The study resulted in giving a conclusive idea about the functional group of different classes of peroxidase and specific characters of all three families. This dry lab study was done using the amino acid sequences of 5 organisms of each class of plant peroxidase superfamily, retrieved from NCBI (http://www.ncbi.nlm.nih.gov/protein) and UniProt proteomic server (http://www.expasy.org). The choice of organisms for each class was based upon their ability produce peroxidase in laboratory conditions with a high yield factor.

Keywords: Amino acids; Peroxidase; Physiochemical properties

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Introduction

Peroxidases widely present in nature, acts as a key natural antioxidant. Peroxidase acts as an oxidizing agent,and facilitates in decomposition aided with decomposition of H_2O_2 [1]. They have different roles in different organisms as in mammals they help in immune system functions or in hormone regulations. In plants, they are implicated in metabolism of auxin, lignin, suber besides cell wall formation and defense against pathogens. Plant peroxidases (peroxidase EC: 1.11.1.7) are haem-proteins, which help in hydrogen peroxide (H_2O_2) mediated catalysis [2]. Peroxidases also play important roles in protection of plant leaves from salt-induced oxidative damage [3]. By the early 1900s, as yet unknown enzymes at work in human body were labeled as "catalases" while the simultaneous observation that plants and animals utilized polyphenols to degrade H_2O_2 lead to the term "peroxidases" [4]. More than 30 different kinds of peroxidases are found in humans whereas *Arabidopsis thaliana* has as many as 130 different peroxidases [5]. Peroxidases from protists, fungi and prokaryotic organisms are known to promote virulence [6]. Although, the most well-known and best studied peroxidase is horseradish peroxidase [7] yet bacterial peroxidases have attracted comparatively more attention than plant peroxidases [8].

In peroxidase, the bound cofactor necessary for its activity is heme [4]. Most heme peroxidases belong to two superfamilies at molecular level, one present in plants, bacteria and fungi [9,10], and the other-one found in animals [11,12]. These have iron (III) protoporphyrin IX (ferriprotoprophyrin IX) as the prosthetic group and heme is a complex between an iron ion and a molecule protoporphyrin IX [13,14]. Catalytic cycle involves distinct intermediate enzyme forms [15].

For most of the peroxidases, the optimal substrate is H_2O_2 but others are much active with organic hyper oxides such as lipid oxides. Peroxidases are used in clinical immunoassay and enzyme biochemistry and for the colorimetric measurement of biological materials [16]. Some novel applications of peroxidases include waste-water (containing phenolic compounds) treatment, removal of peroxide from materials such as industrial wastes and foodstuffs and synthesis of different aromatic chemicals [17]. Peroxidases have potential for bio-remediation of wastewater contaminated with cresols, chlorinated and non-chlorinated phenols, and for bio-pulping, bio-bleaching in paper industry and textile-dye degradation [18].

Based on differences in primary structure, the peroxidase superfamily can be further divided into three classes I, II and III. Class I

peroxidases are intracellular in nature, present in plants, bacteria and yeast such as microbial cytochrome peroxidase (EC1.11.1.5), bacterial catalase-peroxidase (EC1.11.1.6) and ascorbate (EC1.11.1.11) peroxidase. Class II peroxidases, are fungal peroxidases exclusively, that have major role in lignin biodegradation [19]. They include lignin peroxidase (LiPs; EC1.11.1.14) and Mn^{2+} dependent peroxidase (MnPs: EC1.11.1.13). Versatile peroxidases (VP; EC 1.11.1.16), have a molecular structure that is hybrid between MnPs and LiPs [20] and may result in catalysis of non-phenolic and phenolic substrates like LiPs [21]. Class III peroxidases (EC 1.11.1.7), widely distributed in plant kingdom [22], include soybean peroxidase (SBP), horseradish peroxidases (HRP), peanut peroxidase (PNP), *etc.* play a vital role in the life cycle of many plants [23]. In plants class III peroxidases are involved in functions like cell wall metabolism [24], lignification [25], suberization [26], auxins metabolism [27], wound healing [28], release of reactive oxygen species (ROS), reactive nitrogen species (RNS) metabolism [29], fruit growth and ripening [30] and defense against pathogens [31]. Plant peroxidase superfamily is of a high economic value due to its diverse applications [32]. Though a large no of data is present for plant peroxidase superfamily, never an attempt was made to compare and inter-relate different classes of this superfamily. Due to high economic importance, finding the relation and difference among them can help us to understand the nature better which can result in enhancing their economic value. In the present study an attempt has been made to analyze three classes of plant peroxidase superfamily using different bioinformatics tools.

Materials and Methods

Retrieval of amino acid sequences

The amino acid sequences of peroxidases for different classes were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/protein), UniProt proteomic server (http://www.expasy.org/) shown in table 1. Organisms were selected on the basis of their ability to produce peroxidase in laboratory conditions with a high yield factor. Total five organisms were selected for each class so that the data has a high value of significance upon computation.

Sr. No	Microorganisms	Accession number
Class I peroxidases		
1.	*Helicobacter pylori* B8	CBI65628.1
2.	*Escherichia coli str.* K-12	YP_491917.1
3.	*Rhodopirellula baltica* SH 1	NP_863829.1
4.	*Alcanivorax borkumensis* SK2	YP_694094.1
5.	*Campylobacter coli* 15-537360	AGZ20833.1
Class II peroxidases		
1.	*Ceriporiopsis subvermispora* B	EMD32649.1
2.	*Fomitopsis pinicola* FP-58527 SS1	EPT05268.1
3.	*Ganoder malucidum*	ACA48488.1
4.	*Schizophyllum* sp. F17	AGO86670.2
5.	*Cerrena unicolor*	AGS19356.1
Class III peroxidases		
1.	*Gossypium hirsutum*	ACJ11766.1
2.	*Beta vulgaris*	CAK22416.1
3.	*Solanum lycopersicum*	CAG25463.1
4.	*Stylosanthes humilis*	AAB67737.1
5.	*Nelumbo nucifera*	ABN46984.1

Table 1: Different organisms producing plant enzymes.

Analysis of some important physiochemical parameters

The physiochemical data of amino acid sequences were generated from the SwissProt and Expert Protein Analysis System (ExPASy). The data for various parameters was generated by using online tool ProtParam and Compute pI/Mw at the ExPASy. The molecular weight (kDa) of sequences was calculated byaverage isotopic masses addition to amino acids in the protein and then deducing average isotopic mass of one molecule of water. Isoelectic point (pI) was calculated using pKa values of amino acids according [33].

Primary structure analysis

The amino acid compositions of proteases was calculated from the ProtParam tool which is available on ExPASy. Molar extinction coefficient of protease proteins was calculated according to equation; E(Prot) = Numb (Tyr)* Ext (Tyr) + Numb (Trp)* Ext (Trp) + Numb (Cystine)* Ext (Cystine)

Aliphatic index of various sequences was obtained using the formula:

Aliphatic index = X (Ala) + a * X (Val) + b * [X (Leu) + X (Ile)] [34]

Where, X (Ile), X (Val), X (Ala), and X (Leu) are mole percent (100 X mole fraction) of isoleucine, valine, alanine, and leucine. The coefficient a and b are the relative volume of valine side chain and of leu/Ile side chain to the side chain of alanine, with the help of ProtParam tool [34].

Secondary structure analysis

Secondary structure analysis included the analysis of number of β-turn, α-helices, β-sheet and extended strand performed by ExPASy SIB Bioinformatics SOPMA tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page = npsa_sopma.html) [35]. To utilize this tool sequences were submitted in FASTA format [36].

Tertiary structure analysis

PHYRE2 software (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) was used to build the 3D models of different peroxidases and their validation was done using the Ramachandran plot constructed using MOL probity (http://molprobity.biochem.duke.edu/) [37].

Multiple sequence alignment and phylogenetic analysis

The phylogeny of three classes of peroxidases was generated followed by alignment using MEGA-X tool which is the standalone tool for the multiple sequence alignments and phylogenetic analysis [38].

Results and Discussion

Computational analysis of some important physiochemical parameters and amino acids of peroxidases

The present study reports a detailed comparison of various physiochemical parameters and amino acids among the three classes of plant peroxidases. The result of analysis of physiochemical properties and amino acids content has been tabulated in tables 1 & 2. Overall analysis revealed theoretical PI, total number of positively charged residues (Arg + Lys), extinction coefficient, instability index and negative charged residues (Asp + Glu) to be statistically significant among all the groups of serine proteases (Table 1). The total number of amino acid residues in the three classes of peroxidases differed substantially

as the number of aminoacids was found to be in the range of 341.0 to 465.0 for class I peroxidases, 332.0 to 364.0 for class II peroxidases and 319.0 to 331.0 for class III peroxidases. Similarly, molecular weight range was 36861.2 to 51570.5 Dalton for class I peroxidases, 34825.4 to 38643.3 Dalton for class II peroxidases and 33781.1 to 37528.1 Dalton for class III peroxidases. On the other hand, theoretical pI ranged from 4.72 to 9.06 for class I peroxidases, 4.12 to 5.33 for class II peroxidases, 5.38 to 8.98 for class III peroxidases, which was found to be statistically significant. The pI value below 7 indicates that a protein is acidic while above 7 indicates a a protein to have basic structure. The total counts for the negatively charged (Asp + Glu) and positively charged residues (Arg + Lys) were found to be statistically significant and higher in case of class I peroxidases. Extinction coefficient was found to be statistically significant which ranged from 27180.0 to 68675.0 for class I peroxidases, 6000.0 to 21470.0 for class II peroxidases, 13575.0 to 23420.0 for class III peroxidases and higher in case of the class I peroxidases. Other significant physiochemical parameter i.e. instability index was also found to be statically significant and ranged between 24.13 to 52.14 for class I peroxidases, 42.18 to 55.71 for class II peroxidases, 29.12 to 42.62 class III peroxidases. Higher the aliphatic index more is the stability of a given compound. A negative value for GRAVY indicates that a protein is non-polar and thus has a greater interaction with water [36].

Primary structure analysis

The comparison of individual amino acid composition has revealed that in case of class I peroxidases Glu (E), Lys (K), Trp (W) and Tyr (Y) were found to be predominantly higher. Class II peroxidases revealed the presence of Ala (A), Asp (D), Gln (Q), Gly (G), His (H), Ile (I), Phe (F), Pro (P) and Thr (T) to be higher in comparison to other two classes of peroxidases. On the other hand Arg (R), Asn (N), Cys (C), Leu (L), Met (M), Ser (S) and Val (V) to be predominantly higher in case of the class III peroxidases (Table 3).

Secondary structure analysis

An average of the sequence for all three classes of peroxidase superfamily were done and checked for secondary structure analysis. All three classes of peroxidase family were rich in random coils which are known to play an important function in determining proteins flexibility and the conformational changes as turnover. Also for a protein to have higher amino acid residues in random coils is an indication for its enzymatic nature. This was in accordance with our study where all the sequence showed high percentage of random coils with class II having highest random coils and class III possessing the least [36]. In addition, all three classes showed higher number of amino acid residues in α-helix as compared to the β- turn.

Parameters	Microorganisms	1	2	3	4	5
Number of amino acids	Class I	350.0	465.0	424.0	402.0	341.0
	Class II	364.0	332.0	364.0	359.0	360.0
	Class III	323.0	326.0	332.0	319.0	331.0
Molecular weight (Dalton)	Class I	38815.1	51570.5	46289.7	44236.0	36861.2
	Class II	38227.9	34825.4	38110.5	37829.4	37678.3
	Class III	35729.6	35499.1	35856.7	33781.1	37528.1
Theoretical pI	Class I	9.06	5.98	4.72	4.87	8.16
	Class II	4.12	5.33	4.42	4.78	4.47
	Class III	5.38	8.98	7.55	7.06	8.44
Total number of negatively charged residues (Asp + Glu)	Class I	37.0	58.0	63.0	48.0	39.0
	Class II	44.0	34.0	40.0	43.0	46.0
	Class III	39.0	32.0	27.0	27.0	44.0
Total number of positively charged residues (Arg + Lys)	Class I	46.0	52.0	36.0	28.0	41.0
	Class II	14.0	25.0	17.0	24.0	22.0
	Class III	34.0	41.0	28.0	27.0	48.0
Extinction coefficient ($M^{-1}.cm^{-1}$) at 280 nm	Class I	31775.0	68675.0	27765.0	43110.0	27180.0
	Class II	11500.0	17210.0	6000.0	21470.0	6000.0
	Class III	13575.0	16430.0	15065.0	23420.0	17460.0
Instability index	Class I	31.19	38.93	41.04	52.14	24.13
	Class II	55.71	48.71	43.72	42.50	43.55
	Class III	36.48	29.12	36.89	41.48	42.62
Aliphatic index	Class I	83.54	80.19	69.62	71.14	86.72
	Class II	83.96	83.61	78.32	80.78	85.42
	Class III	75.82	92.33	85.69	83.67	84.53
Grand average of hydropathicity (GRAVY)	Class I	-0.234	-0.395	-0.438	-0.433	-0.240
	Class II	0.112	-0.027	0.060	-0.035	0.025
	Class III	-0.167	-0.095	0.026	-0.082	-0.362

Table 2: Physiochemical parameters of various microorganisms calculated using ProtParam tool at ExPASy proteomic server.

Class I peroxidase: 1. *Helicobacter pylori B8* **2.** *Escherichia coli str. K-12* **3.** *Rhodopirellula baltica SH1* **4.** *Alcanivorax borkumensis SK2* **5.** *Campylobacter coli 15-537360*
Class II peroxidase: 1. *Ceriporiopsis subvermispora B* **2.** *Fomitopsis pinicola FP58527SS1* **3.** *Ganoder malucidum* **4.** *Schizophyllum sp. F17* **5.** *Cerrena unicolor*
Class III peroxidase: 1. *Gossypium hirsutum* **2.** *Beta vulgaris* **3.** *Solanum lycopersicum* **4.** *Stylosanthes humilis* **5.** *Nelumbo nucifera*

Amino acid	Peroxidase class	1	2	3	4	5
Ala (A)	Class I peroxidases	5.4	9.9	11.1	7.5	9.7
	Class II peroxidases	12.1	14.5	11.8	12	11.9
	Class III peroxidases	9	5.5	6.3	6	7.6
Arg (R)	Class I peroxidases	2.6	4.1	4	4.5	1.5
	Class II peroxidases	3.3	4.5	2.7	3.3	2.5
	Class III peroxidases	5.3	3.4	5.1	4.1	7.3
Asn (N)	Class I peroxidases	4.6	4.1	5.7	6.7	6.2
	Class II peroxidases	3.3	4.2	4.5	3.6	2.8
	Class III peroxidases	6.8	5.5	5.1	5.3	4.5
Asp (D)	Class I peroxidases	5.1	6.7	6.4	6	4.4
	Class II peroxidases	6.3	6.6	7.2	7.1	8.3
	Class III peroxidases	7.7	5.2	5.4	6.6	8.2
Cys (C)	Class I peroxidases	1.7	1.5	1.4	1.2	1.2
	Class II peroxidases	2.2	1.2	2.2	2.2	2.2
	Class III peroxidases	2.5	3.1	2.4	2.8	3
Gln (Q)	Class I peroxidases	3.1	4.9	3.3	4	2.6
	Class II peroxidases	5.8	3.3	3.6	4.7	4.7
	Class III peroxidases	3.4	2.5	4.5	3.8	2.4
Glu (E)	Class I peroxidases	5.4	5.8	8.5	6	7
	Class II peroxidases	5.8	3.6	4.7	4.7	4.4
	Class III peroxidases	4.3	4.6	2.7	1.9	5.1
Gly (G)	Class I peroxidases	8.3	7.7	8.3	7.7	8.5
	Class II peroxidases	8.5	8.4	8.5	8.6	8.6
	Class III peroxidases	6.8	8.9	9.6	10.7	4.8
His (H)	Class I peroxidases	1.7	2.2	2.4	2.7	1.8
	Class II peroxidases	1.4	2.4	2.8	2.7	2.2
	Class III peroxidases	0.9	2.5	1.2	2.5	3.3
Ile (I)	Class I peroxidases	6.6	4.9	4.2	4	5.3
	Class II peroxidases	6.3	3.9	6.4	5.2	4.7
	Class III peroxidases	5.3	5.8	5.1	3.1	4.8
Leu (L)	Class I peroxidases	8.6	8.6	7.8	9.2	9.7
	Class II peroxidases	6.6	9.3	5.8	6.9	8.3
	Class III peroxidases	6.8	9.8	8.7	10	9.1
Lys (K)	Class I peroxidases	10.6	7.1	4.5	2.5	10.6
	Class II peroxidases	0.5	3	1.9	3.3	3.6
	Class III peroxidases	5.3	9.2	3.3	4.4	7.3
Met(M)	Class I peroxidases	3.4	1.9	2.6	1.7	1.5
	Class II peroxidases	1.9	3	0.3	0.8	0.6
	Class III peroxidases	4.6	1.2	1.8	1.3	2.1
Phe (F)	Class I peroxidases	5.1	3.4	5.2	5.5	4.4
	Class II peroxidases	6.6	4.5	7.2	7.1	6.4
	Class III peroxidases	6.8	4.9	6	4.7	4.8
Pro (P)	Class I peroxidases	5.7	5.8	6.6	7	4.4
	Class II peroxidases	8.8	7.2	8.1	7.7	7.8
	Class III peroxidases	3.1	4.6	3.3	4.7	5.4
Ser (S)	Class I peroxidases	6.9	4.7	3.5	9.7	5.9
	Class II peroxidases	6.3	8.7	9.9	5.3	5.8
	Class III peroxidases	7.1	6.7	9.3	10	5.1
Thr (T)	Class I peroxidases	4.9	4.7	7.5	5.5	6.2
	Class II peroxidases	6.3	3.6	6.6	7	6.9
	Class III peroxidases	5	5.5	8.1	7.2	3.9
Trp (W)	Class I peroxidases	0.9	1.5	0.5	1	0.9
	Class II peroxidases	0.5	0.6	0.8	0.3	0.3
	Class III peroxidases	0.3	0.3	0.6	0.6	0.3
Tyr (Y)	Class I peroxidases	2.9	4.3	2.6	3.5	2.1
	Class II peroxidases	0	1.2	0.8	0	0
	Class III peroxidases	1.2	0	0.8	0	0
Val (V)	Class I peroxidases	6.6	6	4	4.2	6.5
	Class II peroxidases	7.4	6	7.4	7.2	7.8
	Class III peroxidases	6.8	8.9	8.7	9.1	9.1

Table 3: Comparative analysis of amino acid residues in different classes of plant peroxidase.

Class I peroxidase: 1. *Helicobacter pylori B8* **2.** *Escherichia coli str. K-12* **3.** *Rhodopirellula baltica SH1* **4.** *Alcanivorax borkumensis SK2* **5.** *Campylobacter coli 15-537360*

Class II peroxidase: 1. *Ceriporiopsis subvermispora B* **2.** *Fomitopsis pinicola FP58527SS1* **3.** *Ganoder malucidum* **4.** *Schizophyllum sp. F17* **5.** *Cerrena unicolor*

Class III peroxidase: 1. *Gossypium hirsutum* **2.** *Beta vulgaris* **3.** *Solanum lycopersicum* **4.** *Stylosanthes humilis* **5.** *Nelumbo nucifera*

These secondary structures dominated by α-helix region, depicts about the thermal resistance of a protein on basis of their intrinsic stability (Figure 1) [37]. In this case class III peroxidase showed highest % of α-helix while least was depicted by class I peroxidase (Figure 1).

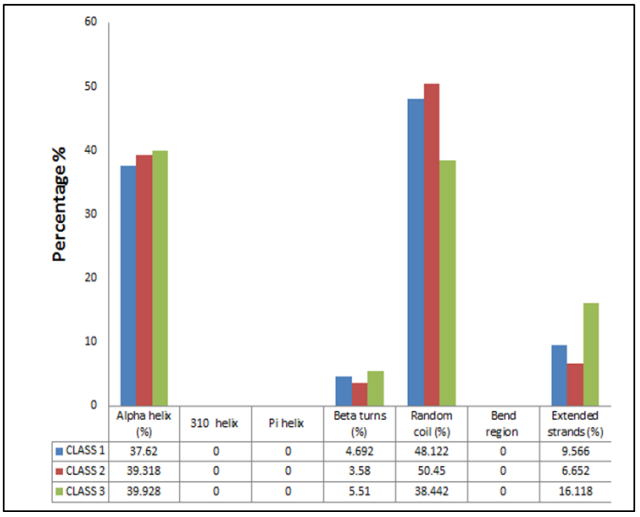

Figure 1: Comparison between secondary structures of different classes of plant peroxidase superfamily.

Tertiary structure analysis

3-D model was prepared to predict the tertiary structure for all three classes of the peroxidase superfamily. For tertiary analysis one structure was developed from each of the class using the PYRE 2 software (Figure 2). The validation of the structure was done using the Ramachandran plot. Amino-acids present in favorable and the allowed region of the Ramachandran-plot were used for the validation of the model [36].

Phylogenetic analysis of three classes of plant peroxidase superfamily

Phylogenetic tree analysis corresponds to the evolutionary distances which may help to estimate the divergence times in genes, proteins, species and in populations. The phylogeny data showed the significant evolutionary distances for all the three classes of

peroxidases (Figure 3). The class II and class III peroxidases were found to be diverged earlier from the ancestor with a shorter branch length as compared to class I. The co-ancestor for class II and class III was found to be same from the root. However, the class I peroxidases were found to be diverged from the root ancestor [37]. The phylogenetic analysis (Figure 3) also explains as to why some properties of class I peroxidase are different from rest classes under comparison as its genetically diverged before the other two classes. A classic example can be as class I peroxidase is intra-cellular in nature while class II and class III are extra-cellular in nature.

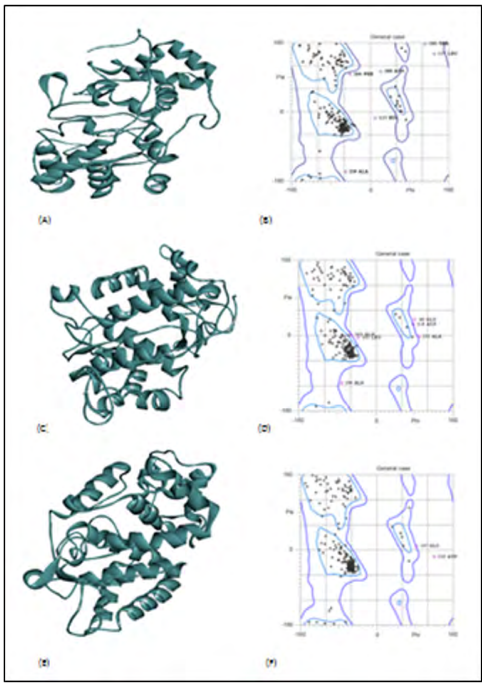

Figure 2: Class I peroxidase (CBI65628.1) (A) 3D model (B) Ramachandran Plot. Class II peroxidase (EPT05268.1) (C) 3D model (D) Ramachandran Plot. Class III peroxidase (ACJ11766.1) (E) 3D model (F) Ramachandran Plot.

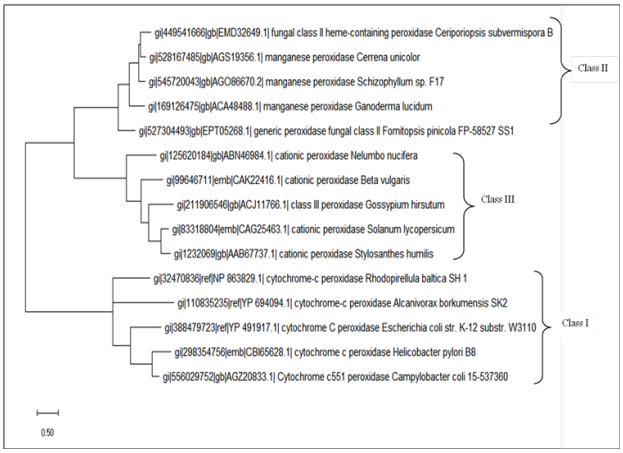

Figure 3: Phylogenetic tree of peroxidases classes obtained by MEGA-X tool. The maximum likelihood method was applied with 1000 bootstrap replications and the Jones–Taylor–Thornton model of amino acid substitutions.

In the present study, an attempt was also made to compare three classes of plant peroxidases based on their physiochemical properties, phylogeny and the predominance of specific amino acids in the individual group. This study gave vital information about characteristics in which the classes of plant peroxidases are alike and in which aspect they differ from each other [38]. The important physiochemical properties such as number of amino acids, molecular weight and aliphatic index tell us that all the three classes are somewhat similar in composition, which makes the three properties to be non-significant as overall difference does not vary. On the other hand negatively charged residues (Arg + Lys) and positively charged residues (Asp + Glu) which have long been known to function as determinants of membrane topology [38] are found to be statistically significant.

Due to diversity of 20 amino acids and due to the incredible number of combinations they afford, proteins differ widely in physiochemical properties, substrate specificity [39] as well as mechanism of their catalytic action. The plant peroxidases share similar overall protein folds and specific features, such as catalytically essential histidine and arginine residues in their active sites [9]. The non-significant value of histidine and arginine in the study showed their common presence in all three classes. Significant difference for amino acid combination were found between the classes of plant peroxidase super family, which is the basic criteria of subdivision of plant peroxidase superfamily [40]. Glycine (G) and Valine (V) amino acids present in large quantity in class III are responsible for compact core packing and functional regulation [41,42]. Cystine content was exponentially high in class II peroxidases. Cystine is said to be responsible for imparting stability to the class II peroxidase as cysteine and methionine form disulphide bonds which affect the stability of the proteins [43]. Cysteine (C) tends to provide flexibility and is capable of making cavities in the core of the psychrophilic protein structure [44] which imparts extra stability to proteins. Asparagine (Asn) and Glutamine (Gln) have interesting hydrogen-bonding properties since they resembled the backbone peptides and hence their more value for class II peroxidase showed that they were structurally more stable [45,46]. The class I peroxidases contains various evolutionary unique features such as no cystine bridges, no carbohydrate, no structural Ca^{2+}, and no endoplasmic reticulum signal sequence, whereas the class II and class III peroxidases, are routed via the endoplasmic reticulum [47,48]. The present study provided a greater insight into the functioning, similarity and dissimilarity among each other.

Conclusion

The members of the plant peroxidases superfamily were compared and contrasted on the basis of their amino acid sequences and physiochemical properties using bioinformatics tools. After comparison, it was observed that three major classes of plant peroxidase superfamily mainly differ in many physiological properties such as theoretical pI, presence of positive and negative charged amino acid and composition of amino acids constituting the protein. This study has clearly worked out prominent differences between three major classes of peroxidases in conserved amino acid residues at several points.

The significant differences in the total number of amino acid residues among these classes were sufficient to determine factors like stability and nature (charge) of the enzyme. It was observed that class III was most stable among plant peroxidase superfamily, and class I

being the least stable. The study helped to get greater knowledge about how closely these classes are related to each other structurally. The phylogenetic studies has signified that class II and class III peroxidase are evolutionary more closely related to each other as compared to class I peroxidase. The pI index being higher for the class I with respect to the other two classes showed that negative amino acids are present in abundance in class I peroxidases when compared with other two classes. Distribution of amino acids also helped in inferring the intracellular nature of class I plant peroxidases, which differed from rest two classes of the plant peroxidase families being extracellular in nature. The results of the present study will indeed be of great help to get an insight of the peroxidase plant superfamily, to understand the role of amino acids to develop practical strategies in engineering these peroxidases and their potential use in different industries, their role in biological and in bioremediation processes.

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Conflict of Interest

Authors declare that they have no conflict of interest

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