

Plant Gene Register

Nucleotide Sequence of the *Nicotiana tabacum* cv Xanthi Gene Encoding 1-Aminocyclopropane-1-Carboxylate Synthase

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Ethylene is a plant hormone that influences many aspects of plant growth and development (10). The biosynthetic pathway of ethylene production in higher plants is well defined. The rate-limiting step in this pathway is the reaction catalyzed by ACC¹ synthase (*S*-adenosyl-L-methionine methyl-thioadenosine-lyase, EC 4.4.1.4), which converts *S*-adenosyl-L-methionine to ACC.

Tobacco (*Nicotiana tabacum*) has been used extensively to study the importance of ethylene in plant defense responses. It has been known for some time that ACC synthase activity is enhanced in tobacco leaves undergoing a hypersensitive response to pathogen attack (3). An EIX produced by the fungus *Trichoderma viride* elicits ethylene biosynthesis in *N. tabacum* cv Xanthi leaf tissue (2). When EIX is applied to the petiole of a detached tobacco leaf, it moves throughout the leaf and, consequently, induces ethylene production and tissue necrosis (1). If the tobacco leaves are first incubated with ethylene, the response to EIX treatment is greatly enhanced. mRNA was prepared from *N. tabacum* cv Xanthi leaves preincubated with ethylene and then challenged with EIX to induce copious ethylene biosynthesis. We amplified from the above tobacco mRNA an ACC synthase gene fragment (450 bp) using the polymerase chain reaction and two degenerate primers based on the noncoding strand of the tomato ACC synthase gene (9). We used this fragment to isolate a cDNA clone (pTACC-13) of an ACC synthase gene. This clone was isolated from a cDNA library prepared from *N. tabacum* cv Xanthi leaves treated as stated above. The clone, pACC-13, was then introduced into *Escherichia coli* (XL1-Blue, Stratagene²), and accumulation of low levels of ACC was observed (data not shown), indicating expression of a functional ACC synthase gene (5).

We report here the ACC synthase gene sequence of *N. tabacum* cv Xanthi (Table I, Fig. 1). The sequence includes the 1473-bp coding region of the *N. tabacum* gene and 153 bp

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1 TCTAGAAGTAGTGGATCCCCGGGCTGCAGGAATTCGGCACGAGAACCCAGCTTTTC
61 TATAGACAGAAGTAGTAGTCACATATATATATATATTTGATCCCAATTTTCATCTTCCTCC
M G F E N E K M S
121 AATATTTTTTTCACCTTAAACTCGTTAAGAAAATGGGATTTGAGAAATGAGAGAACAGC 9
S I L S R L A T N E E L G E N S P T F D
181 TCAATCTTATCTAAGCTCCCTAATGAAAGACTAGGTGAAACTCCGCCATATTTTGGAT 29
G W F A Y D N D P F H P L K N F N G V I
241 GGAATGGAAGCATAGATAAGCATCCCTTTCACCTCTGAEAAACCTTAATGGTGTATC 49
Q M G L A E N Q L C F D L I E E W I K R
301 CAAATGGGTCTTGTGAAATCAGCTTTGTTGACTTATGAGAAATGGATTAAGAGA 69
N P N A S I C T T E G I K S F R A I A N
361 AATCCAAAGCTTCAATTTGCACACAGAAAGAAATCAATCTTTCAGGGCCATGGCCAAC 89
F Q D Y H G L P E F R S A I A K F M E K
421 TTTCAAGATTATCAAGGCTACCTGAATTCAGAAAGGCTATTTGCGAAATTTATGGAGAAA 109
T R G G R V T F D P E R V V M A G G A T
481 ACAAGAGGGGAGGGTTACGTTTGCATCCAGAGAGTAGTTATGGCTGGGTGGCAACT 129
C A N E T I F C L A D T G D A F L V P
541 GGAGCCAAAGCAATTAATTTGTTGGGTGATACAGGCGATGCAATTCCTAGTACT 149
S P Y Y P A F N R D L R W R T G V Q L I
601 TCACCACTATCCAGCAATTAACCGGACTTAAGATGGAAGACTGGAGTACAACTCAIT 169
P I P C D S S N N F Q I T T K A V R E A
661 CCAATTCCTTGGCAGTTCACCAACTTCCAAATCACTAAGAAAGCTGTGAGAGAGCA 189
Y E N A Q K S N I K V K G L I L T N P S
721 TATGAAAATGCCAGAAATCAATCAAACTCAAAAGCTGATTTGACCAACCCATCA 209
N P L G T T L D R D T L K N L L T F T N
781 AATCCATTAGCCCACTTTGGACAGACACACTGAAAAAATCTCTGACCTCCACCAAC 229
Q H N I H L V C D E I Y A A T V F N T F
841 CAACATAACATCCACTCGTTGGGAGGAAATTTACGGCGCAACGCTTTTAAATACCT 249
C F V S I A H E L D D E S F E C N R D I
901 CAATTCCTGACGATTCCTGAAATTCGACGACGAAAGCTGATTTGCAACAAAGATTTG 269
I H I V Y S L S K R D M G L P G F R V G I
961 GTTCATATCGTATACGCTTTCAAAAGACAATGGCTTACAGGATTCAGAGTCGGAATC 309
V Y S F N D A V V N C A R K M S S F G L
1021 GGTATTCATCCAGATGCCCTGCTTAATTTGAGTCAAAAATTCAGGTTTGGTTTA 329
V S T Q T Q Y L L A E M L S D E R F V S
1081 GTTCCACTCAACACAGTATTTGCTGCTGAGATTTATCCGAGCAGAAAGATTCGCTCA 349
N F L T E S S K R L A K R H H F T N G
1141 AATTTCTAATCGAAAGCTGGAAGATTAGCTAAGAACACAACTTCACTAAGCGA 369
L E E V G I K C L R S N A G L F C W M D
1201 CTGGAAGAGTTGGAATTAATGCTTGAAGAGCAATTCGAGACTTTTCGTTGATGGAT 389
L R F L L K E S T F D S E M S L W R V I
1261 TTTCCGACCTTTGAAAGGTCACCTTTCGACTCAGAAATGTCATTTAGGAGAGTGAAT 409
I N D V I L N V S P Q E F G
1321 AATAATGACGTGAAGCTTAAAGTCTCCGGGACTTCTCATTTGATTCAGAGCCAGGA 429
F F F R V C F A N M D D E T V D I A L A R
1381 TTTTTCGAGTTGTTTCGCTAACATGGAATGAAAGACTGGAATTTGCAATGAGGAGG 449
I R S F V G V K K S G D E S T P I L M E
1441 ATTCCGAGTTTTCGGGTGTTAAGAAAATGGAGATGAATGCACTCCAAATTAATGGAG 469
K K Q Q W K K N N L R L S F S K R M Y D
1501 AAGAAGCAGCAATGGAAAAGAACAACTAGACTTATTTCTCGAAAAGAAATGACGAT 489
E S V N L S P L S S P I P H S P L V R A
1561 GAAAGCTTAATTTGTCACCACTTTCATCTCTATCCCTCACTCCCACTGTTGAGCT 491
R T
1621 AGAAGCTTAAAGCGAGGAATTTGTTTTATTTTTATTTTATATAAAGAAGATAAAGG
1681 AAAAAAGAAAGCAATGTAGTAGAATGATCTTTCAGAAATTAATTAATTTGTTATATACT
1741 AAAAAAAAAA
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Figure 1. Nucleotide sequence of the *N. tabacum* cv Xanthi ACC synthase gene. The deduced amino acid sequence is shown in single-letter codes. Active site is underlined.

upstream and 122 bp downstream of the open reading frame. The DNA coding sequence is 62 to 86% similar to that of other plant ACC synthase nucleotide sequences. The deduced amino acid sequence (also shown in Fig. 1) is similar to the ACC synthase protein from other plants as follows: 88.8% with tomato ACC synthase 1 (9), 71.0% with tomato ACC synthase 2 (7), 66.1% with zucchini (4), and 64.4% with carnation (6). The "consensus" active site in tomato ACC

¹ Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; EIX, ethylene biosynthesis-inducing xylanase.

² Mention of trademark, proprietary product, or vendor does not constitute a guarantee of warranty by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products or vendors that may also be suitable.

Table I. Characteristics of ACC Synthase cDNA from Tobacco

Organism:	<i>Nicotiana tabacum</i> L. (cv Xanthi).
Gene Product, Pathway:	ACC synthase, ethylene biosynthesis.
Techniques:	Polymerase chain reaction of 450-bp tobacco ACC synthase gene fragment using degenerate primers TTTG[TC][TC]TNGCNGA[TC]CCNGGNGA[TC]GC corresponding to residues 560 to 584 and A[AG]GA[TC]ATGGGN[TC]TNCCNGGNTT[TC][CA]G corresponding to residues 986 to 1010 of the noncoding strand of tomato ACC synthase (9). Construction of cDNA library in λ Uni-Zap (Stratagene) from mRNA isolated from EIX-treated tobacco leaves. Isolation of clone pTACC-13 from library using the above polymerase chain reaction fragment as a probe. Sequencing by the dideoxynucleotide chain termination method (8). Both strands were sequenced, and each position was determined at least three times.
Method of Identification:	Similarity of deduced amino acid sequence to previously characterized ACC synthase clone (9) from tomato (88.8%). Accumulation of ACC in <i>E. coli</i> transformed with pTACC-13.
Structural Features of Gene:	Open reading frame of 1473 bp with untranslated regions of 153 bp upstream (5') and 122 bp downstream (3').
Codon Usage:	Codons not used are CGT (R), CGC (R), and CCC (P).
(G+C) Content:	40.3% in protein-coding region.
Structural Features of Protein:	Translated region yields a protein of 491 amino acids. Possesses the consensus active site of tomato ACC synthase 1 (9) and zucchini ACC synthase (4).
Antibodies:	Not available.
EMBL Accession No.:	X65982.

synthase 1 and in zucchini genes is also conserved in the *N. tabacum* sequence.

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