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Cloning of a caffeoyl-coenzyme A *O*-methyltransferase from *Camellia sinensis* and analysis of its catalytic activity

Key words:
Tea (*Camellia sinensis*), *O*-methyltransferase, *CsCCoAOMT*, prokaryotic expression, catalytic activity, methylated EGCG

Research purpose

Epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3''Me) present in leaves of *Camellia sinensis* (*C. sinensis*), has many beneficial biological activities for human health. In the following aspects, it showed much stronger activities than EGCG.

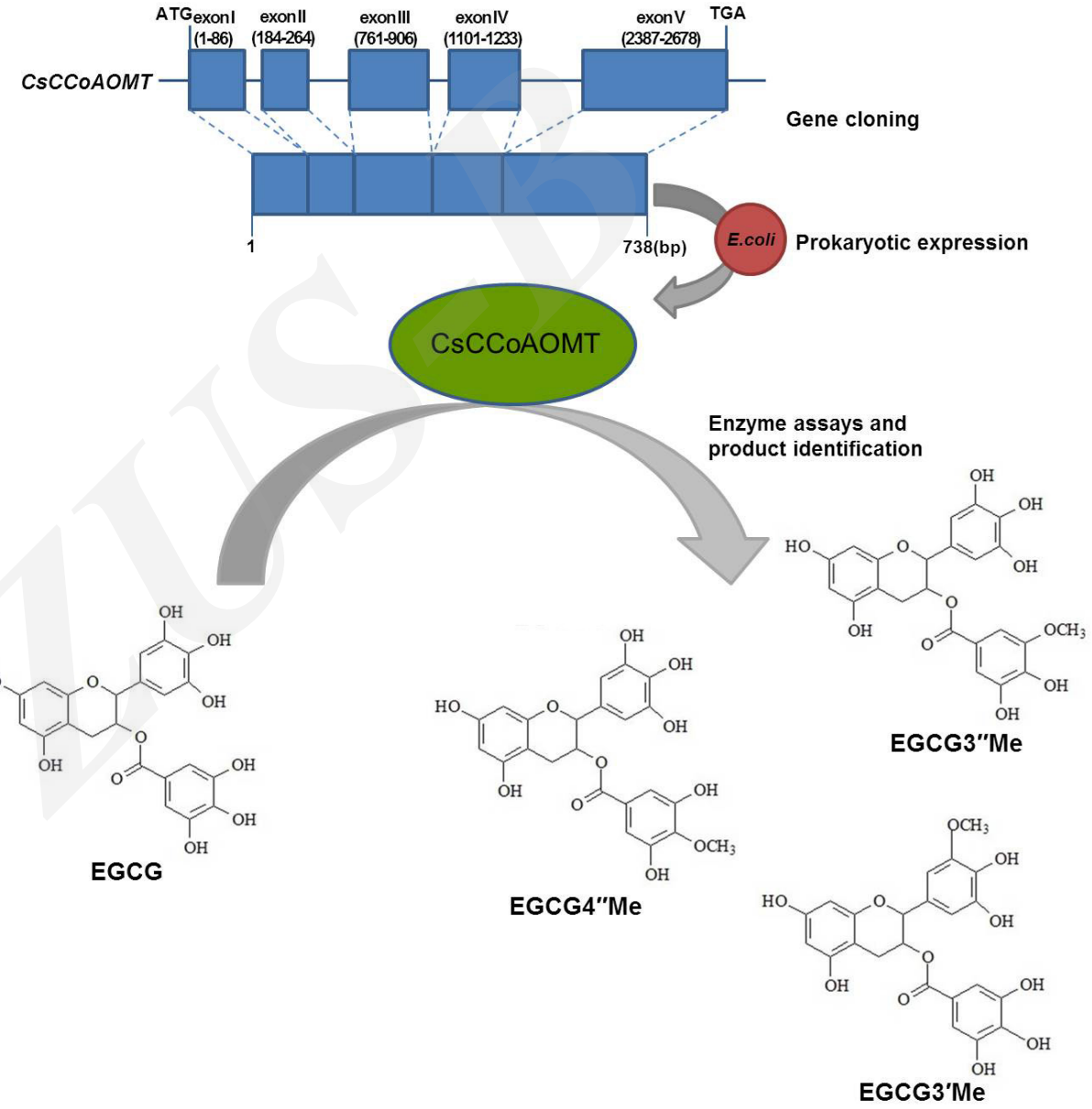
- **anti-allergic (Maeda-Yamamoto *et al.*, 2004; 2007)**
- **anti-hypertensive (Kurita *et al*., 2010)**
- **absorbed more easily (Sano *et al*., 2000)**

However, EGCG3''Me occurs naturally in tea leaves in extremely limited quantities. Finding an enzyme from *C. sinensis* to catalyze the synthesis of EGCG3''Me is an alternative method to make up for the scarcity of EGCG3''Me in natural situations.

Main contents and results

In the present study, a cDNA encoding region and genomic DNA of the *CCoAOMT* gene were isolated from *C. sinensis* (designated *CsCCoAOMT*).

Enzymatic reaction products were analyzed by HPLC-QTOF-MS, and three methylated products (EGCG4″ Me, EGCG3″Me and EGCG3′Me) were detected.

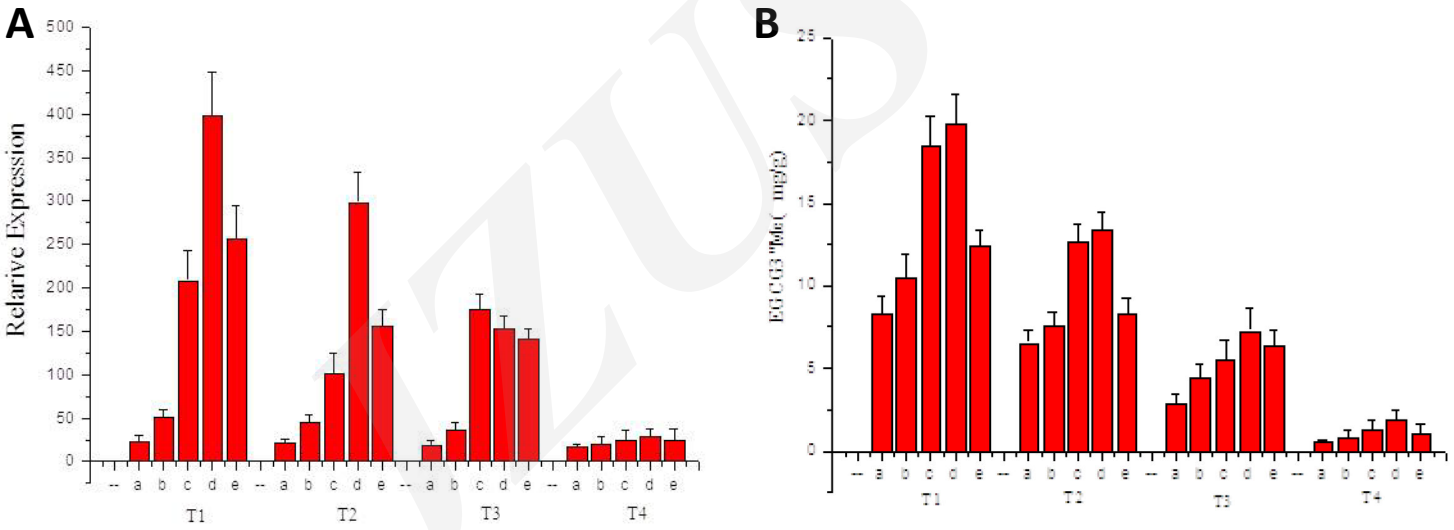


Main contents and results

Four tea cultivars (T1, T2, T3, and T4) that differing greatly in EGCG3”Me contents were selected to analyze the relative gene expression by RT-PCR. Two tendency were discovered.

1) In the four tea cultivars, the relative gene expression levels showed a tendency to increase consistent with increasing EGCG3”Me content in fresh leaves (Fig. A-e and B-e).

2)There was a positive correlation between leaf maturity and EGCG3”Me content detected by HPLC (Fig. B a–d), and with expression of *CsCCoAOMT* (Fig. A a–d).



a–d, different stages of leaf maturity: a, the first leaf and a bud; b, the second leaf; c, the third leaf; d, the fourth leaf; e: the mixed leaves above. (A) Relative expression of *CsCCoAOMT* quantified by RT-PCR. (B) EGCG3’’Me content in leaves detected by HPLC.

Conclusion

In this paper, the cDNA and genomic DNA of *CsCCoAOMT* gene were cloned. Sequence analysis of *CsCCoAOMT* showed that the ORF contained 738 bp, which encoded a polypeptide of 245 amino acid residues. The genomic DNA of *CsCCoAOMT* was 2678 bp in length and comprised five exons and four introns.

By enzyme assays and products identification, it is indicated that CsCCoAOMT catalyzes the synthesis of methylated EGCGs (EGCG4'' Me, EGCG3''Me and EGCG3'Me). Therefore, we believe that the present study contributes effectively to the preparation of methylated EGCG.

Besides, the relative gene expression of four tea cultivars were analyzed by RT-PCR. The results showed that there was a positive correlation between leaf maturity and EGCG3''Me content in fresh leaves. It was also considered to be an evidence for supporting that CsCCoAOMT was associated with biosynthesis of methylated catechin in *C. sinensis.*