

cDNA Cloning of Caffeine (Theobromine) Synthase from Coffee (*Coffea arabica* L.)

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SUMMARY

Caffeine synthase (CS), the *S*-adenosylmethionine(SAM)-dependent *N*-methyltransferase is a key enzyme for caffeine biosynthesis, since this enzyme is involved in the last two-methylation steps. The CS protein was purified from young tea leaves and subsequently the CS cDNA, named TCS, was isolated (Kato et al., 2000) To isolate cDNA clones encoding CS from coffee, we established a cDNA library from young coffee leaves and carried out screening of this. Oligonucleotides corresponding to the consensus sequences, which form the putative SAM binding region of TCS, were synthesized and used for RT-PCR as primers. The resulting PCR products were used to screen approximately 5.0×10^5 plaques from a coffee cDNA library. Finally, independent five cDNA clones, termed CCS clones, were isolated and analyzed those sequences. The predicted amino acid sequences of the CCS clones are over 80% identical among those of the clones and share almost 40% identity with those of TCS and salicylic acid carboxyl methyltransferase from *Clarkia breweri*, respectively. The molecular masses of these proteins were almost the same (approximately 43 kDa). These values agree well with that of TCS protein (41kDa). We have established expression systems of CCS cDNAs in *E. coli*. Some transformants have produced recombinant proteins. Theobromine producing activity was confirmed in some kinds of recombinant proteins.