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병풀로부터 dammarenediol 합성 유전자 확인

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Confirmation of dammarenediol synthase in *Centella asiatica (L.) Urban*

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Objectives

Oxidosqualene cyclases (OSCs), which are situated at the branching step for biosynthesis of phytosterols and triterpenoids including mono-, tri-, tetra-, or pentacyclic types, catalyze the cyclization of 2,3-oxidosqualene. In a plant, different triterpenoid carbon skeletons, such as α-amryin, β-amyrin, lupeol, cycloartenol, and lanosterol, can be produced by various OSCs. In case of *Centella asiatica*, two OSCs encoded cycloartenol synthase (CaCYS) or β-amyrin synthase (CabAS), were isolated and characterized at the level of gene expression. However, the exact identification of these genes has not been achieved in the yeast. In this study, to elucidate the function of *CabAS* gene reported as a gene encoding putative β-amyrin synthase (Kim et al. 2005), the gene was overexpressed through a recombinant vector system and the final product was identified by GC-MS analysis.

Materials and Methods

○ Functional expression in *Saccharomyces cerevisiae* mutant ERG7

The erg7 mutant (*MATa erg7 ura3-1 trp1-1*) was kindly provided by F. Kars, Colmar, France (Karst and Lacroute et al. 1977). Yeast transformation and over-expression of an inserted DNA fragment were carried out as described by Kushiro et al. (1998).

○ GC-MS analysis for products determination

GC-MS analysis was carried out on a mass spectrometer (JMS-AM SUN200, JEOL) connected to a gas chromatograph (6890A, Agilent Technologies) with an Rtx-5MS capillary column (15m×0.25 mm, Restek).

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Results and Discussion

GC spectrum indicated that the isolated dammarenediol of the standard achieved a peak and this signal was not detected in the extract from transformants with empty vector. However, after inducing the CabAS gene by galactose, a peak which was consistent with the standard of dammarenediol was detected. To identify the chemical structure, the extract was purified by silica gel thin-layer chromatography (TLC) developed in acetonitrile:water (95:5, v/v) and the purified extract was analyzed by GC-MS (mass spectrometry). The representative fragmentation ion values by GC-MS of authentic dammarenediol II exhibited m/z 409 as a dominant peak and m/z 427 as the parent ion. These values were consistent with those as described by Han et al. (2006). These peaks also were detected in the purified extracts from transformants with pYES2.1-CabAS vector. Therefore, this result indicates the accumulated product to be dammarenediol. In the previous paper, the characterization of CabAS gene (AY520818) was reported (Kim et al. 2005) and annotated as putative β-amyrin synthase. Its function now is elucidated in this study. Therefore, CabAS should be corrected to *C. asiatica* dammarenediol synthase (CaDDS).

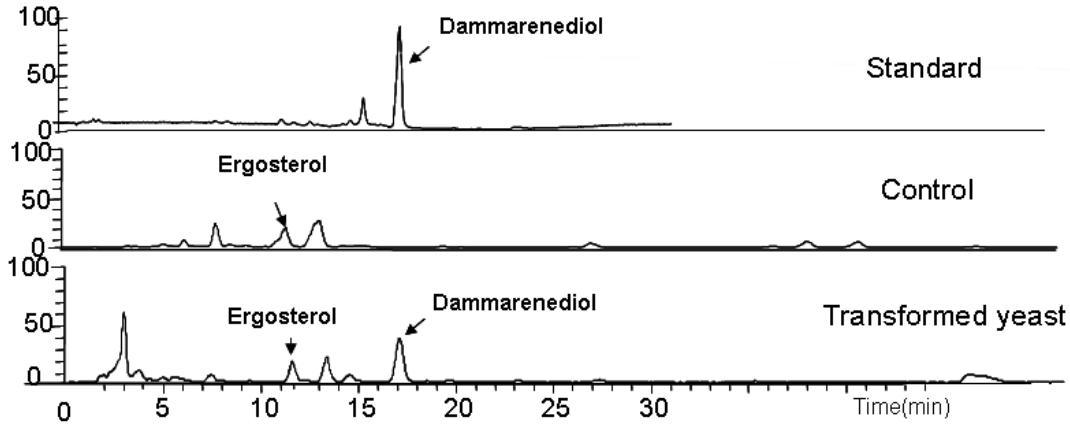

Figure 1. Total ion chromatogram of GC analysis of the pYES2.1-CaDDS production in yeast