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Bioinformatics analysis of catalase gene from different plant species

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Abstract

Catalase is one of the most important enzymes to detoxify reactive oxygen species. In order to determination of catalase gene evolution, we analyzed the 74 different sequences from plants, animals, bacteria and fungal species. Our analyses indicated that in average, this gene has 496 amino acids, 56.806 KDa molecular mass and 6.93 theoretical isoelectric point. phylogeny analyses shown that this sequences divided into two main groups. Our expression analyses indicated catalase expression occurs in response to nitrogen, sulfate and phosphate starvation, abscisic acid, cadmium ion and light stimulus. The results of this study is helpful genetic engineering and production of tolerance plants against stressful conditions.

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Introduction

Oxygen molecule is required for aerobic organisms. Although, some derivatives of oxygen such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydrogen radicals (OH^-) are toxic (Foyer and Noctor, 2005; Modarresi *et al.*, 2012). Superoxide Radicals produce in many biological oxidation such as electron transportation during photosynthesis in chloroplasts, high intensity light, heat stress or toxic metals (Modarresi *et al.*, 2013). Also mitochondrial respiration can produce superoxide radicals. Superoxide anions can be converted to hydrogen peroxide naturally or by superoxide dismutase enzyme. Also hydrogen peroxide is part of the main components of signals in response to pathogen attack or environmental stresses such as cold, drought, salt and ozone stresses (Mittler, 2002). Hydrogen peroxide can be react with nucleic acids, lipids, proteins and other cellular components and it cause serious threats to the plant (Modarresi *et al.*, 2014b). Furthermore, hydrogen peroxide is powerful oxidizing agent and it can oxidize the side chains of amino acids such as methionine (Boonvisut *et al.*, 1982). In plant cells, there are several factors which destroy hydrogen peroxides (Modarresi *et al.*, 2014a). Main these include ascorbate peroxidase, peroxidase and catalase (Halliwell, 1992). Catalase (EC 1.11.1.6) is a tetrameric enzyme, which its subunits have 54-59 KDa weigh in plants. Each monomer contains one heme and NADPH. The NADPH binds with surface of each monomer by 12 amino acids and prevent oxidation the enzyme by H_2O_2 as its substrate. Heme has a porphyrin and an iron core. Catalase exists in Eukaryotes peroxisome which it converts hydrogen peroxide to water and oxygen. Catalase is very active molecule, so that a molecule can convert millions of hydrogen peroxide molecules to water and oxygen. Catalase enzymatic reaction is very fast and it reaction rate constants is K ≈ 107M-1Sec-1.

Catalase gene has an important role in plants defense systems against stressful conditions (Fig. 1), so in this study diversity and polymorphism of catalase gene among different species has been investigated by bioinformatics analyses.

Material and methods

Data collection

In this study, 74 catalase genes from different species, including 40 plants species, 3 bacterial species, 9 fungal species and 23 animal species were selected, and DNA and protein sequences was carried out from the NCBI database. It is noteworthy that all sequences were complete CDs.

Homology and Motif analyses

In this study, protein sequence motifs was identified by the MEME program (available at: http://www.meme.nbcr.net). These analyzes parameters was considered including: number of repeat: any, maximum motif number: 5, optimum motif width ≥15. Also motif profile of each protein is shown schematically. TMHMM transmembrane motifs was detected by using the TMHMM Server (http://www.cbs.dtu.dk/services/TMHMM) with the default settings of the software. Finally, catalase secondary structure and homology examined with Jalview program.

Phylogenetic analyses

Genetic evolution is a scientific process to determine the evolutionary history of a group of organisms or sequences. Evolutionary relationships of a group of sequences can be studied using by phylogenetic methods. For that purpose, alignment for functional domains of all catalase protein sequences done. MAFFT program used for finding L-INS-I as a most accurate MSA (Multiple protein Sequence Alignment) methods. Also Phylogenetic tree considered MEGA5 software Bootstrap analysis was performed with 100 replicates.

Intron- Exon analyses

Gene structure of the data files were analyzed by GFF (only genomic data used), also for graphical display were used Gene Structure Display Server of poking University.

Results and discussion

Our analyses with 74 different catalase genes amino acids indicated that number of amino acids

depending on the species were different and they ranged from 360 to 560 amino acids with average 496. Also PIR data (available at www.pir.georgetown.edu/pirwww/index.shtml)displayed that among 6669 different catalase amino acids sequences including Eukaryotae=992; Bacteria=5641; Archaea=18; Viruses=0; other=18, length range was Minimum=414; Maximum=591 and Average=498; with Standard Deviation=21. The difference in results is due to differences in the samples size used. Different in CDs length occurs by adding a variety of mutations (Insertion), deleted (Deletion) or alternative splicing and it is interpreted as polymorphism protein. Species which have greater CDs length diversity may have more phenotypic variation and it can be used in order to study the genetic basis associated with the corresponding phenotype. Both different in CDs length and terminal amino acid codons between species can serve as a good marker for studies of the analysis of association relationship. Most frequency in amino acids belong to Pro (7.2%) and Asp (6.8%) and lowest Cys (1.1%), respectively. To determine the structure and properties, rice (D86611) catalase sequences used as reference sequence, and utilized ProtParam program in Expasy tools package. This protein has 492 amino acids length, 56.806 KDa molecular mass and its theoretical pI is equal to 6.93. Also Total number of negatively charged residues (Asp + Glu): 60, Total number of positively charged residues (Arg + Lys): 58, Formula: $C_{2563}H_{3858}N_{720}O_{727}S_{13}$, Total number of atoms: 7881, Aliphatic index: 70.73, Grand average of hydropathicity (GRAVY): -0.583 and the instability index (II) is computed to be 34.56.

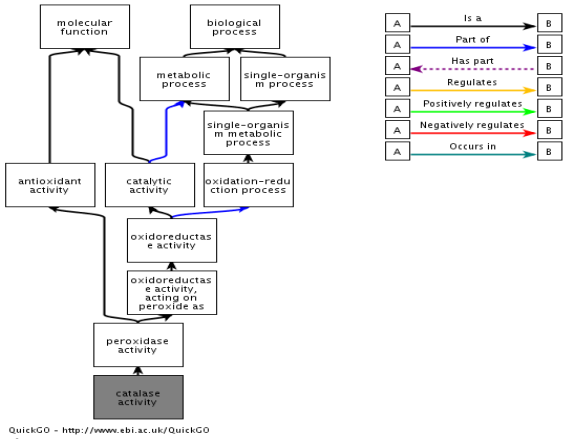

Fig. 1. Catalase activity diagram.

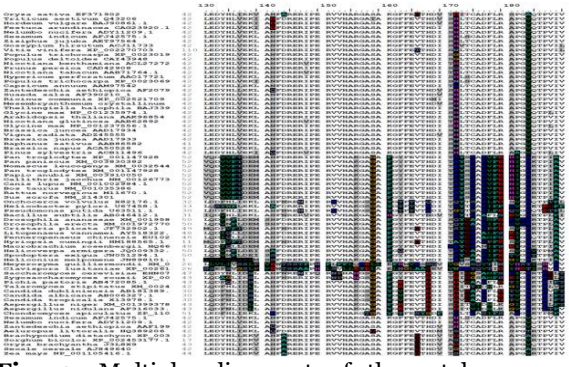

Fig. 2. Multiple alignment of the catalase gene between 74 different species.

Fig. 3. Phylogenetic tree showing the evolutionary relationship among catalase genes from different organisms.

Comparative genomic sequences using the degree of protection, identifies structural and functional sequences of the genome. Our blast search and multiple alignment on the amino acid level, indicated that *Oryza sativa* catalase gene was similar to other catalases in plants specially Gramineae family including *Oryza glaberrima* (XP_003558892.1 with 98 % identity), *Brachypodium distachyon* (ADY11209.1 with 95 % identity), *Hordeum vulgare* (BAJ90861.1 with 91 % identity), *Triticum aestivum* (Q43206.1 with 91 % identity), *Festuca arundinacea* (CAG23920.1 with 91 % identity), *Nelumbo nucifera* (ADY11209.1 with 88 % identity) etc (Fig. 2). Studied species phylogeny tree were divided into two main groups (Fig. 3). The first group includes the plant catalase genes and the second group including related genes in bacteria, fungi and animals, it represents the evolutionary distance between plants catalase gene in comparison with other types of organisms. The second group itself is divided into three separate sub-groups, including fungi, bacteria and animals.

Animals Sub-group divided into several sub-sub groups which mammals are the largest group. Also plant catalase divided into three main branches. It seems that doublication in bacterial ancestors catalase gene cause difference and create distinct branches of this gene (Klotz *et al.*, 1997).

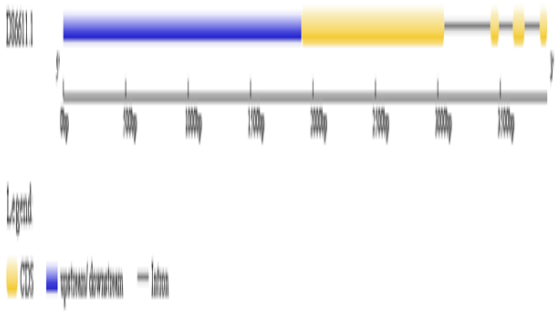

Fig. 4. Intron- Exon analyses by Gene Structure Display Server of poking University. This gene has four Exons and three introns.

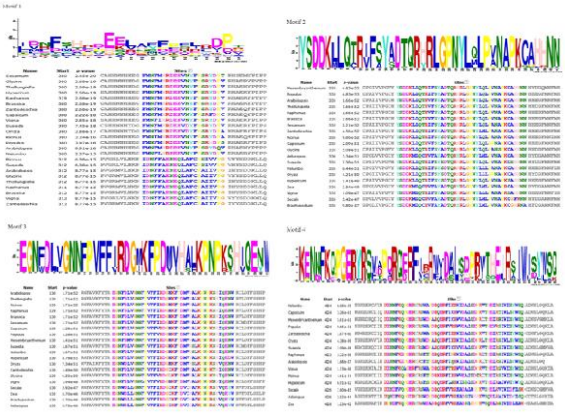

Fig. 5. Sequence-specific MEME motifs for Catalase proteins, Motif 1: [IFL][DV]NFSHxDE[EK][LV]A[FY]FPFLR DPT, Motif 2: YSDDK[LM]LQ[TC]R[IV]F[SA]Y[AS]DTQR[HY]R LGPNYL[QM]LP[VA]NAPKCAHHNN, Motif 3: EGN[FW]DL[VL]GNNFPVFF[IV]RDG[MI]KFPD[MV][VI]H A[LF]KPNPKSH[IV]QE[NY]W and Motif 4: KEN[ND]FKQPGERYRS[WF][ADT]P[DA]RQ[ED]RF [IV][RCK] R[WF][IVA]D [AS]LS[DH]P[RK] [VI] [TS]HE[IL]RSIWI[SD]Y[WL]SQ.

STRING data analyses indicated that catalase expression occurs in almost all aerobically respiring organisms to protect them from the toxic effects of hydrogen peroxide. Also it is cellular response to nitrogen starvation, phosphate starvation, sulfate starvation, abscisic acid, cadmium ion and light stimulus. Also indicated that catalase has functional partners with superoxide dismutase (with score 0.963), glutathione reductase (0.918), peroxisomal multifunctional enzyme type 2 (0.871) and immutans protein (0.839). Uniprot data bank data shown that catalase has active binding sites in positions 65, 138 and metal (iron) binding site in position 348. Prosite database analyses indicated that this gene has two consensus pattern including Catalase proximal heme-ligand signature (R-[LIVMFSTAN]-F-[GASTNP]-Y-x-D-[AST]-[QEH]) and Catalase proximal active site signature ([IF]-x-[RH]-x(4)-[EQ]-R-x(2)-H-x(2)-[GAS]-[GASTFY]-[GAST]). Also this program analyses shown that this gene has three hits in positions 14-492, 54-70 and 344-352. Intron and Exon analyses indicated that this gene has four Exons and three introns. The sizes of the exons, are generally conserved among plant species (Iwamoto *et al.*, 1998). Domain and protein motifs analysis in different species is as a sign that aware of the existence of a specific sequence in the genome. This marker can also indicate genetic differentiation at individual level, gender, family or higher levels (Fig. 5).

Conclusion

To understand that how genetic difference leads to diversity in phenotype, require the use of bioinformatics methods. The results of this study explore proteins key positions and functional sections such as catalytic residues or ligand binding sites. This data can helpful for genetic engineering and transformations.

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