A Comprehensive Bioinformatics Analysis of the Lipoxygenases Superfamily in *Shewanella Woodyi* Strain (Strain ATCC 51908/MS32)

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**Abstract:** Shewanella bacteria have real potential for the bioremediation of contaminated environmental and presents a differential that are three lipoxygenases described in its genome. Lipoxygenases (LOX) are a family of iron-containing enzymes that catalyze the dioxygenation of polyunsaturated fatty acids in lipids. They occur ubiquitously in plants and mammals, and only recently, they have been detected in coral, moss, fungi and a number of bacteria as well. In this work, analyzed three enzymes lipoxygenases described for *Shewanella woodyi* deposited in GeneBank as probable LOX gene in the Swoo_2318 *S. woodyi* ATCC 51908: Proteins Code (ACA86597.1) hypothetical protein, (ACA87192.1) arachidonate 15-lipoxygenase and (AEF01209.1) arachidonate 15-lipoxygenase precursor the strain *S. woodyi* DSM 12036. The bioinformatics tools tend to solve impossible problems to be addressed in the past decades. These analyses identified were the three proteins described as lipoxygenases have different secondary structures, the phosphorylation sites; the protein GRAVY (grand average of hydropathy) and protein isoelectric point are distinct. However, binding site is Fe for three lipoxygenases. This suggested that three lipoxygenases deserve special attention for work in situ, due to peculiar characteristics and still not know what is the need of *S. woodyi* have in its genome three genes encoding the same protein.

**Key words:** Arachidonate, crystallography, hypothetical protein.

1. Introduction

*Shewanella woodyi* (strain ATCC 51908/MS32) was isolated from the squid, sediment and water of the Alboran Sea (mixture of Atlantic and Mediterranean Sea) as a bioluminescent bacterium able to degrade RDX and complete sequence of *S. woodyi* ATCC 51908, Submitted (FEB-2008) to the EMBL/GenBank/DDBJ databases [1]. A massive quantity of protein amino acid and sequences generated from hundreds of complete genomes can be computationally inferred via homology to other sequences which have had experiments performed on them or which we know something about from some other means [2]. Lipoxygenases (linoleate: oxygen oxidoreductase, EC 1.13.11.12) catalyze the region and stereoselective insertion of molecular oxygen into a (1Z, 4Z)-pentadiene system of polyunsaturated fatty acids, forming hydroperoxy fatty acids [3]. In the animal system, preferably using lipoxygenase arachidonic acid as substrate and are involved in the formation of several regulatory components play roles in inflammation, immunity, and hypersensitivity reactions in host defense and in the formation of free radicals and leukotrienes [4]. The LOX of the plant occur in various parts of the plant performing functions in various processes such as growth and development [3], senescence [5], vegetative storage [6], germination [7], response to injury [8] and resistance...
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to insects and pathogens [9]. However, it is not yet defined the role of the lipooxygenase pathway in microorganisms. However, LOX containing manganese was found in the fungus Gaeumannomyces graminis [10]. The LOX enzyme has been detected in some bacterial species: Thermoactinomyces vulgaris [11], Pseudomonas aeruginosa [12-14], Pseudomonas oleovorans [15], Nocardia and Corynebacterium [16], Sphingomonas [17], Flavobacterium [18], Sarcina [19], Bacillus [20], Shewanella woodyi [21]. Efforts have been made to describe the enzymes involved in the biotransformation of fatty acids of mid-chain length [22]. The interest in bacteria LOXs tends to increase because the chiral hydroxy fatty acids can be used as starting materials in the fields of fine chemical production [23]. However, the role of LOX in prokaryotes remains a mystery. The Bioinformatics is a step forward in proteomics of protein method appears as a strategy of choice to generate in silico hypotheses for experimental testing [24-26]. Thus, the bioinformatics analysis incorporating sequence information, gene structures, evolutionary relationships, and motifs of putative functional to identify three gene of LOX activity, which was submitted to bioinformatics analysis, modeling for testing the biological potential.

2. Materials and Methods

We use the three enzymes lipooxygenases described for Shewanella woodyi deposited in GeneBank as probable LOX gene in the Swoo_2318 S. woodyi ATCC 51908: Proteins Code (ACA86597.1) hypothetical protein, (ACA87192.1) [1] arachidonate 15-lipoxygenase and (AEF01209.1) arachidonate 15-lipoxygenase precursor the strain S. woodyi DSM 12036 [21]. The genes functional identification the data were analyzed in ProDom [27] is a comprehensive database of protein domain families generated from the global comparison of all available protein sequences. Pfam [28] is a database of protein families, where families are sets of protein regions that share a significant degree of sequence similarity, thereby suggesting homology, similarity is detected using the HMMER3. The determination homology and the I-Tasser server [29] we used are on-line platform for protein structure and function predictions. 3D models are built based on multiple threading alignments by LOMETS and iterative template fragment assembly simulations, functions in slights are derived by matching the 3D models with BioLip protein function database. PyMOL Molecular Graphics System, version 1.5.0.4 is a program user sponsored molecular visualization system on an open-source foundation [30]. We used the MEMSAT3 and MEMSAT-SVM a novel version of a widely used transmembrane topology prediction method [31] and PSIPRED to identify the signature subfamily [32]. The program used for comparison was Basic local alignment search tools (Blast) [33] and the sequences compared with those online at the GenBank. Sequence alignments were first done using Clustal W (version 1.8) [34], and then adjusted using the BioEdit, version 5.0.9 Program [35]. Phylogenetic relationships were inferred by preferential alignments of the LOX protein sequences obtained from GenBank. This was done using the program MEGA6 (version 2.1) [36]. Bootstrap analysis was performed with 1000 replicates [37]. This was GRAY (grand average of hydrophy) value for the protein sequences [38]. Protein Isoelectric Point calculated the theoretical pi (isoelectric point) for the protein sequences [39].

3. Results and Discussion

The data information of three genes the LOX we analyzed using the ProDom and Pfam programs [27, 28]. We confirmed belong to the superfamily lipooxygenase. Then we submitted the amino acid sequences on I-Tasser serve, has generated protein structure predictions for thousands of modeling requests from more than 35 countries. A scoring function (C-score) based on the relative clustering structural density and the consensus significance score of multiple threading
templates introduced to estimate the accuracy of the I-TASSER predictions. A large-scale benchmark test demonstrates a strong correlation between the C-score and the TM-score (a structural similarity measurement with values in \([0, 1]\)) of the first models with a correlation coefficient of 0.91. Using a C-score cutoff > -1.5 for the models of correct topology, both false positive and false negative rates are below 0.1. Combining C-score and protein length, the accuracy of the I-TASSER models can be predicted with an average error of 0.08 for TM-score and 2 Å for RMSD [29].

The templates protein of similar folds from PDB (Protein Data Bank) library, with the result: 100.0% confidence by the single highest scoring template is a true homology, code template the structure for hypothetical (ACA86597.1) identified by the PDB was similar code 1hu9A is Lipoxygenase-3 of soybean, the structure for arachidonate 15-lipoxygenase (ACA87192.1) identified by the PDB was similar code 1no3A is Lipoxygenase-3 of soybean and the structure for (AEF01209.1) arachidonate 15-lipoxygenase precursor PDB code 1hu9A is Lipoxygenase-3 of soybean in all structures has the ligand as Fe (III) ion. After predictions of structure and function of the Lipoxygenase gene, the determination of proteins 3D structures was predicted using TASSER the 3D models are built based on multiple-threading alignment using COFACTOR is a structure-based method for biological function annotation of protein molecules [29] we used the PyMol [30] to visualize the image in Fig. 1 structure all structures has the ligand as Fe (III) ion. We found in sequence LOX hypothetical (ACA86597.1), 2 Iron; catalytic (By similarity), 6 for LOX for arachidonate 15-lipoxygenase (ACA87192.1) and 2 for (AEF01209.1) arachidonate 15-lipoxygenase precursor. The iron atom in lipoxygenases is bound by four ligands, three of which are histidine residues [40]. Six-histidine are conserved in all lipoxygenase sequences, five of them are found clustered in a stretch of 40 amino acids.

The structure for (ACA86597.1) hypothetical proteins shows identity with lipoxygenase-3 identified by the PDB code 1hu9A. Cscore\textsuperscript{LB} is the confidence score of predicted binding site was the 0.62, BS-score is a measure of local similarity (sequence & structure) between template binding site and predicted binding site in the query structure was the 1.05. TM-score is a measure of global structural similarity between query and template protein was the 0.931. IDENa is the percentage sequence identity in the structurally aligned region was the 0.181. Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein was the 0.950 (Fig. 1A).

The structure for (ACA87192.1) arachidonate 15-lipoxygenase showed identity with Lipoxygenase-3 identified by the PDB code 1no3A. Cscore\textsuperscript{LB} was the 0.70. BS-score was the 1.65.

Fig. 1 The structure for three Lox studied similar Lipoxygenase-3 of soybean.
TM-score was the 0.876, IDENa was the 0.220. Cov. was the 0.879 (Fig. 1B). The structure for (AEF01209.1) arachidonate 15-lipoxygenase precursor also shows identity with lipoxygenase-3 identified by the PDB code 1hu9A. CscoreLB was the 0.67. BS-score was 1.12. TM-score was the 0.850. IDENa was the 0.198. Cov. was the 0.874 (Fig. 1C).

The first crystal structure of a prokaryotic lipoxygenase, from *Pseudomonas aeruginosa* (Pa_LOX) the most striking difference is an insertion in the catalytic domain of a pair of long antiparallel alpha helices, contributing to an enlarged binding pocket in Pa_LOX containing a bound phospholipid: a phosphatidylethanolamine with well-defined chains of 18 and 14 carbons in length [14]. This demonstrates would be very interesting to try to crystallize the three proteins lipoxygenases of *S. woodyi* order to compare with the protein structure of Pa_LOX.

The MEMSAT3 and MEMSAT-SVM program were used [31] to identify transmembrane helices in Fig. 2 shown for (ACA86597.1) hypothetical protein 3 regions the transmembrane. The N-terminal located at amino acid 166 being in membrane cytoplasmic, was shown 3 regions. The pore-lining located between amino acids 118-136; 403-418; 580-595 and C-terminal located at amino acid 595 domain that is being transported to the extracellular space passing through the pore in Fig. 2A. It was shown for (ACA87192.1) arachidonate 15-lipoxygenase 3 regions the transmembrane, the N-terminal is located at amino acid 61 domain that is being transported to the extracellular space passing through the pore, was shown 3 regions the pore-lining is located between amino acids 61-76; 404-419; 565-602 and C-terminal located at amino acid 166 being in membrane cytoplasmic in Fig. 2B. It was shown for (AEF01209.1) arachidonate 15-lipoxygenase precursor 3 regions the transmembrane, the N-terminal is located at amino acid 184 being in membrane cytoplasmic, was shown 3 regions the pore-lining is located between amino acids 184-204; 441-456; 621-637 and C-terminal is located at amino acid 637 domain that is being transported to the extracellular space passing through the pore in Fig. 3C, see more in supplemental material S1.

Fig. 2 The diagram produced by the MEMSAT-SVM algorithm available via the PSIPRED server. A cartoon of the transmembrane helix topology summarizing the linear coordinates for the helices and indicating where the protein is extra- and intercellular regions. (A) Hypothetical protein (ACA86597.1), (B) Arachidonate 15-lipoxygenase (ACA87192.1), (C) Arachidonate 15-lipoxygenase precursor (AEF01209.1), none of the three proteins present signal peptide.
The evolutionary tree of lipoxygenase was constructed utilizing sequences found in the NCBI protein databases for several lipoxygenase structures known including: barley L1, rice L2 soybean lipoxygenase L4, coral 8-lipoxygenase, human 5-lipoxygenase, human 15-lipoxygenase and porcine leukocyte 12-lipoxygenase catalytic domain and others, the evolutionary history was inferred using the Neighbor-Joining method [41]. The optimal tree with the sum of branch length = 7.17637587 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches [37]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree in Fig. 3. The evolutionary distances were computed using the p-distance method [42] and are in the units of the number of amino acid differences per site. The analysis involved 43 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 487 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [36].

The sequence in the tree was selected of verified that three lipoxygenases the S. woodyi were grouped with sequences of prokaryotes, however, are distinct.

The results for protein isoelectric point were pH 4.85 for Hypothetical protein (ACA86597.1), pH 5.00 for Arachidonate 15-lipoxygenase (ACA87192.1) and pH 4.73 for Arachidonate 15-lipoxygenase precursor (AEF01209.1). Protein GRAVY results were -0.481; -0.285 and -0.433, respectively. A previous study presented in 2012 [43] were described differences between the isoelectric points, candidate membrane-spanning segments and aliphatic index: GRAVY, the authors suggested that arachidonate 15-lipoxygenase precursor with special features.

We computationally predict catalytic kinase-specific phosphorylation sites for three LOX, the enzymes must be sufficiently specific and act only on a defined subset of cellular targets to ensure signal fidelity. Proteins can be phosphorylated at serine, threonine and tyrosine residues [44]. The hypothetical protein (ACA86597.1) showed 18 phosphorylated sites being: 6 Serine (S), 5 Threonine (T) and 7 Tyrosine(Y), the arachidonate 15-lipoxygenase (ACA87192.1) showed 18 phosphorylated sites being: 8 (S), 3 (T) and 7 (Y) and arachidonate 15-lipoxygenase precursor (AEF01209.1) showed 16 phosphorylated sites being: 5 (S), 5 (T) and 6 (Y). Catalytic Kinases Mitogen-activated protein kinase (MAPK), more was shared by the three LOX in Fig. 4, all sites see supplementary material (S2). MAPK cascades are among the most thoroughly studied of signal transduction systems and have been shown to participate in a diverse array of cellular programs, including cell differentiation, cell movement, cell division, and cell death [45].

Protein phosphorylation, which is an important mechanism in post-translational modification, affects essential cellular processes such as metabolism, cell signaling, differentiation and membrane transportation. According Rodrigues [26], using the alignment of the predicted amino acid sequence for identifies active site and the ligands are highly conserved.

4. Conclusions

Our results corroborate with the hypothesis the high unexplored that protein amino acid and sequences generated from hundreds of complete genomes can be computationally inferred via homology to other sequences.

The use of different bioinformatics tools supports the predictions of the three lipoxygenases the S. woodyi are distinct, this suggests a special feature that, our work in situ will be cloning the gene expression vector for later kinetic characterization and crystallization for better understanding of the function the lipoxygenases in prokaryotes.
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Fig. 3  Evolutionary relationships of taxa for lipoxygenases.

Fig. 4  The logo of the phosphorylated threonine site of catalytic kinases similar three LOX.

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