

## Isolation and Characterization of cDNA Clones Involved in Catechin Biosynthesis of *Camellia sinensis*

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### Objectives

Catechins are powerful antioxidants with beneficial effects on human cardiac health and immunity. Since tea (*Camellia sinensis*) is a commercially important crop that is valued for its catechins, we isolated genes involved in the catechin biosynthesis from the subtractive cDNA library specific for young leaves stage. In this study, to understand the molecular regulation of catechin biosynthesis, a full-length of cDNA encoding Catechin synthase (CS) was identified and cloned, and its enzymatic characteristics are being studied in detail.

### Materials and Methods

1. Plant materials : young and old leaves of tea plant
2. Methods : The genes involved in the catechin biosynthesis were isolated from the subtractive cDNA library enriched for differentially expressed cDNA (young and mature tea leaves as tester and driver, respectively). A full length cDNA involved in catechin biosynthesis was isolated using RACE cDNA Amplification kit (Clontech). The cDNAs were ligated into pET-32a(+) vector (Novagen) and transformed into *E. coli* BL21 (DE3).

### Results and Discussion

According to the results of HPLC analysis, the young tea leaves accumulate much higher amounts of catechins than mature leaves do (1.72-, 2.29-, and -fold for EgC, EgCg, and Ecg). And the results encouraged us to isolate the genes involved in catechin biosynthesis from young leaves.

EST clones for a catechin synthase (CS), key enzyme for catechins biosynthesis, were isolated from the subtracted cDNA library. RACE PCR using the cDNA library led to the identification of a full-length about 1.04 kb cDNA with 70.2% identity of amino acid sequence to the key enzyme of catechin biosynthesis in *legume Medicago truncatula* and 58.4% of *Arabidopsis thaliana*. The recombinant CS was highly expressed in *E. coli*. To functionally characterize CS, the *E. coli* lysates will be assayed with the substrates (Cyanidin chloride, Pelargonidin chloride, Delphinidin chloride, etc).