27P-am007

Cloning and characterization of differentially expressed genes involved in quinolizidine alkaloid biosynthetic pathway between bitter and sweet forms of *Lupinus angustifolius*

◯Somnuk BUNSUPA[1], Emi IKEURA[1], Kazuki SAITO[1,3], Mami YAMAZAKI[1,2]
([1]Graduate School of Pharmaceutical Sciences, Chiba University, [2]CREST, JST, [3]RIKEN, PSC)

[Introduction] Quinolizidine alkaloids (QAs) or lupin alkaloids occur mainly in the genus *Lupinus*. QAs are supposed to be formed from **L**-lysine via decarboxylation by lysine decarboxylase (LDC) and produced cadaverine is oxidized and cyclized to form various molecules of QAs.

[Method] The bitter form specific cDNA fragments between alkaloid-containing 'bitter' form and alkaloid-free 'sweet' form in *Lupinus angustifolius* have been profiled by PCR-select cDNA subtraction. Several fragments show the homology with ornithine decarboxylase (ODC), amine oxidase (AO), and acyltransferase (AT) from plants. Using the sequence information of these fragments, we performed 5'- and 3'-RACE to obtain the full length cDNAs of *L .angustifolius LDC*, *AO* and *AT*.

[Results and discussion] The purified recombinant LDC expressed in *E.coli* exhibits decarboxylase activity with **L**-ornithine and **L**-lysine at the same optimal pH value of 7.5. Although the *K*m value with **L**-ornithine (1.20 mM) is lower than **L**-lysine (3.84 mM). The comparison of the catalytic efficiency (*k*cat/*K*m) revealed that LDC exhibits a preference for **L**-lysine ca. 5 times over **L**-ornithine. LaAT contains the conserved HXXXD and DFGWK motifs, which are characteristic features of the acyltransferases BAHD superfamily. LaAT might be involved in the ester formation of QA. The functional analyses of LaAT are now in progress. The first 100 amino acid of *LaDC* was fused to N-terminal region of GFP. The full-length of *LaDC* was fused either to the N- or C-terminus of region of GFP. These fusions were used in particle gun bombardment of *A. thaliana* leaves. Only LaDC100-GFP fusion protein was observed transiently expressed in chloroplast on laser microscopy of *A. thaliana* leaves. Transgenic study in *A. thaliana* and Tobacco BY-2 cells are now in progress.