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Plant Gene Register

Molecular Cloning and Nucleotide Sequence of a cDNA Encoding Catalase from Tomato[1]

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Catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) is a peroxysomal enzyme involved in the degradation of H_2O_2 in aerobic cells. In plants, catalase plays an important role in the scavenging of H_2O_2 released during the β-oxidation of fatty acids, particularly in the cotyledons of germinating seedlings. In addition, the enzyme is an important component of the system involved in detoxification of reactive oxygen species that increase following biotic and abiotic stresses (8). In this system, superoxide dismutase reacts with superoxide radicals, yielding H_2O_2, which is subsequently converted to water and oxygen. Catalase is a tetrameric enzyme that contains a heme group buried in a hydrophobic pocket as the active domain (1, 4) and has been extensively characterized. In higher plants, catalase cDNAs have been reported from maize (6), pea (3), sweet potato (7), and cotton (5). Native catalase is often composed of subunits that vary in mol wt and charge. The genetic basis of this heterogeneity has been extensively studied in maize, in which catalase has been shown to be encoded by three different structural genes that exhibit differential patterns of expression (6).

Tomato catalase was partially purified and characterized from pericarp tissue of green fruit (2). The purified holoenzyme has a molecular mass of 225 kD with four subunits of 55 kD. Here we report the isolation of a full-length cDNA clone that encodes a tomato catalase subunit. Our strategy was to use the polymerase chain reaction to isolate a catalase cDNA. Two oligonucleotide primers were synthesized based on the sequence of sweet potato catalase (7). The sense primer was 5′-TTCTTTGAGGTCACTCATG-3′, and the antisense primer was 5′-CTTGAAGTTGTTCTCCTTC-3′. Total RNA extracted from mature green tomato fruit was used as a template for cDNA synthesis and the resulting cDNA used in a polymerase chain reaction with the synthesized primers. The resulting product was of expected size (1068 bp) and was subcloned into the *Sma*I site of pGEM7Zf(+) to serve as a template for double-stranded sequencing. Preliminary sequence analysis revealed extensive homology of the amplified tomato cDNA with sweet potato catalase. The partial cDNA clone was subsequently used to screen a cDNA library prepared in λZAP from polyadenylated RNA isolated from young tomato leaves. Several putative catalase cDNAs were identified, and the longest insert (pTOMCAT1) was sequenced. The sequence of pTOMCAT1 is 1822 bp and contains an open reading frame of 492 amino acids (Fig. 1). The nucleotide sequence of pTOMCAT1 is 73, 69, 67, and 66% identical with catalases from cottonseed (subunit 1), pea, sweet potato, and maize (CAT1), respectively (3, 5–7). The predicted protein of pTOMCAT1 shares extensive homology with other catalases (Table I). Of the 10 amino acids reported to be involved in catalytic activity (His^{74}, Ser^{113}, and Asn^{147}) and direct association with the heme group (Val^{73}, Arg^{111}, Tyr^{114}, Phe^{150}, Pro^{335}, Arg^{353}, and Tyr^{357}), all are conserved in

Table I. *Characteristics of a Tomato Catalase Subunit cDNA (pTOMCAT1)*

Organism:
Tomato (*Lycopersicon esculentum* cv Rutgers).
Function:
Encodes a subunit of the tetrameric enzyme catalase (EC 1.11.1.6), which catalyzes the conversion of H_2O_2 into water and oxygen.
Clone type, Designation:
cDNA, full-length; pTOMCAT1.
Source:
cDNA library in λZAP vector constructed using polyadenylated RNA isolated from young tomato leaves.
Method of Identification:
Library was screened with a 1068-bp tomato polymerase chain reaction-derived catalase cDNA (Fig. 1).
Sequencing Strategy:
Double-stranded plasmid-based sequencing of exonuclease III deletions was used to sequence both strands.
Expression Characteristics:
Transcript of approximately 1900 nucleotides; high steady-state mRNA concentration in stem and moderate levels in leaf and fruit tissue. Low, but detectable, transcript levels in roots.
Structural Features of Deduced Protein:
492 amino acids (M_r 56,505); isoelectric point of 7.01; 78 and 74% identical with predicted catalase subunits from cottonseed (5) and maize CAT1 (6), respectively.
Antibodies:
None available.
Subcellular Location:
Not determined.
GenBank Accession No.:
M93719

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   1  CTCTCTTCAATTCTTCTTTCATTTCCATCACCATGGATCCCTCTAAGTATCGCCCATCAA
                                        M  D  P  S  K  Y  R  P  S  S   10
  61  GCGCATACGACACCCCTTTCTTGACAACAAATGCTGGTGGTCCTGTGTACAACAATGTTT
        A  Y  D  T  P  F  L  T  T  N  A  G  G  P  V  Y  N  N  V  S   30
 121  CTTCCTTGACTGTTGGACCTAGAGGGCCTGTTCTGCTTGAGGATTACTATCTAATTGAGA
        S  L  T  V  G  P  R  G  P  V  L  L  E  D  Y  Y  L  I  E  K   50
 181  AGCTCGCGACATTTGATCGCGAGAAGATACCTGAACGTGTTGTTCATGCTAGAGGTGCTA
        L  A  T  F  D  R  E  K  I  P  E  R  V  V  H  A  R  G  A  S   70
 241  GTGCTAAGGGATTCTTTGAAGTTACTCATGACATTTCTCATCTTACTTGTGCTGATTTTC
        A  K  G  F  F  E  V  T  H  D  I  S  H  L  T  C  A  D  F  L   90
 301  TCCGAGCTCCTGGCGCTCAAACGCCTGTTATTTGTCGATTCTCTACTGTTGTCCATGAAC
        R  A  P  G  A  Q  T  P  V  I  C  R  F  S  T  V  V  H  E  R  110
 361  GTGGAAGCCCCGAGTCTATCAGGGACATTCGTGGTTTTGCTGTCAAGTTCTACACCAGAG
        G  S  P  E  S  I  R  D  I  R  G  F  A  V  K  F  Y  T  R  E  130
 421  AGGGTAACTTTGATCTTGTTGGAAACAATGTCCCCGTGTTCTTTAATCGTGATGCTAAGT
        G  N  F  D  L  V  G  N  N  V  P  V  F  F  N  R  D  A  K  S  150
 481  CGTTCCCTGACACGATTCGTGCATTGAAACCAAATCCAAAGTCACACATTCAGGAGAACT
        F  P  D  T  I  R  A  L  K  P  N  P  K  S  H  I  Q  E  N  W  170
 541  GGAGGATACTTGATTTCTTCTCGTTCCTTCCTGAGAGTTTGCATACATTCGCCTTCTTCT
        R  I  L  D  F  F  S  F  L  P  E  S  L  H  T  F  A  F  F  Y  190
 601  ACGATGATGTTTGTCTCCCAACGGATTACAGACACATGGAAGGTTTTGGCGTTCACGCGT
        D  D  V  C  L  P  T  D  Y  R  H  M  E  G  F  G  V  H  A  Y  210
 661  ATCAATTGATTAACAAAGAGGGGAAAGCACATTATGTGAAGTTCCACTGGAAGCCAACTT
        Q  L  I  N  K  E  G  K  A  H  Y  V  K  F  H  W  K  P  T  C  230
 721  GTGGTGTGAAATGTATGTCTGAGGAAGAAGCTATTAGAGTCGGTGGTACTAATCATAGCC
        G  V  K  C  M  S  E  E  E  A  I  R  V  G  G  T  N  H  S  H  250
 781  ACGCGACCAAGGATCTTTACGATTCAATTGCTGCTGGAAACTATCCTGAGTGGAAGCTTT
        A  T  K  D  L  Y  D  S  I  A  A  G  N  Y  P  E  W  K  L  F  270
 841  TTATCCAAACAATGGACCCCGAGGATGTAGACAAGTTCGATTTTGATCCTCTGGATGTAA
        I  Q  T  M  D  P  E  D  V  D  K  F  D  F  D  P  L  D  V  T  290
 901  CCAAGACATGGCCTGAGGATCTCTTGCCGTTGATCCCAGTTGGTCGATTGGTGTTGAACA
        K  T  W  P  E  D  L  L  P  L  I  P  V  G  R  L  V  L  N  R  310
 961  GGAACATTGATAACTTCTTCGCAGAGAATGAACAACTCGCGTTTAACCCTGGACATATTG
        N  I  D  N  F  F  A  E  N  E  Q  L  A  F  N  P  G  H  I  V  330
1021  TCCCTGGTATTTACTATTCCGAGGATAAGCTTCTCCAGACTAGGATATTCGCGTATGCTG
        P  G  I  Y  Y  S  E  D  K  L  L  Q  T  R  I  F  A  Y  A  D  350
1081  ATACTCAGAGACACCGTATTGGACCAAACTATATGCAGCTCCCAGTTAATGCTCCCAAGT
        T  Q  R  H  R  I  G  P  N  Y  M  Q  L  P  V  N  A  P  K  C  370
1141  GTGGTCATCACAACAATCATCGCGATGGTGCTATGAACATGACACATCGCGATGAAGAGG
        G  H  H  N  N  H  R  D  G  A  M  N  M  T  H  R  D  E  E  V  390
1201  TGGATTATTTGCCCTCGAGGTTTGATCCTTGTCGTCCTGCTGAGCAGTACCCGATTCCTT
        D  Y  L  P  S  R  F  D  P  C  R  P  A  E  Q  Y  P  I  P  S  410
1261  CTTGTGTCTTGAATGGAAGGCGTACAAATTGTGTCATTCCGAAAGAAAACAACTTCAAAC
        C  V  L  N  G  R  R  T  N  C  V  I  P  K  E  N  N  F  K  Q  430
1321  AGGCAGGGGAGAGGTACAGATCATGGGAACCTGACAGGCAAGACAGATACATCAACAAAT
        A  G  E  R  Y  R  S  W  E  P  D  R  Q  D  R  Y  I  N  K  W  450
1381  GGGTTGAGTCTTTATCCGATCCACGAGTCACTCATGAGATTCGCAGCATATGGATATCAT
        V  E  S  L  S  D  P  R  V  T  H  E  I  R  S  I  W  I  S  Y  470
1441  ACTTGTCTCAGGCTGACAAGTCCTGTGGTCAGAAGGTCGCTTCTCGTCTCACTGTGAAGC
        L  S  Q  A  D  K  S  C  G  Q  K  V  A  S  R  L  T  V  K  P  490
1501  CTACAATGTGAAAAATCAATGAAAATAGTTGAAATGGTTTCAAGCTGCAAATGTTGAAGG
        T  M                                                        492
1561  ACTAATGCAAAAAAAACGTCCGCGTTGTGCTATAAACTGTACTTCTTTTTCAATCGTAATG
1621  TTGTATTTTGTATCGAATTTCGATGTCTTGTGTTTTTACTATAATGATGTTGGAACCTGA
1681  ATAAGTTCACAGTTGTATGTTCAATGTTTCACTTTCTAAAGTTATGTAATTATGTTGAGT
1741  TCTTGTTCACTTTGGTGCTTGAAGAACACACTCTCAATTTCAATAATATCACTTTCATTT
1801  CAGTAAAAAAAAAAAAAAAAA
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Figure 1. Nucleotide and deduced amino acid sequence of tomato pTOMCAT1.

the predicted protein of pTOMCAT1 (1, 4). Therefore, we conclude that pTOMCAT1 encodes a subunit of tomato catalase.

LITERATURE CITED

1. **Fita I, Rossmann MG** (1985) The active center of catalase. J Mol Biol **185:** 21–37
2. **Inamine GS, Baker JE** (1989) A catalase from tomato fruit. Phytochemistry **28:** 345–348
3. **Isin SH, Allen RD** (1991) Isolation and characterization of pea catalase cDNA. Plant Mol Biol **17:** 1263–1265
4. **Murthy MRN, Reid TJ III, Sicignano A, Tanaka N, Rossmann MG** (1981) Structure of beef liver catalase. J Mol Biol **152:** 465–499
5. **Ni W, Turley RB, Trelease RN** (1990) Characterization of a cDNA encoding cottonseed catalase. Biochim Biophys Acta **1049:** 219–222
6. **Redinbaugh MG, Wadsworth GJ, Scandalios JG** (1988) Characterization of catalase transcripts and their differential expression in maize. Biochim Biophys Acta **951:** 104–116
7. **Sakago S, Nakamura K, Ashai T** (1987) Molecular cloning and nucleotide sequence of a full-length cDNA for sweet potato catalase mRNA. Eur J Biochem **165:** 437–442
8. **Scandalios JG** (1990) Response of plant antioxidant defense genes to environmental stress. Adv Genet **28:** 1–41