論文の内容の要旨

生産・環境生物学専攻 専攻

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論文題目 Studies on the ability of the Asian corn borer *Ostrinia furnacalis* to catabolize DIMBOA, a host antibiotic

（アワノメイガ *Ostrinia furnacalis* の DIMBOA 異化代謝能力に関する研究）

Maize contains an allelochemical, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which functions as a feeding deterrent, growth inhibitor, and toxin against many herbivorous insects. Therefore, insects that feed on maize are considered to have developed adaptive mechanisms to cope with this compound. The adaptations of insects to toxic compounds involve modified feeding behavior, physiology, and metabolism. The Asian corn borer *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) is an important pest of maize in the Asia. Although nine *Ostrinia* species are reported to inhabit Japan, *O. furnacalis* is the only *Ostrinia* species in the Asia that feeds on maize. Among the sympatric congeners, the adzuki bean borer *Ostrinia scapulalis* (Walker) is particularly interesting in terms of host plant usage, because this species, although very polyphagous, does not utilize maize as a host. Comparison of the two congeners, *O. furnacalis* and *O. scapulalis*, may shed light on the mechanisms of the differentiation of host plant usage, sympatric speciation that may have occurred after this differentiation, and many other aspects of evolutionary biology.

Previous studies in our laboratory suggested that UDP-glucosyltransferase (UGT), which catalyzes glucosylation of lipophilic compounds and thereby expediting its excretion from insect body, is involved in the catabolism of DIMBOA; however, the glucosylation product of DIMBOA was not detected. In this thesis, I aimed to further clarify the physiological adaptations of *O. furnacalis* to its host, by focusing on the genetic basis of its ability to catabolize DIMBOA and, subsequently, on the UGT enzyme involved in the catabolism of this allelochemical. This dissertation consists of two chapters.

In **Chapter 1**, I compared the ability of *O. furnacalis* and its congener *O. scapulalis* to tolerate DIMBOA, with reference to the tolerance of their hybrids. The tolerance of *O. furnacalis*, *O. scapulalis*, and their F1 hybrids to DIMBOA was evaluated by the growth, development, and survival rate of larvae that were fed on an artificial diet containing DIMBOA. In laboratory assays, the addition of 0.3 mg/g of DIMBOA to an artificial diet markedly affected the survival of *O. scapulalis* larvae, but not that of *O. furnacalis* larvae. Besides the survival rate, the growth and development of *O. scapulalis* larvae were significantly retarded as compared with those of *O. furnacalis*. Hybrids of *O. furnacalis* and *O. scapulalis*, crossed in both directions, tolerated DIMBOA to the same extent as *O. furnacalis*, indicating that this tolerance was conferred by a single or a few autosomal genes that are dominant to those of *O. scapulalis*.

Subsequently, I investigated the contribution of UGT to the catabolism of DIMBOA in *Ostrinia furnacalis*. *In vitro*, DIMBOA was rapidly catabolized when incubated with the homogenate of the digestive tract of *O. furnacalis* in the presence of UDP-glucose. The UDP-glucose-dependent DIMBOA-catabolizing activities of the homogenate of the digestive tracts of *O. scapulalis* and hybrids correlated with

their tolerance; low in *O. scapulalis* and high in the hybrids. These results reconfirmed that UGT or other UDP-dependent enzymes are involved in the catabolism of DIMBOA in *O. furnacalis*; however, consistent with our previous findings, DIMBOA-2-*O*-glucoside, the expected product of UGT, was not detected in the products of *in vitro* assays. This study reconfirmed the contribution of UGT in the catabolism of DIMBOA, but the whole picture of DIMBOA catabolism in *O. furnacalis* remains to be clarified.

In **Chapter 2**, I aimed to identify *O. furnacalis* UGT responsible for the catabolism of ingested DIMBOA. Based on RNA-seq analysis of genes expressed in the pheromone gland of *Ostrinia zaguliaevi*, another congener of *O. furnacalis*, I selected four UGT gene candidates that may be responsible for the catabolism of DIMBOA (comp3666, comp37547, comp36019, and comp37715). Among these genes, RT-PCR experiments using the midgut of *O. furnacalis* larvae have shown that *O. furnacalis* homolog of comp37547 possessed characteristics required for the genes involved in the catabolism of maize allelochemicals. Those are, 1) high expression levels in the midgut and Malpighian tubules, 2) its expression level in these tissues is increased in the larvae that had fed on corn or artificial diet containing DIMBOA, and 3) higher expression level in *O. furnacalis* as compared with the non-maize feeder *O. scapulalis*. Accordingly, I cloned this gene and named it OfurUGT1. The full length OfurUGT1 comprised 1733 bp with an open reading frame of 1563 bp encoding a protein of 520 amino acids. The molecular mass of OfurUGT1 protein was estimated as 58.33 kDa. OfurUGT1 belongs to insect UGT40 family, and primary structure analysis has shown that OfurUGT1 protein all structures characteristic of UGT. For example, OfurUGT1 had N-terminal substrate binding domain and the C-terminal sugar-donor binding domain. In N-terminal, the signal peptide cleavage sites and

catalytic residue were identified. In C-terminal, the UGT signature motif, donor binding region 1 (DBR1), donor binding region 2 (DBR2), and negatively charged region were identified.

Phylogenetic analysis of the amino acid sequences of OfruUGT1 and other UGTs belonging to insect UGT40 family suggested that OfruUGT1 is relatively closely related to UGT40R and UGT40Q. However, since OfurUGT1 does not form a compact clade neither with UGT40R nor UGT40Q, OfurUGT1 may belong to a yet undescribed subclass of UGT40 family.

I subsequently aimed to perform functional assay of OfurUGT1 heterologously expressed in Sf9 insect cells using Baculovirus expression system. Although I confirmed the expression of OfurUGT1 protein in Sf9 cells, enzymatic activity of this protein toward DIMBOA has not yet been demonstrated. Optimization of recombinant protein expression and improvements in the design of functional assay are required before drawing any conclusion about the activity of OfurUGT1.

In conclusion, I obtained further evidence that UGT is involved in the enhanced tolerance of the larvae of *O. furnacalis* to DIMBOA. The nucleotide and amino acid sequences of OfurUGT1, which is a good candidate of UGT responsible for the catabolism of DIMBOA in *O. furnacalis*, were disclosed for the first time.