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Isolation and characterization of linalool UDP-Glc glycosyltransferases from *Coffea arabica*

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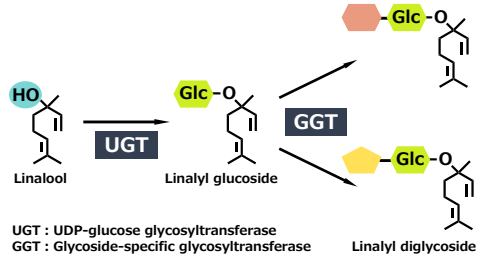
<RATIONALE> The aroma is principal to decide the value of coffee beans. Linalool, which is one of the volatile terpene compounds, is constitutive in the coffee aroma. Since terpenoids are generally accumulated as glycosides in plants, the glycosylation is catalyzed by the UDP-glucose glycosyltransferases (UGTs). To reveal the mechanism of accumulation of those volatile compounds in coffee, the functional analysis of the UGT genes that work terpenoids such as linalool was performed. We expect to develop coffees with a rich aroma using this information. In this study, we identified those UGT genes from *Coffea arabica* and analyzed them with recombinant enzymes. Here, we report the isolation and characterization of UGT genes that have activity towards linalool.

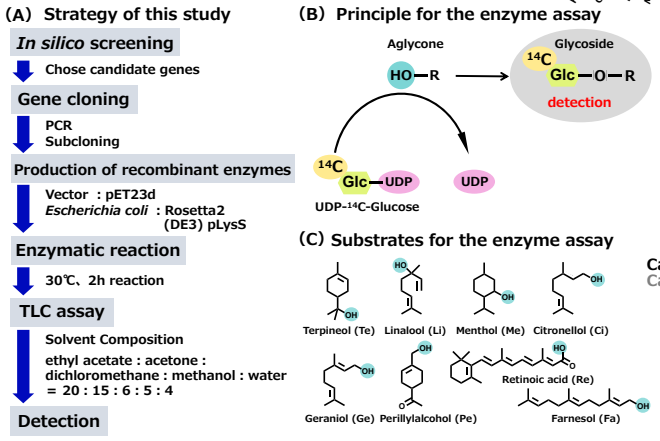
▶Fig1. Glycosylation of linalool

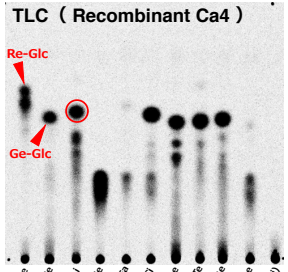
In coffee, linalool accumulates as a non-volatile glycoside catalyzed by UGT and GGT. UGT85K11, which catalyzes the glycosylation of geraniol and linalool, was isolated from *Camellia sinensis* in a previous study (Shoji et ai.,(2015)). In this study, we chose six genes based on the sequence of UGT85K11 to identify and functional analysis.



▶Fig2. Methods of this study

(A) We performed *in silico* screening of the genes from *C. arabica* based on the nucleotide sequence of *UGT85K11*. Then, six genes were candidates to identify and analyze(termed *Ca4*, *Ca5*, *Ca10*, *Ca14*, *Ca15* and *Ca20*) . To prepare the cDNAs, total RNAs were extracted from young leaves and flower buds. Those six genes were amplified by RT-PCR using specific primers and those cDNAs as a template. After sequence analysis, those recombinant enzymes were produced using a pET-system. Those enzyme activities were measured by radioisotope mediated TLC assay. (B) UGTs catalyze the transfer of ^{14}C-glucose to the aglycone. The radioactivity is detected by TLC. (C) These terpenoids were used as substrates with reference to Caupti et al.,(2008).





◀Fig3. Activity of recombinant enzymes

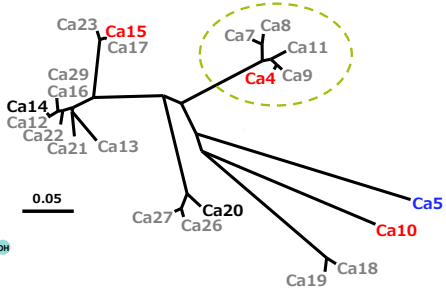
Lanes on the left side were positive controls which revealed that has the activities towards retinoic acid and geraniol. Transition of ^{14}C glucose was detected as a radioactivity. Then, black signals were detected as the activity.

▼Table1. Results of the functional analysis

Three recombinant enzymes showed activity linalool and the other substrates. Ca10 had same activities as Ca4. Furthermore, Ca4 showed most clearly signal towards linalool.

◎ : Clearly signal
○ : Medium signal
△ : Weak signal
– : Not detected

name	Li	Me	Fa	Ci	Pe	Te	Ge	Re
Ca4	◎	–	–	◎	◎	◎	◎	–
Ca5	–	–	–	–	△	△	△	–
Ca10	◎	–	–	◎	◎	◎	◎	–
Ca15	△	○	–	◎	◎	○	◎	–



◀Fig4. A phylogenetic tree of candidate genes from *C. arabica*

These *UGT* genes of *C. arabica* were selected by *in silico* screening with BLAST. In this study, Ca4, Ca5, Ca10 and Ca15 showed activity towards terpenoids. After this, we will analyze the genes which are classified as the same clade of Ca4.

Red : Accept linalool
Blue : Accept to except linalool
Gray : Not yet be analyzed
Black : No activity

<CONCLUSION> We identified six genes, *Ca4*, *Ca5*, *Ca10*, *Ca14*, *Ca15* and *Ca20*, encode polypeptide of 483, 479, 489, 483, 494 and 503 amino acid residues respectively. The amino acid sequence identities among these enzymes with UGT85K11 were 60.8, 60.1, 59.3, 59.0, 58.5 and 58.1%, respectively. Although Ca4, Ca10 and Ca15 can catalyze glycosylation of linalool, the activity was not the dominant activity (Table1). Now, we proceed to produce recombinant enzymes of Ca14 and Ca20. These recombinant enzymes will be used for enzyme assay too. The structure-function relationship of these isolated UGT genes would be clarified. Hereafter, to develop high-quality coffee with a rich aroma, we will utilize that information.

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