博士論文

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 異化代謝能に関する研究)

TRAN THI THU PHUONG

トラン　ティ　トゥ　フーン

博士論文

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 異化代謝能に関する研究)

TRAN THI THU PHUONG

トラン　ティ　トゥ　フーン

Graduate school of Agricultural and Life Sciences

The University of Tokyo

March 2016

ACKNOWLEDGEMENTS

To complete my doctoral course and dissertation, I received great supports from many individuals and organizations.

First of all, I would like to express greatest appreciation to my supervisor, Professor Yukio Ishikawa, for his endless supports, valuable guidance, encouragements and provide accessibility to the best facilities to perform excellent research. I would like to thank Associate Professor Takashi Matsuo for his comments and supports for my research. I would like to thank Dr. Masabonu Yamamoto and Takeshi Fujii for technical training, their cooperation and conducting experiments, their valuable suggestions and comments from which this thesis would be possible. I would like to thank Mr. Yu Rong for his technical supports at laboratory and his help in my life in Tokyo. I appreciate very much assistance from all of lab members of Laboratory of Applied Entomology, Graduate school of Agricultural and Life Sciences, The University of Tokyo.

My kindest gratitude of Prof. Tran Duc Vien, the President of Vietnam National University of Agriculture, and Prof. Nguyen Van Dinh, Dean of Graduate School, Vietnam National University of Agriculture for giving me the opportunity and support me during my doctoral course in Japan.

I also take this opportunity to grateful the financial support from the Japanese Government for providing MEXT scholarship during 3 years (2012- 2015).

Last but not least, I would like to devote this successful dissertation to my daughter and son, Vo Thi Phuong Linh and Vo Huu Quan, my husband, Vo Huu Cong, for their inspiration, supports, and encouragements, and especially, my parents, my sister and brother for all kinds of supports for me.

LIST OF CONTENTS

GENERAL INTRODUCTION

In nature, insect herbivores and plants are involved in complex biochemical and ecological interaction networks (Fürstenberg-Hägg et al., 2013). In the course of evolution, plants developed a diverse array of chemical defenses in order to protect themselves from herbivores (Berenbaum, 1995; Fürstenberg-Hägg et al., 2013; Schoonhoven et al., 2005). Many secondary metabolites of plants are known to work as feeding deterrents, growth inhibitors, or toxins against insects (Fürstenberg-Hägg et al., 2013; Hartmann, 2004; Howe and Jander, 2008). To overcome these defenses, herbivorous insects have evolved countermeasures such as modified feeding behavior, physiology, and metabolism (Després et al., 2007; Pentzold et al., 2014). These reciprocal processes between insect herbivores and their hosts are considered to have driven coevolution (Ehrlich and Raven, 1964).

Cyclic hydroxamic acids (cHx) are known as secondary metabolites in several Poaceae plants such as maize and wheat (Cambier et al., 1999; Hofman and Hofmanová, 1969; Niemeyer, 1988; Tipton et al., 1967). cHx are biosynthesized during the first 10 days after seed germination and then decrease as plant ages, and thus the concentration of cHx is highest in youngest leaf tissue (Cambier et al., 2000). The main cHx in maize is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which is stored in plant tissues as non-toxic glucoside (Cambier et al., 1999; Hofman and Hofmanová, 1969). Upon disruption of tissues, DIMBOA and other aglucones are released by the action of plant β-glucosidase (Niemeyer, 1988; Woodward et al., 1978).

DIMBOA is known to work as feeding deterrent and growth inhibitor against many insects. This compound has been reported to decrease the growth,

development, and the survival rate of various insects, such as the European corn borer *Ostrinia nubilalis* (Hübner) (Campos et al., 1989; Feng et al., 1992), Asian corn borer *Ostrinia furnacalis* (Guenée) (Yan et al. 1999), corn stalk borer *Sesamia nonagrioides* (Lefebvre) (Ortego et al. 1998), and the bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus) (Mukanganyama et al., 2003). In addition, this allelochemical has been demonstrated to influence the activities of various enzymes, for instance, nervous system enzymes, digestive proteases, and detoxification enzymes of insects (Mukanganyama et al., 2003; Ortego et al., 1998; Yan et al., 1995). DIMBOA inhibits the activities of carboxypeptidases, aminopeptidases, glutathione S-transferase and esterases in *S. nonagrioides* (Ortego et al., 1998) and *R. padi* (Mukanganyama et al., 2003). Besides that, this compound has been shown to inhibit the activities of acetylcholinesterase and general esterase of *O. furnacalis* larvae (Yan et al., 1995).

Many herbivores have developed physiological and metabolic adaptations to overcome toxins in the host plants (Després et al., 2007; Pentzold et al., 2014). Detoxification enzymes such as cytochrome P450 monooxygenases, glutathione S-transferases, and UDP-glucosyltransferases (UGTs) play important roles in these adaptations (Ahmad and Hopkins, 1993a; Després et al., 2007; Pentzold et al., 2014). Insect UGTs catalyze glucosylation of small lipophilic compounds by using UDP-glucose as the main donor of glucose (Ahmad and Hopkins, 1993b, 1992; Ahn et al., 2012). Many UGT genes have been found in a single insect species, and form a large multiple gene family (Ahn et al., 2012). UGTs have been suggested to play an important role in detoxification of DIMBOA in several lepidopteran species, such as *Spodoptera littoralis* (Boisduval), *Spodoptera frugiperda* (Smith), and *Mythimna separate* (Walker) (Maag et al., 2014; Sasai et al., 2009*;* Wouters et

al., 2014). Interestingly, DIMBOA-glucoside found in the frass of insects was an epimer of plant DIMBOA-glucoside, indicating the occurrence of stereoselective reglucosylation of DIMBOA in herbivorous insects (Wouters et al., 2014).

In addition to detoxification of toxic compounds by enzymatic activities, alkalinity in the gut lumen of lepidopteran insects has been shown to inhibit the activities of ingested plant β-glucosidase, hence contributing to the reduction of toxic aglucones (Pentzold et al., 2014). Larvae of some insect species have highly alkaline pH conditions in the midgut lumen, which may inhibit plant β-glucosidases and prevent activation of ingested defense compounds. A direct link between an alkaline midgut and reduced plant β-glucosidases activity towards benzoxazinoid glucosides was reported in the generalist fall armyworm *S. frugiperda* (Pentzold et al., 2014). The larval midgut lumen with a pH of 10 was shown to reduce plant β-glucosidases activity by more than 80%, which strongly reduced the release of toxic aglucones (review by Pentzold et al., 2014). Insect herbivores with an alkaline midgut may have been pre-adapted to feed on plants protected by allelochemicals. Thus, both detoxification enzymes and alkalinity of gut lumen are considered to be the measures to counter plant chemical defenses.

The Asian corn borer *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) is an important pest of maize in the Asia (Ishikawa et al., 1999; Mutuura and Munroe, 1970). Although nine *Ostrinia* species are reported to inhabit Japan, *O. furnacalis* is the only *Ostrinia* species in Japan that feeds on maize (Ishikawa et al., 1999; Mutuura and Munroe, 1970). Among the sympatric congeners, the adzuki bean borer *Ostrinia scapulalis* (Walker) is particularly interesting in terms of host plant usage, because this species, despite very polyphagous, does not utilize maize as a host (Ishikawa et al., 1999). 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.

In the previous study (Kojima et al., 2010), *O. furnacalis* was shown to be better adapted to maize chemical defense than the congener adzuki bean borer *O. scapulalis*. The homogenate of digestive tract of *O. furnacalis* degraded cHx more rapidly than the *O. scapulalis* counterpart. The degradation of cHx by *O. furnacalis* was considered to involve UGT; however, the glucosylation product of cHx was not detected in the previous study (Kojima et al., 2010). The objectives of my research are to clarify the genetic background (inheritance) of the tolerance of the Asian corn borer *Ostrinia furnacalis* to cyclic hydroxamic acids (cHx), evaluation of the contribution of UDP-glucosyltransferase (UGT) to the detoxification of cHx, cloning of genes encoding UGT from *O. furnacalis*, and to perform functional assays of these genes.

CHAPTER 1:

COMPARISON OF THE ABILITY TO CATABOLIZE DIMBOA, A MAIZE ANTIBIOTIC, BETWEEN *Ostrinia furnacalis* AND *Ostrinia scapulalis,* WITH REFERENCE TO THEIR HYBRIDS

1.1. Introduction

In this chapter, I aimed to further clarify the mode of detoxification of DIMBOA by *O. furnacalis* upon the basis of the results of previous studies conducted in our laboratory (Kojima et al., 2010). I first reinvestigated the resistance of *O. furnacalis* to DIMBOA in detail. I examined the growth and survival of *O. furnacalis*, *O. scapulalis*, and hybrids of these two species on an artificial diet containing DIMBOA in order to obtain information on the genetic background of this resistance. I subsequently evaluated the contribution of UDP-glucosyltransferase (UGT) to the catabolism of DIMBOA *in vitro* using digestive tract homogenates.

1.2. Materials and methods

1.2.1. Laboratory culture of *Ostrinia*

Wild female moths of the genus *Ostrinia*, mostly *O. furnacalis* (Fur) and *O. scapulalis* (Sca), were collected at Mastudo, Japan (35.5°N, 139.6°E) in June 2014. They were brought to the laboratory, and maintained singly in 430-ml plastic cups in order to allow them to lay eggs. Their offspring were reared by family on an artificial diet (Silkmate 2M, Nosan, Corp., Yokohama, Japan) under a photoperiod of 16L: 8D at 25°C and 60–70% relative humidity. Since female moths of the *Ostrinia* species are very similar, species identification of the collected female

moths was impractical. Therefore, the species of each family was identified by the sex pheromone of the virgin females (**Fig. 1.1**) and thickness of the midlegs of male moths. The female sex pheromone of *O. furnacalis* is a blend of (*Z*)-12- and (*E*)-12-tetradecenyl acetates, whereas that of *O. scapulalis* is a blend of (*Z*)-11- and (*E*)-11-tetradecenyl acetates (Ishikawa et al. 1999). The midleg of male *O. furnacalis* is thin, whereas that of *O. scapulalis* is thick (Mutuura and Munroe, 1970).

1.2.2. Sex pheromone analysis

The pheromone glands of 10 virgin females were collected and female sex pheromone components were analyzed using a gas chromatograph coupled to a mass spectrometer (QP2010 SE GC-MS, Shimadzu) equipped with a capillary column (DB-Wax, 0.25 mm i.d. × 30 m; Agilent Technologies, Santa Clara, CA). The initial column oven temperature of 80°C was maintained for 2 min, then raised at 8°C/min to 240°C, and maintained at this temperature for 4 min. The flow rate of the carrier gas (He) was 1.0 ml/min.

1.2.3. Crossing

In order to obtain F_1 hybrids (Fur × Sca), 20 virgin females of *O. furnacalis* and 25 males of *O. scapulalis* (2–3 days old) were housed in a mesh cage (20 × 20 × 20 cm) for 7 days. Reciprocal crossing (Sca × Fur) was conducted in a similar manner. F_1 eggs were collected every 24 h and reared as described above. The female sex pheromones of F_1 hybrids (Fur × Sca and Sca × Fur) used in the feeding test were analyzed to confirm their hybrid status (Sakai et al., 2009). F_1 females of the both reciprocal crosses produced the sex pheromone components of both parents, namely, (*Z*)-11-, (*E*)-11-, (*Z*)-12-, and (*E*)-12-tetradecenyl acetates (Fig. 1.1). Male

hybrid moths are expected to have the thick midlegs of *O. scapulalis* (Frolov et al. 2012). We confirmed that male hybrids had thick midlegs (data not shown).

1.2.4. Maize

The seeds of dent corn *Zea mays* (variety KD640) were obtained from Kaneko Seeds Co., Ltd., Gunma, Japan. Maize seedlings were grown on moist paper towels in a plastic tray (30 cm × 23 cm × 4.5 cm), and kept in the dark at 25–28°C. Seedlings were cut 7 days after germination and stored frozen at −20°C. Maize plants were cultivated in the field under natural conditions in July and August 2014 at the Yayoi Campus of the University of Tokyo, Japan. Maize plants were harvested 35 days after germination.

1.2.5. Purification of DIMBOA, DIMBOA-2-glucoside, and MBOA

DIMBOA-2-glucoside and DIMBOA were extracted from 7-day-old maize seedlings by the method of Lyons et al. (1988) and Larsen and Christensen (2000), respectively, and purified by high-performance liquid chromatography (HPLC; LC-9A, Shimadzu, Kyoto, Japan) equipped with an ODS column (10 mm × 250 mm; YMC-Pack Pro C18, YMC Co., Ltd., Kyoto, Japan). The mobile phase for HPLC was as described by Lyons et al. (1988). The flow rate of the mobile phase was 2.0 ml/min and the eluates were monitored by UV absorption at 254 nm. 6-Methoxy-2-benzoxazolinone (MBOA) was obtained in the previous study (Kojima et al., 2010). Purified DIMBOA-glucoside, DIMBOA, and MBOA were analyzed by NMR (^{1}H) spectroscopy (ECA-II 500 MHz, JEOL RESONANCE Inc., Tokyo, Japan) in order to verify their chemical structures (**Table 1.1**).

1.2.6. Feeding test

The third-instar larvae of *O. furnacalis* were fed on an artificial diet containing 0, 0.3, 0.5, and 0.7 mg of DIMBOA/g according to the method of Kojima et al. (2010). The effects of DIMBOA on the growth of *O. scapulalis*, F_1 (Fur × Sca), and F_1 (Sca × Fur) were only examined at 0 and 0.3 mg of DIMBOA/g. The duration of larval development, growth rate, pupal weight, and survival rate were used to evaluate the effects of DIMBOA on larvae.

1.2.7. *In vitro* assays

We slightly modified the method of Kojima et al. (2010) for *in vitro* enzymatic assays. The digestive tracts of larvae were isolated and washed in phosphate-buffered saline [PBS (+), 2,5 mM KCl, 141 mM NaCl, 8.1 mM Na_2HPO_4, and 2.5 mM KH_2PO_4 (pH 7.8), with 0.9 mM $CaCl_2$, 0.03 mM $MgCl_2$]. Twenty digestive tracts were homogenized in 6 volumes (V/W) of PBS (+) and used as the enzyme solution. Reaction mixtures consisted of PBS (+), 0.3 mM DIMBOA, 0.6 mM UDP-glucose, and enzyme solution in a final incubation volume of 0.3 ml. In the control experiments, UDP-glucose was removed from the reaction mixture or the enzyme solutions were boiled for 15 min before the enzyme assay. After being incubated at 37°C for 90 min, the reaction was stopped by the addition of methanol (0.1 ml) and centrifuged at 20,000 g for 15 min. The supernatants were analyzed by HPLC (ODS 4.6 mm × 250 mm column, GL science, Tokyo, Japan). The flow rate of the mobile phase was 1.0 ml/min. After a 5-min isocratic elution at 5% **A** (acetonitrile), 95% **B** (0.1% formic acid in water), the column was eluted with a linear gradient to 20% **A**, 80% **B** over 25 min followed by a second linear gradient to 100% **A** over 20 min. The eluates were monitored by UV absorption at 254 nm.

Calibration curves for DIMBOA-glucoside and DIMBOA were obtained using the standards prepared as described above.

1.2.8. Effects of pH on the catabolism of DIMBOA

In order to determine the effects of pH on the catabolism of DIMBOA, the digestive tracts of *O. furnacalis* were homogenized in 6 volumes (V/W) of PBS (−) [2,5 mM KCl, 141 mM NaCl, 8.1 mM Na_2HPO_4, and 2.5 mM KH_2PO_4] at four different pH values: 5.3, 7.2, 7.8, and 9.1. The enzyme solutions, reaction mixtures, and method applied for the analysis were the same as those described above.

1.2.9. Gut pH measurement

The midgut was quickly dissected out from a fifth-instar larva of *O. furnacalis*, and placed on the flatbed sensor of pH meter B-71X (Horiba, Kyoto, Japan). The measurement of pH was repeated three times using different samples.

1.2.10. Statistical analysis

Statistical analyses were performed using IBM SPSS software (version 22.0). An analysis of variance (ANOVA) or generalized linear model (GLM) was employed to analyze the effects of DIMBOA on the developmental time, growth rate, and pupal weight of *O. furnacalis*, *O. scapulalis*, and their F_1 hybrids. Comparisons between the treatment and control were made separately for *O. furnacalis*, *O. scapulalis*, and F_1 hybrids. The survival curves of *O. furnacalis*, *O. scapulalis*, and F_1 were analyzed by Kaplan-Meier estimates and the Log-rank test. The catabolism of DIMBOA in *in vitro* enzymatic assays was analyzed by ANOVA.

In all experiments, differences between treatments were compared using Tukey's multiple comparison test.

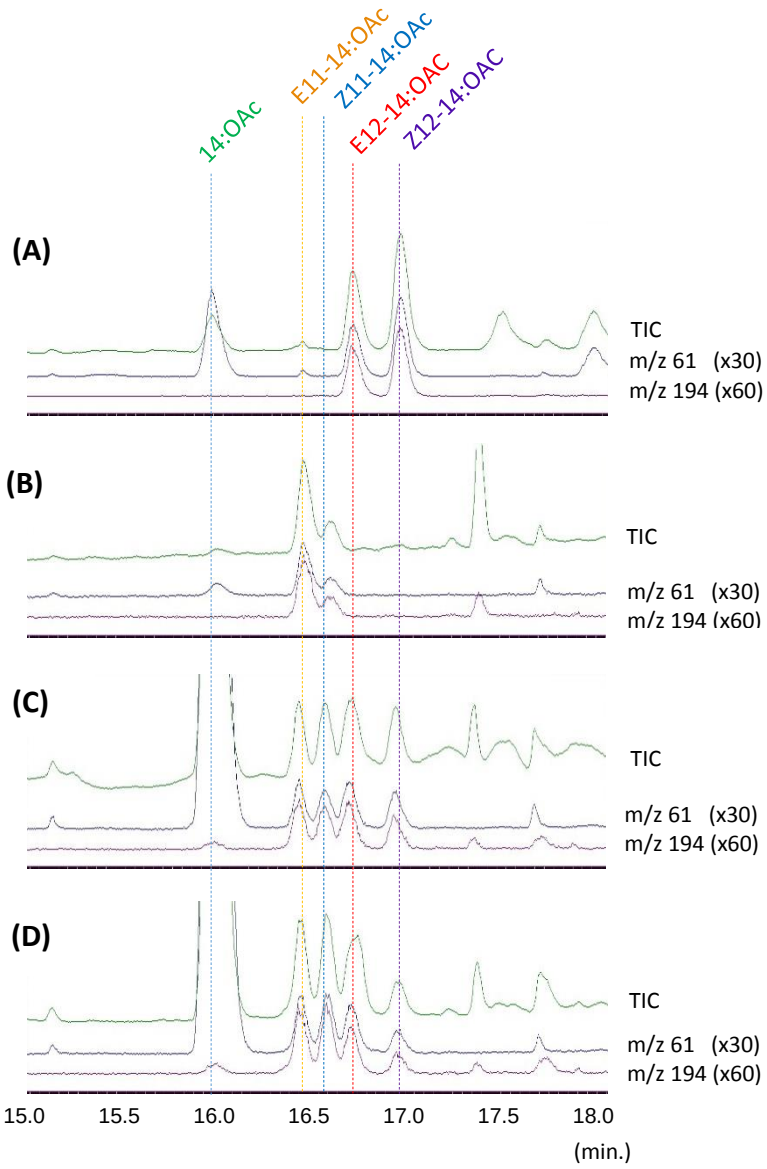
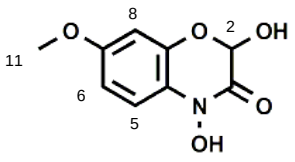


Figure 1.1. Typical GC-MS chromatograms of female sex pheromone gland extracts of (**A**) *O. furnacalis*, (**B**) *O. scapulalis*, (**C**) F_1 (Fur × Sca), and (**D**) F_1 (Sca × Fur). Consistent with the findings of Sakai et al. (Insect Biochem. Mol. Biol. 39: 62-7, 2009), in addition to the pheromone components of both parents, an extremely large amount of 14:OAc was detected in hybrids. Retention time of female sex pheromone components: E11: 16.44 min, Z11: 16.57 min; E12: 16.70 min, Z12: 16.93 min, and saturated OAc: 15.99 min.

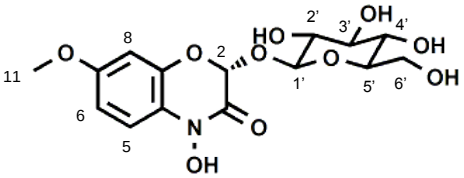
Table 1.1. ^{1}H NMR data of DIMBOA, DIMBOA-glucoside, and MBOA

Position	δ_H (multiplicity, J)		
	DIMBOA	DIMBOA-glucoside from maize seedlings	MBOA
2	5.72 (s)	5.93 (s)	
4			7.02 (d, J = 8.6)
5	7.25 (d, J = 8.8)	7.27 (d, J = 8.7)	6.74 (dd, J = 2.8, 8.4)
6	6.68 (dd, J = 2.7, 8.8)	6.70 (d, J = 8.7)	
7			6.9 (d, J = 2.4)
8	6.61 (d, J = 2.7)	6.74 (d, J = 2.2)	
10			3.80 (s)
11	3.78 (s)	3.79 (s)	
1’		4.79 (d, J = 7.5)	
2’		3.18 (s)	
3’		3.41 (dd, J = 8.5, 10.5)	
4’		3.22 (dd, J = 8.4, 8.8)	
5’		3.41-3.45 (m)	
6’a		3.89 (d, J = 10.7)	
6’b		3.68 (dd, J = 5.1, 11.2)	

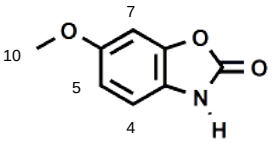
^{1}H NMR (500 MHz, acetone-d6) δ (ppm), J (Hz)



DIMBOA



DIMBOA-glucoside



MBOA

DIMBOA: 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one

DIMBOA-glucoside: 2-*β*-*D*-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one

MBOA: 6-methoxy-2-benzoxazolinone

1.3. Results

1.3.1. Feeding tests

In the no-choice feeding test, *O. furnacalis* and its F_1 hybrids were affected less by DIMBOA than *O. scapulalis* in terms of the growth rate, duration of larval development, and pupal weight (**Table 1.2** and **Fig. 1.2**). The survival rate of *O. scapulalis* were significantly decreased when fed on a diet containing 0.3 mg/g of DIMBOA, whereas the decrease observed in the survival rate of *O. furnacalis* became significant when larvae were fed on a diet containing higher concentrations (0.5 and 0.7 mg/g) of DIMBOA (**Table 1.2**). The degree of retardation of development in *O. furnacalis* fed on 0.7 mg/g of DIMBOA was similar to that in *O. scapulalis* fed on a diet containing 0.3 mg/g of DIMBOA. The growth and survival rate of F_1 (Fur × Sca) and F_1 (Sca × Fur) on diet containing 0.3 mg/g of DIMBOA were similar to those of *O. furnacalis* (**Table1.2** and **Fig. 1.2**).

1.3.2. Catabolism of DIMBOA *in vitro*

The involvement of UGT in the catabolism of DIMBOA in *O. furnacalis* was reinvestigated. DIMBOA was decreased when it was incubated with the homogenate of the digestive tract of *O. furnacalis* in the presence of, but not in the absence of, UDP-glucose (**Fig. 1.3 B, C**). Thus the involvement of UGT was also suggested in the present study; however, consistent with previous findings (Kojima et al. 2010), no peak corresponding to DIMBOA-glucoside, the expected glucosylation product, was observed (**Fig. 1.3**). Although DIMBOA is known to spontaneously degrade into MBOA under alkaline conditions (Woodward et al., 1978), no significant amount of MBOA was detected in *in vitro* enzyme assays (**Fig. 1.3 B, C**).

1.3.3. Fate of DIMBOA

In the above enzyme assays, a few new peaks, which are likely to be catabolites of DIMBOA, appeared close to DIMBOA and DIMBOA-glucoside as DIMBOA was catabolized (**Fig. 1.3C**). Among these compounds, my preliminary ^{1}H NMR analyses suggested that product 1 and product 2 in **Fig. 1.3C** are lactam-glucoside **(Fig. 1.4**) and lactam (**Fig. 1.5**), respectively. Since the presence of lactam-glucoside is the evidence of UDP-dependent glucosylation activities in the enzyme assay system, we considered a possibility that DIMBOA-glucoside is once produced but immediately disappeared because it was subject to further degradation. To test this possibility, I added DIMBOA-glucoside in place of DIMBOA in the enzyme assay (**Fig. 1.6**). Interestingly, DIMBOA-glucoside was rapidly degraded by the homogenate of the digestive tract of *O. furnacalis* not only in the presence of, but also in the absence of, UDP-glucose (**Fig. 1.6**). These results clearly indicated that in addition of UGT, other unidentified detoxification enzymes, which degrade DIMBOA-glucoside but not DIMBOA, are involved in the catabolism of DIMBOA in *O. furnacalis* (**Fig. 1.7**).

1.3.4. Optimum pH and tissue distribution of DIMBOA-catabolizing activity

I hereafter refer to the enzymatic activity that decreases DIMBOA as UDP-glucose-dependent DIMBOA-catabolizing activity. The optimum pH for the catabolism of DIMBOA lay between 7.2 and 7.8 (**Fig. 1.8**), slightly lower than the pH within the digestive tract of *O. furnacalis*, 8.3–8.8. Among the tissues of *O. furnacalis* larvae tested, a high UDP-glucose-dependent DIMBOA-catabolizing activity was observed in the midgut and Malpighian tubules (**Fig. 1.9**).

1.3.5. UDP-glucose-dependent DIMBOA-catabolizing activities of F_1 hybrids

We determined whether the mode of inheritance of UDP-glucose-dependent DIMBOA-catabolizing activity in *Ostrinia* was consistent with that of tolerance to DIMBOA. The UDP-glucose-dependent DIMBOA-catabolizing activities of F_1 (Fur × Sca) and F_1 (Sca × Fur) were not significantly different from that of *O. furnacalis*, whereas that of *O. scapulalis* was very low (**Fig 1.10A**). These results were consistent with the assumption that tolerance to DIMBOA in *O. furnacalis* was conferred by genes dominant to those of *O. scapulalis*.

1.3.6. Induction of UDP-glucose-dependent DIMBOA-catabolizing activities

We examined the induction of UDP-glucose-dependent DIMBOA-catabolizing activities by the previous ingestion of DIMBOA. The catabolism of DIMBOA in *O. furnacalis* that had been fed for 5 days on a diet containing 0.1 mg/g or maize plants, which contained 0.19 mg/g fresh weight, occurred significantly more rapidly than that in larvae fed on a control diet (**Fig. 1.10B**). In contrast, such an enhancement in the UDP-glucose-dependent catabolism of DIMBOA was not observed in *O. scapulalis* (**Fig. 1.10B**).

Table 1.2. Growth indices of *O. furnacalis*, *O. scapulalis*, and their F_1 Hybrids fed on an artificial diet containing DIMBOA

Species	DIMBOA concentration (mg/g diet)	Growth rate[1] (mg/2day)	Duration of larval development[2] (days)	Pupal weight[3] (mg)	Survival rate[4] (%)
O. furnacalis	0	2.46 ± 0.09a (n = 50)	14.7 ± 0.4a (n = 42)	60.3 ± 2.5a (n = 35)	76.5d (n = 98)
	0.3	2.10 ± 0.06b (n = 53)	15.7 ± 0.5a (n = 43)	51.9 ± 1.6b (n = 43)	74.2cd (n = 97)
	0.5	1.99 ± 0.05b (n = 41)	17.7 ± 0.5b (n = 31)	45.3 ± 1.2c (n = 30)	46.3ab (n = 95)
	0.7	1.72 ± 0.04c (n = 43)	18.4 ± 0.7b (n = 31)	38.7 ± 0.8d (n = 31)	40.2ab (n = 102)
O. scapulalis	0	2.17 ± 0.06a (n = 50)	15.8 ± 0.5a (n = 55)	56.0 ± 1.3a (n = 34)	76.1d (n = 109)
	0.3	1.44 ± 0.03c (n = 52)	18.1± 0.9b (n = 22)	40.9 ± 1.1c (n = 22)	30.0a (n = 110)
F_1 (Fur ♀ x Sca ♂)	0	2.18 ± 0.04a (n = 49)	15.7 ± 0.4a (n = 46)	56.4 ± 1.4a (n = 39)	75.4cd (n = 61)
	0.3	1.88 ± 0.05b (n = 47)	16.1 ± 0.6a (n = 38)	48.9 ± 1.0b (n = 37)	59.4bcd (n = 64)
F_1 (Sca ♀ x Fur ♂)	0	1.97 ± 0.04a (n = 46)	15.5± 0.4a (n = 46)	54.9 ± 1.2a (n = 39)	77.4cd (n = 62)
	0.3	1.75 ± 0.06b (n = 40)	16.4 ± 0.4a (n = 34)	47.1 ± 1.2b (n = 27)	53.1bc (n = 64)

Means in the same column with the same letter are not significantly different at $p < 0.05$. Data are the mean ± standard error. Tukey's multiple comparison test for proportions was used for the analysis of the survival.

[1] Larval weight post 48-h feeding/initial larval weight. Third-instar larvae weighting 14–16 mg were inoculated.

[2] Days from the start of the treatment with DIMBOA to pupation.

[3] Weight of pupae within 48 h of pupation.

[4] The percentage of larvae that pupated successfully.

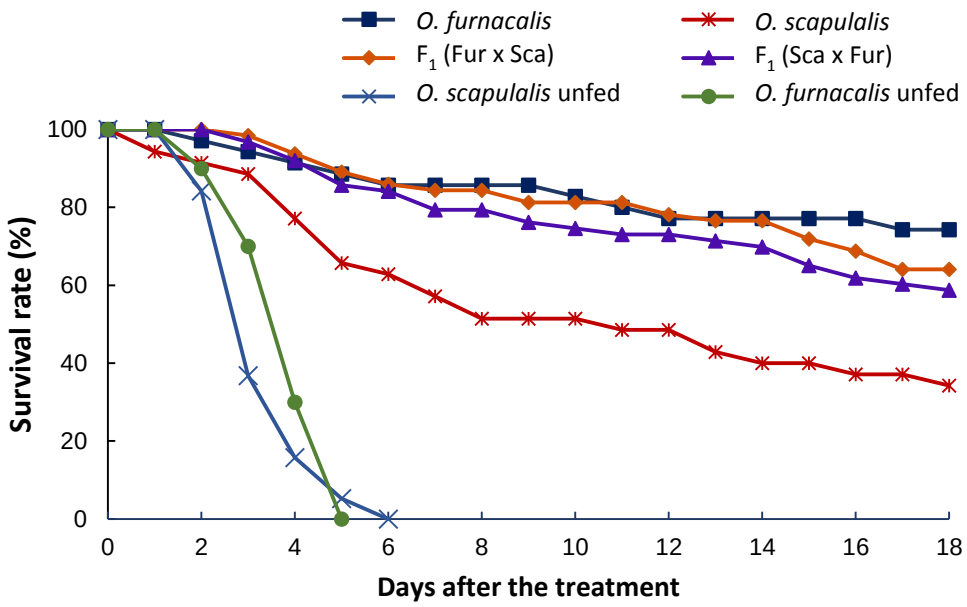


Figure 1.2. Survival curves of *O. furnacalis, O. scapulalis*, and their F_1 hybrids in the no-choice feeding test on an artificial diet containing 0.3 mg/g of DIMBOA. The survival curve of *O. scapulalis* was significantly different from those of *O. furnacalis* and F_1 hybrids at $p < 0.05$ by the Log-rank test. Unfed larvae of *O. furnacalis* and *O. scapulalis* died within 5 and 6 days, respectively (n = 30).

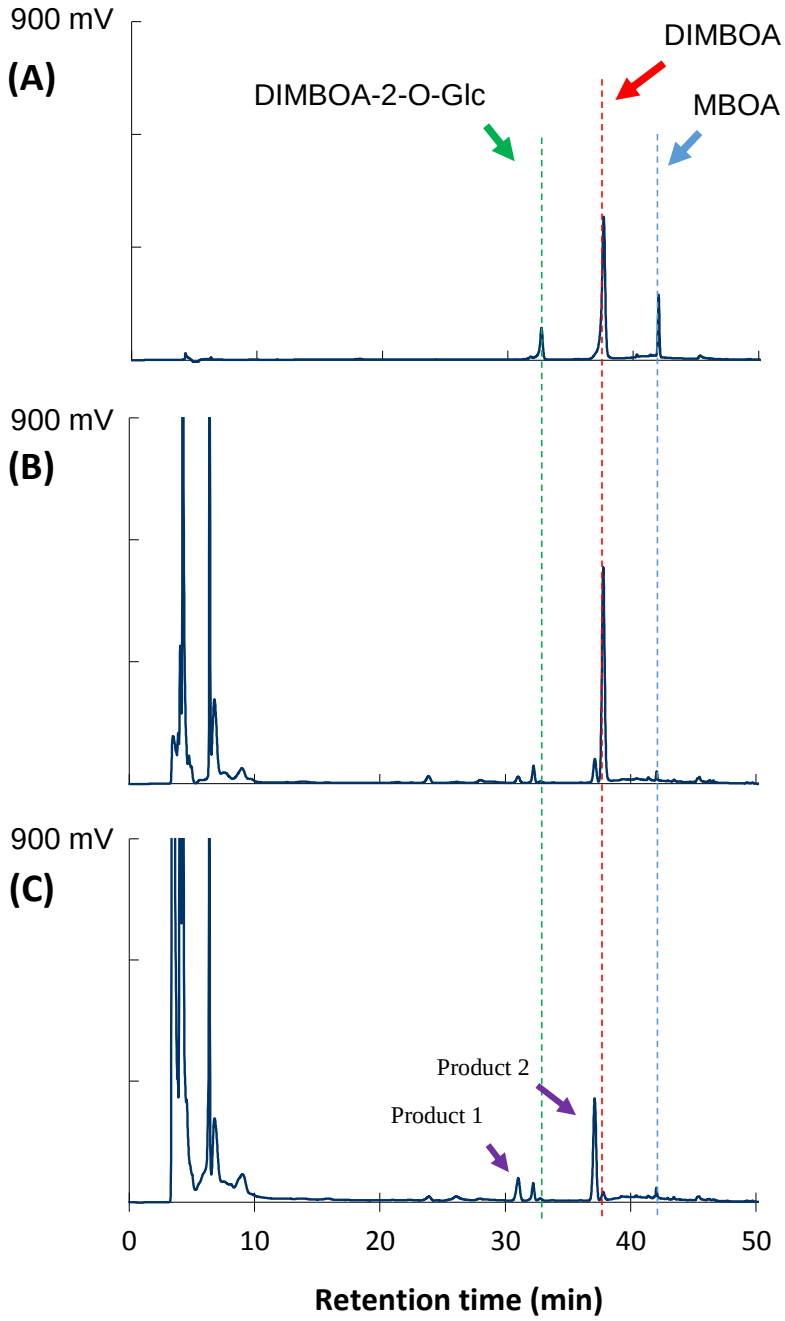
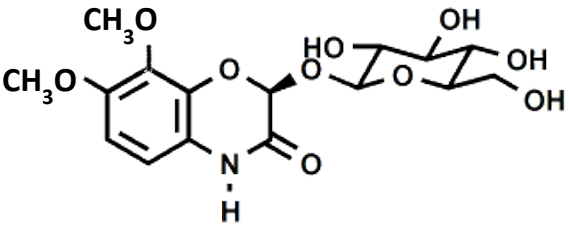


Figure 1.3. An HPLC chromatogram showing DIMBOA-glucoside (32.69 min), DIMBOA (37.65 min), and MBOA (42.03 min) standards prepared from maize seedlings (**A**). HPLC chromatograms of products after the incubation of DIMBOA with the homogenate of the digestive tract of *O. furnacalis* in the absence (**B**) and presence (**C**) of UDP-glucose. In the presence of UDP-glucose, a few peaks appeared close to that of DIMBOA and DIMBOA-glucoside (see text).



2-*O*-*β*-glucopyranosyloxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one

(**HM$_2$BOA-Glc**)

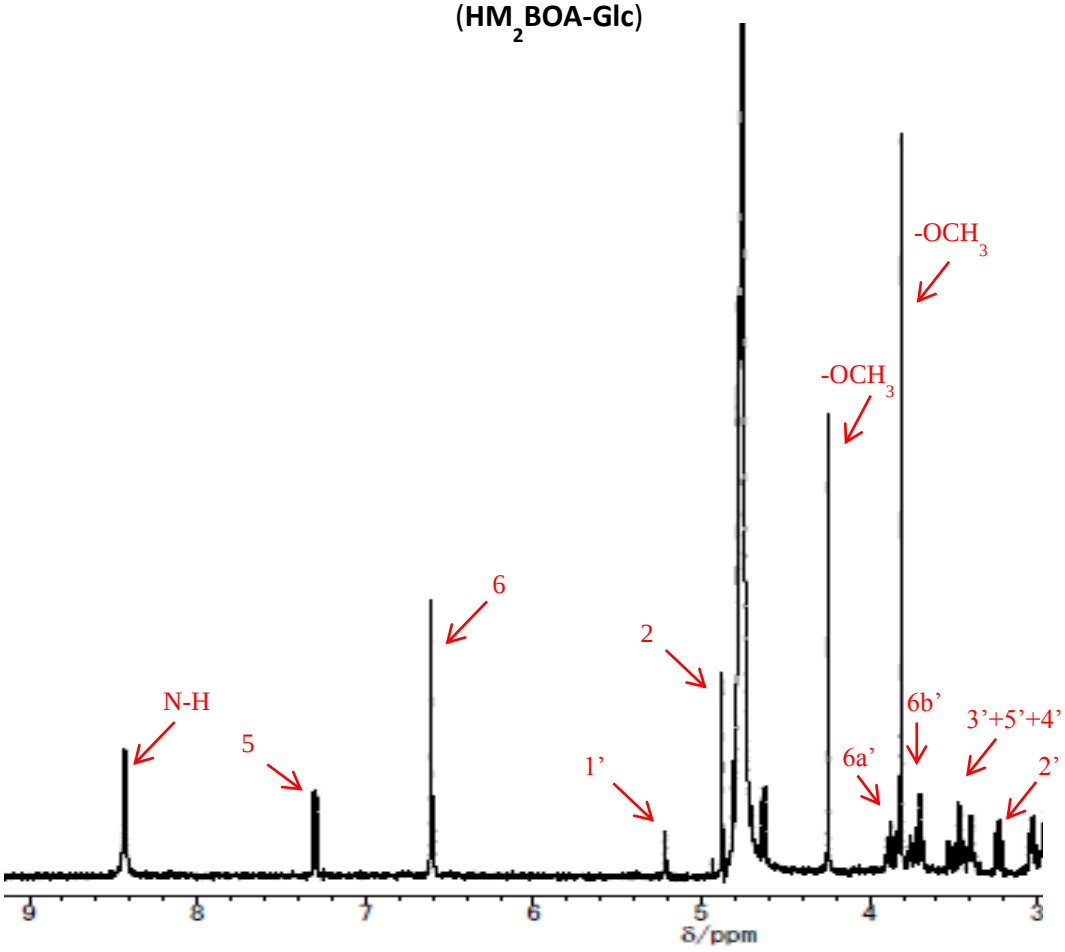
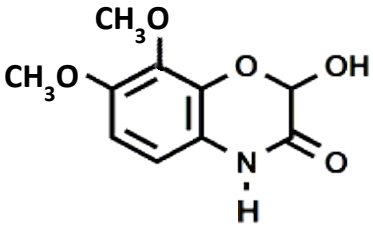


Figure 1.4. ^{1}H NMR spectra of product 1 (HM_2BOA-glucoside) in *in vitro* enzymatic assays.



2-hydroxy-7,8-dimethoxy-1,4-benzoxazin-3(4H)-one
(**HM$_2$BOA**)

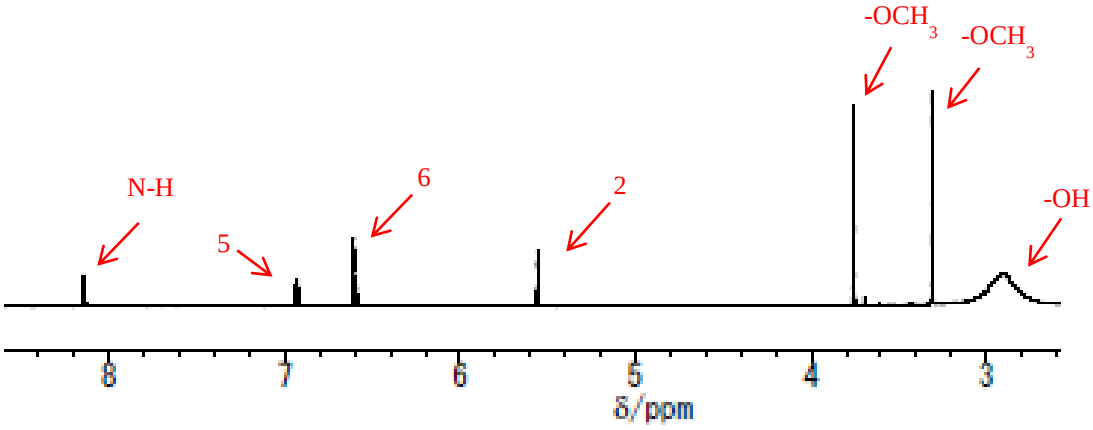


Figure 1.5. ^{1}H NMR spectra of product 2 (HM_2BOA) in *in vitro* enzymatic assays.

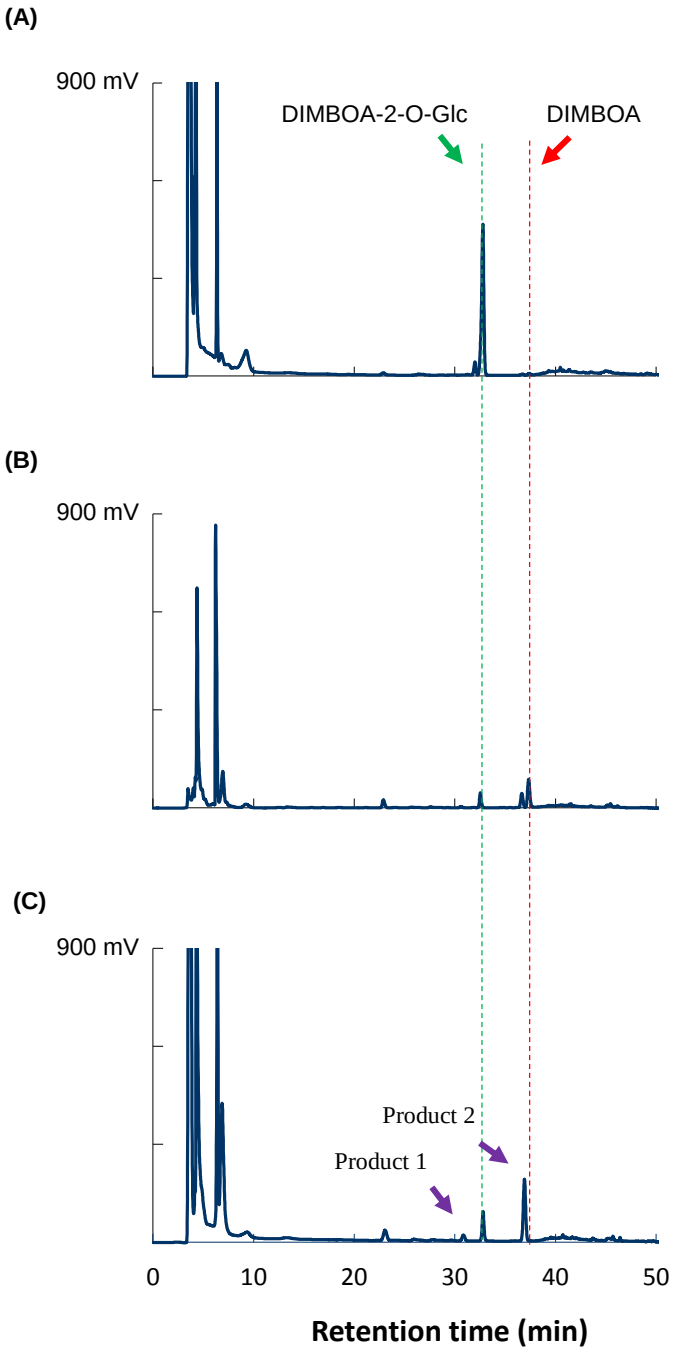


Figure 1.6. HPLC chromatograms of the catabolites of DIMBOA-glucoside in the enzyme assay. DIMBOA-glucoside was added to the homogenate of digestive tract of *O. furnacalis* with (+) or without (–) UDP-glucose. (A) No incubation (reaction was immediately stopped by adding MeOH), + UDP-glucose. (B) After incubation for 30 min, – UDP-glucose. (**C**) After incubation for 30 min, + UDP-glucose.

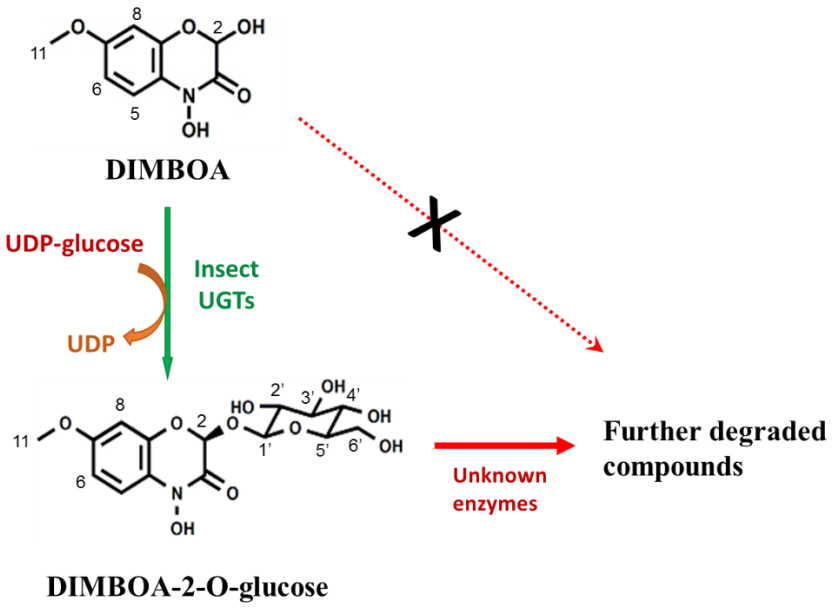


Figure 1.7. Possible fate of DIMBOA *in vitro*. In addition of UGT, other unidentified detoxification enzymes, which degrade DIMBOA-glucoside but not DIMBOA, are involved in the catabolism of DIMBOA in *O. furnacalis*.

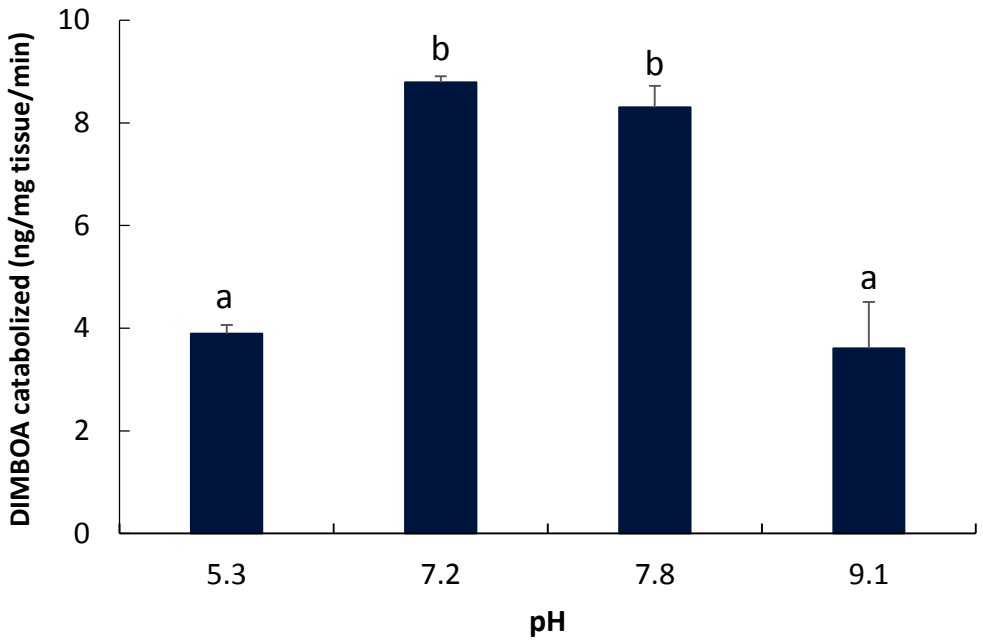


Figure 1.8. Effects of pH on the catabolism of DIMBOA by the homogenate of the digestive tract of *O. furnacalis*. Bars with the same letter are not significantly different at $p < 0.01$. Data are means ± SE (n = 3).

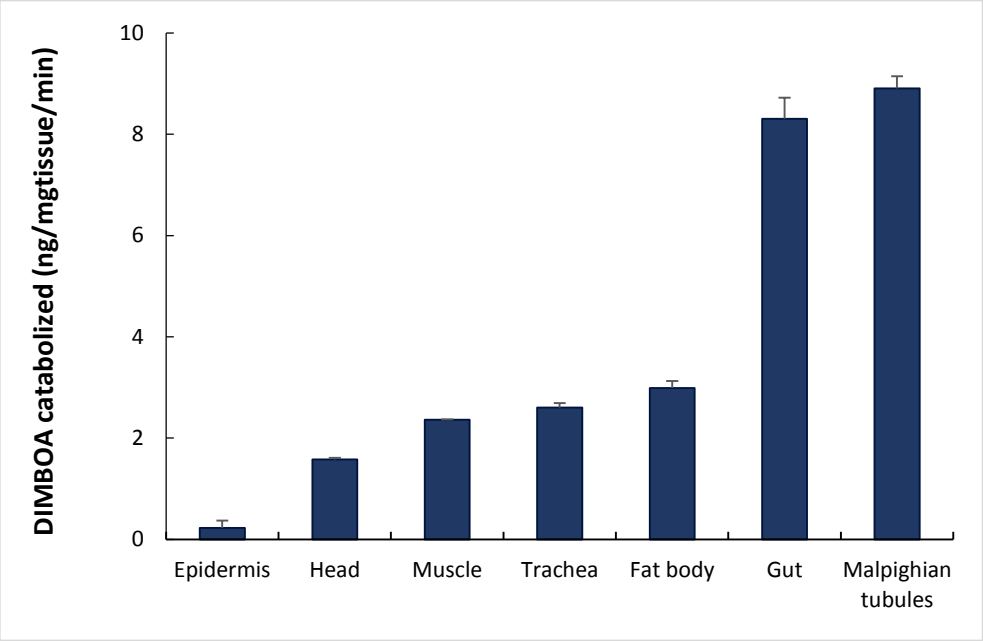


Fig. 1.9. Comparison of UDP-glucose-dependent catabolism of DIMBOA in various tissues of *O. furnacalis* larvae. Data are means ± SE (n = 3).

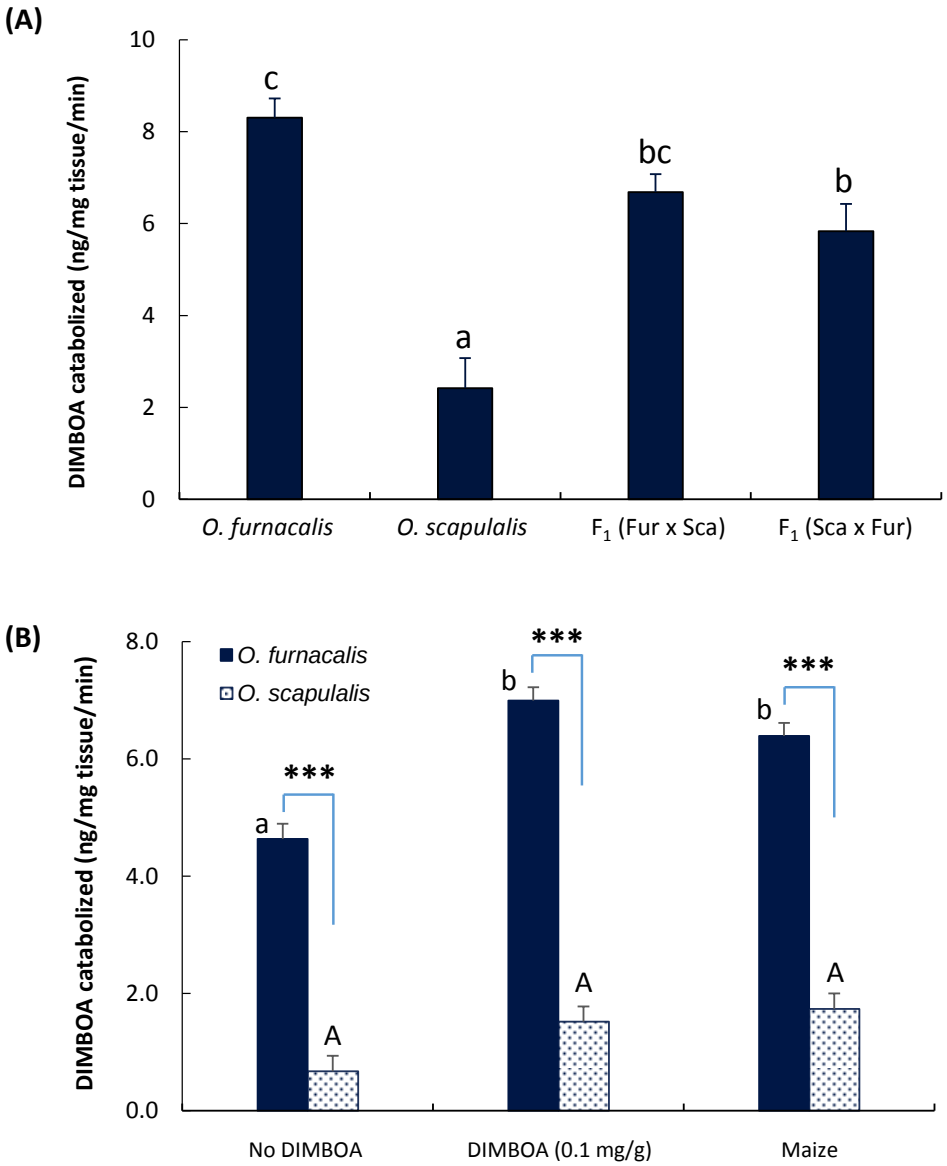


Figure 1.10. (A) UDP-glucose-dependent catabolism of DIMBOA by the digestive tracts of *O. furnacalis*, *O. scapulalis*, and F_1 hybrids. The homogenates of the digestive tract of fifth-instar larvae fed on maize plants for 5 days were used as the enzyme solution. Bars with the same letter are not significantly different at $p < 0.05$. Data are means ± SE ($n \geq 3$). (B) UDP-glucose-dependent catabolism of *O. furnacalis* and *O. scapulalis* larvae that had been fed on an artificial diet containing DIMBOA or maize. The homogenates of the digestive tracts of the fifth-instar larvae of two species fed on an artificial diet containing no or 0.1 mg/ml of DIMBOA, or maize plant, which contained approximately 0.19 mg/g FW of DIMBOA, for 5 days were used as the enzyme solution. Bars representing the same species with the same letter are not significantly different at $p < 0.05$. Asterisks indicate significant differences between species (***$p < 0.001$). Data are means ± SE ($n \geq 3$).

1.4. Discussion

The larvae of *O. furnacalis*, a maize feeder, tolerated higher concentrations of DIMBOA than its congener *O. scapulalis*, which does not feed on maize in nature. The European corn borer *Ostrinia nubilalis*, another congener feeding on maize in Europe and USA, also shows tolerance to DIMBOA; the survival rate of *O. nubilalis* that fed on a 0.5 mg DIMBOA/g diet (49.2%; Campos et al., 1989) is similar to that of *O. furnacalis* (46.3%; the present study). These results suggest that adaptation to the toxicity of DIMBOA is a prerequisite for insect herbivores to be able to utilize maize as their host plant. However, maize defenses against herbivores are not limited to DIMBOA. The major chemical and physiological defenses of maize include, in addition to DIMBOA, flavonoids such as maysin and chlorogenic acid, terpenoids such as (*E*)-β-caryophyllene, and protease inhibitors (Meihls et al., 2012). Comparisons of the abilities of *O. furnacalis* and *O. scapulalis* to cope with these defenses may lead to a better understanding of the co-evolution of maize and the maize feeder *O. furnacalis*.

In the present study, we for the first time examined the effects of DIMBOA on the F_1 hybrids of *O. furnacalis* and *O. scapulalis*. The larvae of F_1 hybrids, both Fur × Sca and Sca × Fur, showed tolerance to DIMBOA, similar to that of *O. furnacalis*. The biological significance of this result is considered next. Since the male moths of *O. scapulalis* as well as its hybrid with *O. furnacalis* bear thick midlegs (Phuong, pers. obs.), they are easily distinguished from the male moths of *O. furnacalis*, which bear thin midlegs (Mutuura and Munroe, 1970). Therefore, the presence of hybrids in maize fields should be very rare because only males with thin midlegs have been recognized in maize fields in Japan (Hattori and Mutuura,

1987). Three possibilities can be considered for this rarity. One is that even though *O. furnacalis* and *O. scapulalis* mated easily when they were confined in a cage under laboratory conditions, natural hybridization rarely occurs because highly species-specific sex pheromones assure the attraction of conspecific mates only. Furthermore, even though hybrids are produced at a low rate in nature, the hybrid female may have difficulty in attracting mates because of its unusual sex pheromone. In addition, oviposition of female hybrid moths may not be tuned for maize, and, accordingly, they may lay eggs on plants other than maize. The last two possibilities need to be examined both under laboratory and field conditions because we currently have no information.

Our study reconfirmed the involvement of enzyme(s) that require UDP-glucose as a co-factor, most likely UDP-glucosyltransferase (UGT), in the catabolism of DIMBOA; however, we have consistently been unable to detect the glucosylation product of DIMBOA, DIMBOA-2-glucoside, in *in vitro* assays. Regarding the peak (retention time ≈ 36.8 min) that appeared as DIMBOA diminished in *in vitro* assay (**Fig. 1.3C**), this compound is probably not the sole catabolite of DIMBOA since the peak was considerably small as compared with that of added DIMBOA. Further efforts are required to identify all the catabolites of DIMBOA. Alternatively, identification of the UGT gene and its silencing by RNAi or other methods may provide an insight into catabolism of DIMBOA in *O. furnacalis*.

In our preliminary observations, the larvae of *O. furnacalis* and F_1 hybrids rapidly consumed the stems of 35-day-old maize, which contained approximately 0.19 mg/g fresh weigh of DIMBOA, while the larvae of *O. scapulalis* consumed much less. Therefore, DIMBOA may function as a feeding deterrent to *O.*

scapulalis, and, hence, the growth retardation of *O. scapulalis* larvae feeding on a diet containing DIMBOA may be partly attributed to reduced food intakes. However, since *O. scapulalis* larvae fed on a diet containing DIMBOA survived significantly longer than those completely starved in the no-choice feeding test (**Fig. 1.2**), it is clear that DIMBOA did not totally inhibit the food intake of larvae. In order to evaluate the feeding deterrence of DIMBOA, we need to develop a method that estimates the amount of food ingested by larvae.

CHAPTER 2:

MOLECULAR CLONING OF A CANDIDATE UGT GENE INVOLVED IN DIMBOA CATABOLISM

2.1. Introduction

<UDP-glucosyltransferase>

Sequences of over 310 putative UDP-glucosyltransferase (UGT) genes have been reported from nine different insect species: *Helicoverpa armigera*, *Bombyx mori*, *Drosophila melanogaster*, *Anopheles gambiae*, *Aedes aegypti*, *Tribolium castaneum*, *Apis mellifera*, *Nasonia vitripennis*, and *Acyrthosiphon pisum* (Ahn et al., 2012; Huang et al., 2008; Luque and O'Reilly, 2002; Luque et al., 2002). The silkworm *B. mori* possesses 45 UGT genes (**Table 2**), which is the largest number among the species investigated to date (Ahn et al., 2012; Huang et al., 2008). Lepidopteran UGTs are conventionally classified into 13 families, i.e., UGT33, UGT34, UGT39–44, UGT46–48, UGT50, and UGT340 (Ahn et al., 2012). Molecular cloning and functional characterization of UGTs of *B. mori* (*BmUGT1* and *BmUGT10286*) and *D. melanogaster* (*DmUgt37a1*) have been reported (Daimon et al., 2010; Luque and O'Reilly, 2002; Luque et al., 2002). The full lengths of *BmUGT1*, *BmUGT10286*, and *DmUgt37a1* are 1.60, 1.60, and 1.65 kb, and the predicted protein comprises 520, 520, and 525 amino acids, respectively. BmUGT1 (=UGT40A1) and DmUgt37a1 proteins were shown to catalyze glucosylation of a wide range of phenolic and phenol-derived compounds, in addition to flavonoids, coumarins, and terpenoids (Luque and O'Reilly, 2002; Luque et al., 2002). In contrast, *BmUGT10286* (=*UGT40K1*) was shown to be

responsible for *green b* locus, which is involved in the formation of green cocoon, and BmUGT10286 protein is virtually the sole source of UGT activity toward the 5-*O* position of quercetin, one of flavonoids in mulberry leaves (Daimon et al., 2010). These results are consistent with the presumed role of UGTs in detoxification processes, such as minimizing the harmful effects of ingested plant allelochemicals. However, the substrate specificities of UGTs have been studied in only a few insect species, and very few reports have been published on the detoxification functions of insect UGTs.

Re-glucosylation of ingested DIMBOA, which is produced via hydrolysis of DIMBOA-2-*O*-glucoside by plant β-glucosidase, was reported in a few insect species, e.g., *Spodoptera* spp. (Wouters et al., 2014) and *Mythimna separata* (Sasai et al., 2009); however there was no report about the molecular cloning and functional characterization of UGT genes, which are presumed to be involved in the glucosylation of DIMBOA. In **chapter 1**, I obtained a line of evidence demonstrating the involvement of UGT in the catabolism of DIMBOA in *O. furnacalis*; however, I was not able to detect the expected product, DIMBOA-2-*O*-glucoside in the *in vitro* assay. Moreover, I was also not able to detect this product in the frass of *O. furnacalis* in a preliminary study using HPLC-MS (data not shown). This may appear inconsistent with the involvement of UGT in the catabolism of DIMBOA. It is interesting to know whether DIMBOA-2-*O*-glucoside is produced by the heterologously expressed *O. furnacalis* UGTs. In this chapter, I aimed to perform molecular cloning and functional characterization of UGTs expressed in the midgut and Malpighian tubules of *O. furnacalis*.

<Strategy of research>

In our laboratory, a comprehensive analysis of genes expressed in the pheromone gland of the butterbur borer *Ostrinia zaguliaevi*, a congener of *O. furnacalis*, has been conducted by using RNA-sequencing. With the availability of RNA-seq data for *O. zaguliaevi*, I thought of the utilization of these data for the analysis of UGT genes in *O. furnacalis*, because sequences of homologous genes in the genus *Ostrinia* generally show very high similarity at the nucleotide level, and thus PCR primers designed based on the sequences of *O. zaguliaevi* are expected to work for the amplification of homologous genes in *O. furnacalis*.

Regarding the lepidopteran UGT genes involved in glycosylation of allelochemicals, Daimon et al. (2010), as mentioned above, had shown that a UGT gene, *Bm-UGT10286* (= UGT40K1) catalyzes glucosylation of a flavonoid, quercetin, which is contained in the mulberry. In this chapter, I focused on *Ostrinia* UGT genes that have a relatively close relationship to *Bm-UGT10286*. I found that a homolog of *O. zaguliaevi* contig comp37547 is highly expressed in the midgut tissues of *O. furnacalis*. Therefore, I subsequently cloned this gene from *O. furnacalis* and aimed to perform functional assays of the protein encoded by this gene.

Table 2
Summary of *B. mori* UGT sequences.

Name	GenBank Accession	BGI number	Length (aa)	No. exons	Chr.
UGT33D1	JQ070229	BmUGT013830*	513	4	28
UGT33D2	JQ070230	BmUGT013831*	515	4	28
UGT33D3	JQ070231	BmUGT013833*	515	4	28
UGT33D4	JQ070232	BmUGT013859	520	4	28
UGT33D5	JQ070233	BmUGT013860-1*	520	4	28
UGT33D6	JQ070234	BmUGT013860-2*	515	4	28
UGT33D7	JQ070235	BmUGT013861*	515	4	28
UGT33D8	JQ070236	BmUGT013829	514	4	28
UGT33K1	JQ070237	BmUGT013836-2P*	516	4	28
UGT33N1	JQ070238	BmUGT013836-1*	519	4	28
UGT33Q1	JQ070239	BmUGT013858*	(419)	4(3)	28
UGT33R1	JQ070240	BmUGT007327*	504	4	3
UGT33R2	JQ070241	BmUGT009788P*	510	4	2
BmUGT009787P*		BmUGT009787P*	(271)	4(2)	2
UGT340C1	JQ070242	BmUGT013834-2*	521	4	28
UGT340C2	JQ070243	BmUGT013834-1*	524	4	28
UGT34A2	JQ070244	BmUGT004965	525	4	25
UGT39B1	JQ070245	BmUGT005443*	520	4	8
UGT39C1	JQ070246	BmUGT005442*	525	4	8
UGT40A1	JQ070247	BmUGT010294	520	8	7
UGT40B1	JQ070248	BmUGT010098*	(499)	8(7)	7
UGT40B2P	JQ070249	BmUGT010099-1*	518	8	7
UGT40B3	JQ070250	BmUGT010099-2P*	(474)	8(7)	7
UGT40B4	JQ070251	BmUGT010295*	518	8	7
UGT40G1	JQ070252	BmUGT010287-1	514	8	7
UGT40G2	JQ070253	BmUGT010287-2P	514	8	7
UGT40H1	JQ070254	BmUGT010289-1	516	8	7
UGT40K1	JQ070255	BmUGT010286	522	8	7
UGT40N1	JQ070256	BmUGT010100	519	8	7
UGT40P1	JQ070257	BmUGT010288	519	8	7
UGT40S1	JQ070258	BmUGT010289-2	516	8	7
UGT41A1	JQ070259	BmUGT001338*	518	9	24
UGT41A2	JQ070260	BmUGT003817*	517	9	24
UGT41A3	JQ070261	BmUGT003835*	516	9	24
UGT42A1	JQ070262	BmUGT008508-3*	512	4	18
UGT42A2	JQ070263	BmUGT014622*	509	4	Un.
UGT42B1	JQ070264	BmUGT008508-2*	508	4	18
UGT43B1	JQ070265	BmUGT008508-1*	516	4	18
UGT44A1	JQ070266	BmUGT008508-4*	525	4	18
UGT46A1	JQ070267	BmUGT010432*	527	4	12
UGT46A2	JQ070268	BmUGT010433*	525	4	12
UGT46C2	JQ070269	BmUGT083789*	(448)	4(3)	12
UGT47A1	JQ070270	BmUGT005046*	536	6	25
UGT48C1	JQ070271	BmUGT002854*	506	8	10
UGT50A1	JQ070272	BmUGT008381*	540	6	18
Total 45					

Asterisks denote genes that are corrected from the BGI automatic annotation. Parentheses indicate partial sequences, or partially identified exon numbers.

BmUGT1

BmUGT10286

Excerpted from Ahn et al. (2012)

2.2. Materials and methods

2.2.1. RNA-seq data for the pheromone gland of *Ostrinia zaguliaevi*

I utilized the results of *de novo* RNA-seq analysis of the pheromone gland of *O. zaguliaevi*, a congener of *O. furnacalis*, which were available in our laboratory. Total RNA had been extracted from the pheromone gland of virgin *O. zaguliaevi* females by Dr. Fujii of our laboratory, and all the processes of RNA-seq analysis, i.e., preprocessing of RNA, sequencing using HiSeq 2000, *de novo* assembling of short reads to construct contigs, and annotations of inferred genes, had been performed by Takara-Bio (Kusatsu, Japan).

2.2.2. Isolation of total RNA

Tissues of interest were dissected from the fifth instar larvae of *O. furnacalis* (or *O. scapulalis*) in phosphate-buffered saline [PBS (-), 2.5 mM KCl, 141 mM NaCl, 8.1 mM Na_2HPO_4, and 2.5 mM KH_2PO_4 (pH 7.4)]. Total RNA was prepared from these tissues using RNAiso (Takara Bio) and DNase I (Takara Bio) according to the instructions of the manufacturer.

2.2.3. Screening of candidate UGT genes

Total RNA (16 μg) prepared from the midgut of *O. furnacalis* was reverse-transcribed with an oligo-dT adaptor primer using a PrimeScript™ II First Strand cDNA synthesis Kit (Takara Bio) under the following conditions: 65°C for 5 min, 30°C for 10 min, 42°C for 60 min, and 95°C for 5 min. Four pairs of primers were designed to amplify partial sequences of *O. zaguliaevi* contigs, comp36666, comp37547, comp36019, and comp37715 (**Table 2.1**) by using Primer3plus

(http://www.bioinformatics.nl/ cgi-bin/primer3plus/primer3plus.cgi), and their sequences are listed in **Table 2.2**. PCR was conducted under the following conditions: 94°C for 3 min, 30 cycles of 94°C for 30 s, 50°C for 30 s, and 68°C for 2 min, and finally 72°C for 10 min.

2.2.4. Cloning of UGT candidate genes

PCR fragments of comp3666 and comp37547 homologs were ligated into pGEM-T easy vector, and cloned using competent cell *E. coli* DH5α via a conventional method. Sequencing of the PCR fragments was conducted by FASMAC Co. Ltd (Kanagawa, Japan). After confirming that these fragments were *O. furnacalis* homologs of comp3666 and comp37547, we aimed to directly obtain the "coding DNA sequences (CDS)" of both genes by using primers designed to amplify them (**Table 2.2**). The PCR conditions used to amplify UGT "CDS"s were as follows: 94°C for 3 min, 30 cycles of 94°C for 30 s, 52°C for 30 s, and 68°C for 2 min, and finally 72°C for 10 min. UGTs amplified using "CDS"-primers were cloned and sequences were analyzed by the same method described above.

2.2.5. Tissue distribution of *O. furnacalis* homolog of comp37547

Total RNAs extracted from midgut, fat body, and Malpighian tubules of fifth-instar larvae of *O. furnacalis* and *O. scapulalis*, and reverse transcribed as described above (**2.2.3**). RT-PCR analysis for UGT expression was performed using primers listed in **Table 2.2**. PCR conditions were as follows: 94°C for 3 min, 30 cycles of 94°C for 30 s, 50°C for 30 s, 68°C for 3 min, and finally 72°C for 10 min.

2.2.6. Phylogenetic analysis

Amino acid sequences of interest were aligned using CLUSTAL W (Thompson et al., 1994) and phylogenetic tree was constructed either by the neighbor-joining method or maximum-likelihood method using MEGA6 (Tamura et al., 2013).

2.2.7. Expression of recombinant OfurUGT1

The CDS of OfurUGT1 gene with His-tag was cloned into pFastBac™ 1. The recombinant vector pFastBact-OfurUGT1 was transformed into MAX Efficiency® DH10Bac™ according to the manufacturer's protocol of Bac-to-Bac® Baculovirus Expression System (Invitrogen). The recombinant bacmid OfurUGT1 was isolated and analyzed by PCR using pUC/M13 primers. The recombinant bacmid OfurUGT1 was transfected into insect cells Sf9 cultured in 60-mm dishes using Cellfectin II reagent. After incubation at 27°C for 72 hours, P1 baculoviral stock was collected and kept at 4°C. P2 viral stock was obtained by infection of insect cell with 100 µl P1 and incubation at 27°C for 72 hours. P2 baculoviral stock was applied to the Sf9 infect insect cells, and subsequently assayed for the expression of recombinant OfurUGT1 protein. Total proteins of insect cells infected with UGT recombinant virus were collected and analyzed by SDS/PAGE and Western Blotting. The samples were separated on 10% gels by SDS/PAGE, and transferred to polyvinylidene fluoride membranes (Immobilon-P; Millipore). Expression of the recombinant UGT in Sf9 cells was detected with a monoclonal antibody Anti-His-tag (Medical & Biological Laboratories Co., Ltd, Nagoya, Japan).

2.2.8. Extraction of total protein from midgut of *O. furnacalis*

Midguts of *O. furnacalis* fed on a normal artificial diet and diet containing 0.5 mg/g of DIMBOA were dissected in PBS (-) buffer as described. Five midguts were homogenized in 50 µl cell lysis buffer pH7.8 containing complete mini (1 ×) proteinase inhibitors. After incubation on ice for 60 min, the homogenates were centrifuged at 20,400 × *g* at 4°C for 5 min. The supernatants were added 50 µl 2× SDS sample buffer and boiled for 5 min. The samples were centrifuged again for 2 min and the supernatants were applied for SDS/PAGE to analyze the presence of protein bands specifically induced by the ingestion of DIMBOA.

2.3. Results

2.3.1. Screening of UGT gene candidates responsible for DIMBOA catabolism

The above mentioned RNA-seq data suggested that at least 18 UGT genes (comp15776–comp38172, see APPENDIX for their sequences) are expressed in the pheromone gland of *O. zaguliaevi*. To characterize these UGT genes, their deduced amino acid sequences were aligned with those of representative lepidopteran UGT genes retrieved from the public data bases (UGT33D1–UGT340C1, see APPENDIX for their sequences), and provisional phylogenetic tree was constructed by the neighbor joining method (**Fig. 2.1**). It was found that the UGT genes expressed in the pheromone gland represent a wide range of UGT families reported for lepidopteran species (**Fig. 2.1**).

Among the 18 UGT genes expressed in *Ostrinia*, we tentatively focused on comp3666, comp37547, comp36019, and comp37715 (**Fig. 2.1**; **Table 2.1**), because these genes are relatively closely related to Bm-UGT10280 (UGT40K1), which has been identified from the silkmoth *Bombyx mori* and demonstrated to

exhibit UGT activity against quercetin, a flavonoid allelochemical contained in the mulberry leaves (Daimon et al., 2010). Among the genes tested, fragments of comp36666 and comp37547 were specifically amplified by the PCR experiments of the cDNA prepared from the midgut of *O. furnacalis* , although an extra band was also observed for comp36666 (**Fig. 2.2**). Subsequently, we examined the expression levels of comp36666 and comp37547 in the midgut and fat body of *O. furnacalis* larvae that had fed on artificial diet and corn by using the primer pairs designed to amplify the "CDS" of comp3666 and comp37547 (**Tables 2.1, 2.2**). Although the "CDS" of comp37547 was successfully amplified, that of comp3666 was not (**Fig. 2.3**). Interestingly, the expression levels of comp37547 "CDS" in both midgut and fat body were elevated in the larvae that had fed on corn as compared with those fed on a normal artificial diet lacking DIMBOA (**Fig. 2.3**). I therefore considered that comp37547 is a good candidate of UGT responsible for the catabolism of DIMBOA.

2.3.2. Determination of the full sequence of OfurUGT1, the *O. furnacalis* homolog of comp37547

Our quick investigation using additional primers designed in the middle of comp36666 suggested that 5'-end sequence of comp36666 is not representing true sequence due to inaccurate *de novo* assembling (data not shown). Accordingly, I hereafter focused only on the *O. furnacalis* homolog of comp37547, and I referred to this homolog as OfurUGT1 in this thesis. Because the predicted "CDS" of comp37547 was substantially shorter than that of known UGTs, we suspected that the 5'-end sequence of comp37547 is also not perfectly accurate. We therefore performed RACE experiments to determine the full length sequence of *OfurUGT1*

gene. The full length OfurUGT1 sequence was found to comprise 1733 bp with a CDS of 1563 bp encoding a protein of 520 amino acids (**Fig. 2.4**). The predicted molecular mass of OfurUGT1 protein is approximately 58.23 kDa. As we suspected, the N terminal of the amino acid sequence of comp37547 protein is truncated as compared with that of OfurUGT1 (**Fig. 2.5**). Comparison of the nucleotide sequences of OfurUGT1 and comp37547 indicated that an erroneous frame shift, which caused erroneous estimation of the CDS, occurred due to inaccurate *de novo* assembling of the sequence of comp37547 (**Fig. 2.6**).

2.3.3. Tissue distribution pattern of *OfurUGT1*

The expression levels of *OfurUGT1* (1575 bp) in various tissues of *O. furnacalis* larvae were compared by semi-quantitative RT-PCR (**Fig. 2.7**). Among the tissues examined, *OfurUGT1* was most highly expressed in the midgut. Relatively high levels of expression were also observed in the Malpighian tubules and fat body.

2.3.4. Comparison of the expression levels of OfurUGT1 in *O. furnacalis* and *O. scapulalis*

The expression levels of OfurUGT1 and its *O. scapulalis* homolog (referred to as UGT1 for brevity) in the midgut, fat body, and Malpighian tubules were compared between the two species, *O. furnacalis* and *O. scapulalis* (**Fig. 2.8**). The expression levels of UGT1 in the midgut and Malpighian tubules of *O. furnacalis* were higher than the levels in the corresponding tissues of *O. scapulalis* (**Fig. 2.8**). In particular, the expression level of UGT1 in the midgut of *O. furnacalis* was

remarkably higher than the level of UGT1 in the midgut of *O. scapulalis*. These results suggest that OfurUGT1 in *O. furnacalis* may be responsible for the catabolism of maize allelochemicals in this species.

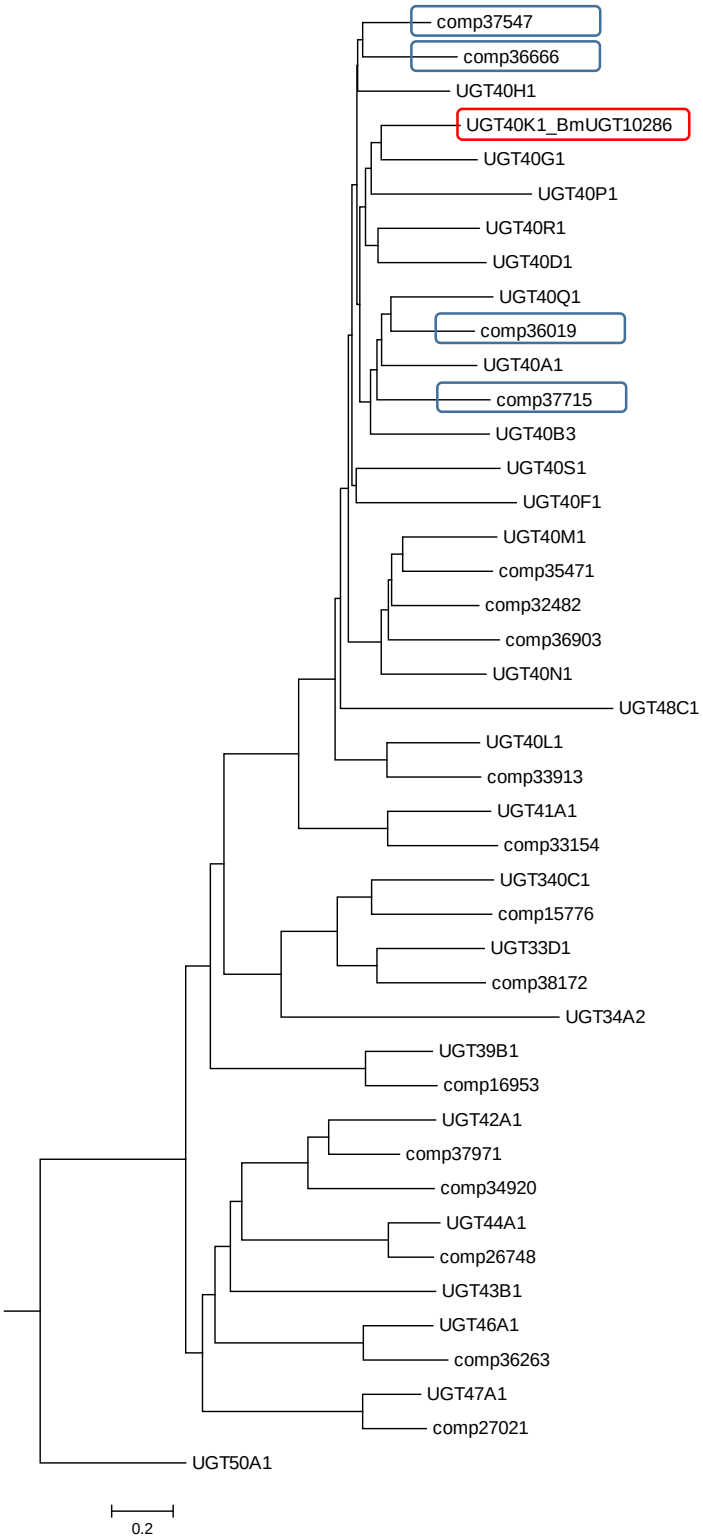


Figure 2.1. Neighbor-Joining tree of amino acid sequences of *Ostrinia zaguliaevi* UGT proteins (comp15776-comp38172) and those of representative lepidopteran UGT proteins obtained from public databases (UGT33D1-UGT340C1). Red rectangle indicates the gene known to catalyze glucosylation of quercetin. Blue rectangles indicate genes I focused on in this thesis. Refer to the APPENDIX for the sequences and GenBank Accession Numbers of the referenced genes.

Table 2.1. Sequences of *Ostrinia zaguliaevi* UGT contigs obtained by RNA-seq analysis, homologs of which may be involved in the catabolism of DIMBOA in *O. furnacalis*.

```
>comp36019
   1 ATGGATCTAA CAAAACTATT GTTTCTTCTA TTGTTTGGGT TTTCAAGTGC
  51 GTACAAAATA CTAGTGGTGT TTCCGTACCC AGGGAAAAGC CATACGATCC
 101 TGGGTGAGGG ATTTGTGAAA CATCTCGTGA GGGCTGGACA TGAGGTCACA
 151 TACATAACTC CGATACCGAT AAACAATCCG CCTAAAGGGC TTCGACAAAT
 201 TGATGTGTCA AGCAATATCA AAACATTTGA ATCAATGTCT TCTTCATTAA
 251 GTTTTAAAAC GGTGTTAAAC AAAGAAGCAG ACCTAAAGGA CACAAGAGCA
 301 TGGGTGGGCG TCATAAACAA CATCGCCAAC CAAACGATAT GGCACCATAA
 351 CGTTCAGAAG CTGATGTATG ACGACAATGA GGAGTTTGAC CTGGTGATCG
 401 CAGAGTGGCT GTATACGGAA CTTTATTGTG GATTCGCAGC CGTCTTCAAC
 451 TGCCCGTTTA TATGGTCTTC CTCCATCGAC CCCCACGGGC TAGTCTTAGG
 501 GCTGATCGAT GAGGAACCCA ACCCGGCCTA CACAGCCAAC CACATGTCGT
 551 CCTTTGAGGC ACCCTTCACA TTCTCACAGC GGCTCGAAGA ACTGTGGGAA
 601 GTGATCTACT TGAAGTACAT GAAATGGGCA ATATACGACC ATGAAAACCG
 651 TATTTTCCAA GAGGGGTATG GTCCAGCTGT AGCCAAAAGA GGTCGAACAA
 701 TTCCCTCACT GTATGAAGTC AGCCATAACG CTTCTCTAAT GTTCGGGAAC
 751 TCGCACTTCT CGTCTGGTAG ACCAGTGAGG TTGCCGCAGA ATTATATCCC
 801 AATAGCTGGA TATCATATTG ATGAAGAGGT TGACAAACCA TTGCCAACGG
 851 ATATTCAAAA GATAATGAAT AACGCGCAAC ACGGCGTCAT ATACTTCAGC
 901 ATGGGATCCA TGATCAGGAG CAGCTCCATG CCTGATGGAA TAAAGCAAGG
 951 GTTCCTGAAA ATGTTCGGCA GTCTCAAGCA AACTGTCATC TGGAAGTTCG
1001 AGGAAGTATT GCCAAATCTG CCCAAAAACG TGCACATCCT GAAATGGGCT
1051 CCTCAGCAAA GTATTTTAGC TCATCCCAAC TGCCTCGTAT TCATCTCCCA
1101 CGGGGGCCTG CTCTCAACCT CCGAGGCGCT TCACTACGGC GTGCCCATCA
1151 TTGGGATCCC AATGTTCGCG GACCAGTTTA TCAATGTGGA TCGCGCCATG
1201 AAGAAAGGCT TCGCCCTAAA GGTCGACATC GCAGAAGACA TGACAGTTCA
1251 CTTGAAAGCA GCGATTGAAG AGATTTTGGG AAACCCCAGA TACCATGAGC
1301 GGATGAAGGA ACTGTCATTT ATCTATCACC ACCGCACTAC GACTCCTGGG
1351 CAAGAGATTC TGCACTGGGT GGACCACGTC GTCAAGACAA GAGGTGCCTT
1401 GCACCTTCGG TCTCCAGCAC TGGACGTGCC CTTCTACCAG AAGATATACC
1451 TGGATCTGAT AACTTTGATA GCTGTCGCAA CTATTGTACT GTTTAGAATT
1501 GCGAAAAGAC TGGTTTGTAA AAGTGCGGTG ACGAAGAAAG TTAAGAAGAA
1551 TTAAACAAAG

>comp37715
   1 ATGCTCGCTC GCGCAGTGGT CCTATACTTG GTGTGCGCAG GCGCAAGTGC
  51 CCTGCGCCTG CTGCTGGTGT TTCCAGTACC GGGACCCAGC CACGCCATCC
 101 TGGCTGGGGG GCTCAGCAAG CACTTGATTG GGGCTGGACA TGAGATCACA
 151 TGCATCACCC CGCTCCCAAG CAAAAACGCC TCGAAGAACC TCCGTCAAGT
 201 CGATATTTCA GCAAACTTCC AACTCGTCCC ATTGGGAGAT GTCCTTCAAC
 251 TCGAGAAGAT AATGTCAAAG GAAATAAACA TGAAAGATTT GGCGTTCATA
 301 AAATCGCTGA TGATTTCCCT CGCCAACGCC ACTCTGACCA ATCCGAACGT
 351 CAAGAGGCTG ATGGAAGACC CGGCTGAACG CTTCGACGCT GTCATTGCTG
 401 AGTGGATGTA CACTGAACTT TTCGCTGGAA TCTCAGCCGT CTTCAACTGC
 451 CCCCTAATCT GGTTTTCCTC CATGGACCCC CAAGCTCTGG TCCTTCGTCT
 501 GATCGACGGG ACCCCCAGCC CGGCGTACTT CGCCGACCCA ATGTCTGCAG
 551 AACACCCTCC TTTTGACTTC TGGCAGAGAA TAAAAGGACT CTGGCTTCTT
 601 TTTCGAAGGA TGAAGCTGGA ATGGTCTACA AGAAGCATTG AAGACTCAAT
 651 CTACAACTCA GAATATGGAC CAGTAGCGGC CGTACGAGGT ATCACCCTTC
 701 CCCCTCTAAC GGTGATGAGG TACAACGCTT CCCTCATGCT GGGGAACTCC
 751 CACATATCCA TGGGACAGTC CATCAGTCTG CCGCAAAATT ATAAAGAAAT
 801 ACTCGGGTAC CACATAGCGG ATAAGGTGCA GCCGTTGCCT GATAACATCA
```

```
 851 AAAAGATAAT GGACGAAGCG AAACATGGCG TGATATACTT CAGCATGGGG
 901 TCCATGCTTA AAAGCACAAC GTTCCCCGAA GCGCTGAAGA GGGAACTCTT
 951 AGACATGTTC CGAGGTCTCA AGCAGACTGT TCTCTGGAAA TTCGAGGACG
1001 TACCACCGAA ATTGCCTGCG AACGTCCATG TTGTCAAGTG GGCTCCACAG
1051 CAAGACGTTT TGGCTCATCC CAATTGTGTG CTGTTCATCA CCCACGGAGG
1101 TCTTCTGTCC ATCACTGAGG CGATTCATCA CGCGGTCCCC ATCATAGGGA
1151 TCCCGATGTT CGCAGACCAG TTCCTGAACA TCAATCGCGC GGTCAGAAAG
1201 GGGTTCGGGA TCAAGGTCAG CCTGGACTGG GATTTGACGA AGAATTTGAA
1251 GTCGGCTATT GAAGAAATAT TTCGGAACTT TAGCTACCAA GAGAAAGTGA
1301 AGGAGGTTTC ATTTGTCTAC CACCACCGTC CAGCGCCACC TGGTGCAGAA
1351 CTCGTGCACT GGATAGAACA CGTGGTCAAA ACCCGCGGGG CGTTGCATCT
1401 GAGGTCTCCA GCACTGAACG TAGCGTTCTA CCAGAAGATG TACCTAGATC
1451 TAGCAGCAGT AGTGGTGGTA GTTCTTGTAG TGGTAGTAAA AGTTGTAAAG
1501 AGTATTCTGA AGTCGAAGAA AGGAAGTGAG AAATCGAAGG AGAAACAGAG
1551 ATGA

>comp36666
   1 ATGTCTCCGC CAATTTCGTC TTCTTGCAAA CTAAAGAAAT TTTCAATCGT
  51 ATGTCTACTG CTGGCATCCC TCCAAGTAGG GTTTGCCTAC AAGATCCTCG
 101 TGGTGTTCCC GATGCCAGGG AAGAGCCACA CAATCCTTGG GGAAGGAGTC
 151 GTCCGACACT TGGCAAATGC TCAGCATGAT GTTACATATA TAACTCCAAT
 201 TCTTCTGAAA TCTCCGCCGA AAAATGTGAG ACAAATAGAT GTAACTTCCA
 251 ATTTCGACTT CATGAAAAGC AATGATATGT TGAATCTCAA GACTCACATG
 301 GACAATAATG GTGAAATGGA TTTGACCATG GTCTTCAACA TGATGATGCA
 351 AATCCACAAC ATGACGTACC ACAACCCGAA CGTGCAGAAA CTGCTGTCAG
 401 ACACTAGCGA GCAGTTCGAC GTCGTCGTCG CTGAGTGGAT GTTCAGTGAA
 451 CTGTACTCTG GATTCTCAGC AATTTTCAAC GTTCCACTCA TCTGGGTGTC
 501 CACCATCGAA CCCCACTGGC TGGTGCTGCG CCTGATGGAC GAAGTCTGTA
 551 ACCCTGCTTA CACTTCGGAT ACACTGTCCG CCAATATTCC TCCTTTCTCA
 601 TTCATTACTC GGCTTCAACA ACTCGGAAGC CAAATATTTG GATTTGGTTT
 651 AAAGAAATTT CTTATAGAAG GCTTCGAGGA GAAGGCATAC GCTGAACTCA
 701 CTCCATATTT CAAAATGAGA GGTCGAGAGG CTCCAGCATT TAAAGAGCTG
 751 GCGTTCAACG CTTCTCTCAT GCTTGGAAAT TCCCACGTGT CATTAGGCCA
 801 GCCTATGTCG TTGCCACAGA GCTACATAAA CGTTGGTGGA TACCATATTG
 851 AGACGAACTT GGCACCTCTT CCTAAGGACT TACAGATCCT GATGGACAAC
 901 GCCAAGCACG GCGTCATATA CTTCAGCTTG GGGTCCAACA TCCAAAGTAA
 951 GGACTTGCCG GACGAACTGA AGCAGAGTCT CCTGAAGATG TTCGGAGAAC
1001 TAAAACACAC AGTTATTTGG AAATTCGAAG AGACGTTGCC TGGACTGCCG
1051 AGCAACGTGC ATATCCTTAA ATGGGCTCCT CAGCCTAGCA TCTTGGCGCA
1101 CCCCAACTGT ATCCTTTTCA TCACGCACGG TGGTCTCCTC TCCACCACCG
1151 AGACCATCCA CTTTGGAAAG CCGATCATTG GAATTCCAGT GTTCGCCGAT
1201 CAGTTCGTCA ACGTGAACAG AGCCGTAGCA AAGGGATTTG CCAAGAGAGT
1251 CGACCTGTCC TACGGCATGG CCCCCGAGCT TGGAGCAGCC ATCAAGGATA
1301 TTATCGGGGA TCCAAAATAC TCCAACAACG TGAAACAACT ATCACTGATA
1351 TACCACGACC GCCCAGTGCC TCCTGGTAAG GAGCTGGTGC ACTGGGTGGA
1401 GCACGTGGTC AAGACTAACG GCGCCCCCCA TCTTCGCTCA CCAGCATTAA
1451 GCGTACCTTT CTACCAGAAA ATGTACCTCG ATCTCCTTGC CTTGATAGTA
1501 GTTATCTTAC TAGGAATAAG AGCAATATTT AGAAGAATAT TCAAGAAGAA
1551 ATCAAGTAAA GTAAAGAAAG AGTGA

>comp37547
   1 CTTTAGTAGA TAGTGCGTTG CGGTGCGTGG TGTAAAAAAC ACGCGTGTCC
  51 ACAGCTGTCG TGATAAGAAC TGTAATCAGT AACTTTTATT AATAGAGTTC
 101 AAAAAGAATC AGTAATAAAG GATTGTGTGT GACTAATGTT TGTTGGGTGT
 151 CGATGGTGTT GAAGTGTGAA GTGATGATAT TTGAGGATTA TTATTAATAA
 201 CAAAGTATTC ACATCAACGC GTCGTGTTTT GTATTTACTT ATATAATGAA
 251 TCTTCTAGGA AAATTCCTGC TAAGTGCAGC TTTATGCTGG AGTATCAGCG
 301 AGGCGTATAA GATTCTGGTG GTGTTCCCTC TACCAGGCCC GAGCCACGGC
 351 ATCCTGGGAG AAGGCGTGGT GCGGCATCTG CTGAATGCTG GACATGAGGT
```

```
 401 CACTTACGTC ACTCCTTTCC CGAAAGACAG CAAGAATCCG AAGTTGAAGC
 451 AGATAGATGT CTCAGTCGAT GAGGCAGCTA TGCCTAAGAT GAACCTCAAG
 501 GACATATTGA ACAAGGAGCA GAGCGCGTTT GATCCGAACA AATTCTTCGA
 551 TTTCACCATT GGGACGCACC AAAGGGCTAT ACAGAATGAA AACATGCAGA
 601 AGATACTGAA TGATCCTCAA CAGACGAGGT GGTGGTGGCT GAGTGGATGG
 651 TGTGCGAACT CTACACTGGG CTCGCGGCTT TCTACGGCTG TCCCTTCATC
 701 TGGGTATCAA CTATTGAGCC TCACTCCACA ATCCTGTCAT TGATCGACGA
 751 CAGCTTGAAC CCAGCTTACA ACCCTGGCCT ATTCTCCAAT ACTATTCCTC
 801 CATACAACTT TGTGGAGCGC GCGAAGGAAT TGTTAATGTC CGTCGCAAAT
 851 GTTGTGTTGA AAGATGTGGT CTTAGTCACA TATTACGAGC AAGCAGCGTA
 901 CGACGAATTG TACGTGCCTC TTTTGAAGAA GAAGGGCCGT CCTGTCCTCA
 951 CATACGAAGA AGTGAGGTAC AACGTGTCGC TGGTTTTGGG CAACTCGCAC
1001 GTATCCTTGG GCCAGGCCAC CAGGCTGCCG CAGAACTACA AACCCATTGG
1051 TGGATATCAT ATTGACACTA ATTTCAAACC GCTACCCGAG GATCTAAAAA
1101 ATCTGCTAGA TAATGCTAAA AATGGCGTAA TATACTTCAG CATGGGATCC
1151 AATATAAAGA GTAAGGACAT GCCAGAGGAA CTGAAGAGGA GCCTCCTCAA
1201 AATGTTTTCT GGACTCAAGC AGACGGTCTT GTGGAAGTTC GAAGAAGTCC
1251 TGACAGATTT GCCCGAAAAT GTGCACATAG TGAAATGGGC GCCGCAGCCT
1301 GCCATCCTTT CGCATCCAAA CTGCATCCTC TTTATAACGC ACGGTGGTCT
1351 CCTTTCGTAC ACTGAAGCAG TCCATTTCGG GAAGCCCACA GTTGGGATTC
1401 CAGTATTCGC CGATCAGTTC CTCAACGTGG AGCGAATTGG GAAGAAAGGC
1451 TTGGGGAAGA GAGTAGACCT TTCTTATACA ATGGCTGATG ATTTGAAGAT
1501 CGCTATTAAC GAAGTCCTTT CCAATCCAAG CTACATGACC AAAGCGAAGG
1551 AACTCTCCCT GATCTACCAC GACCGGCCAA CGCCCCCTGG TGGAGAGTTA
1601 GTACACTGGG TGGAGCACGT CATCAAGACT GCTGGCGCCC CCCACCTGAG
1651 GTCACCTGCT TTAAACGTGC CCTTCTACCA GAAAATGTAC CTGGACCTAG
1701 CAGCCTTAGT AGTTGTAGTT ATTATTACCC TTAGATTAAT TGTGAAACGT
1751 CTGTGCAATA GTTGTAGGAA AAAGAAAATA AGCAGCGAAA AGAAAAATAA
1801 GTGAATAGTT AATGTTGGTG ATACTCGTAT CATGGTGATA TTGTGATATG
1851 ATTTTGTACA ATAAAATTAA ATAATGTAGG ATATTGTTGT TTAAAAATAA
1901 AC
```

Table 2.2. List of primer sequences used in this study

Primer Name	Nucleotide sequence (5' to 3' end)	expected product size
		bp
comp36019F2	TCCTTTGAGGCACCCTTCACA	233
comp36019R2	AACCTCACTGGTCTACCAGAC	
comp37715F2	TGCAGAACACCCTCCTTTTGAC	234
comp37715R2	AGACTGATGGACTGTCCCATG	
comp36666F2	AACGTGAACAGGGCCGTAGC	189
comp36666R2	ACCCAGTGCACCAGCTCCTT	
comp37547F1	CAACGTGGAGCGAATTGGAAAG	191
comp37547R1	CCACCCAGTGTACTAACTCTCC	
UGT36666-"CDS"-F1	ACGCCGATACCTCTGAAAACTC	1624
UGT36666-"CDS"-R1	CCGATGTGTCAAGTTCGTTCAC	
UGT37547-"CDS"-F1	ACGCACCAAAGGGCTATACAG	1203
UGT37547-"CDS"-R1	ACAACTATTGCACAGACGTTTCAC	
UGT-RACE-5'	CCTCGGGTAGCGGTTTGAAATTAG	
UGT-RACE-5'nested	CTTCGCGCGCTCCACAAAGTTGTAT	
UGT-RACE-3'	GGGCCGTCCAGTCCTCACTTATGA	
UGT-RACE-3'nested	CAAGCAGACGGTCTTGTGGAAGTT	
pFB-OfurUGT1-F1	CTCGAGATGAATCTTTTAGGAAAATTCCTGCTAAGTGC	
pFB-OfurUGT1-R1	GGTACCCTATTCACTTATTCTTCTTCTCACTGC	
pFB-OfurUGT1-R2	GGTACCTCAGTGATGGTGATGGTGATGCTTATTCTTCT TCTCACTGC	
Actin F1	GAGGCCCAGAGCAAGAGAGGTAT	784
Actin R1	GTGATTTCCTTCTGCATACGGTC	
Actin F2	CCCGCCATGTACGTCGCCATCCA	565

PCR conditions:

10 x buffer:	1
dNTP:	1
Primer F	0.5
Primer R	0.5
Ex Taq	0.1
cDNA template	0.5
mQ (DW):	6.5
Total:	**10.1 µl**

Temperature cycling:

	1 cycle	30 cycles			1 cycle	
Temperature:	94ºC	94ºC	50ºC	68ºC	72ºC	4ºC
Time:	3 min	30 s	30 s	2 min	10 min	∞

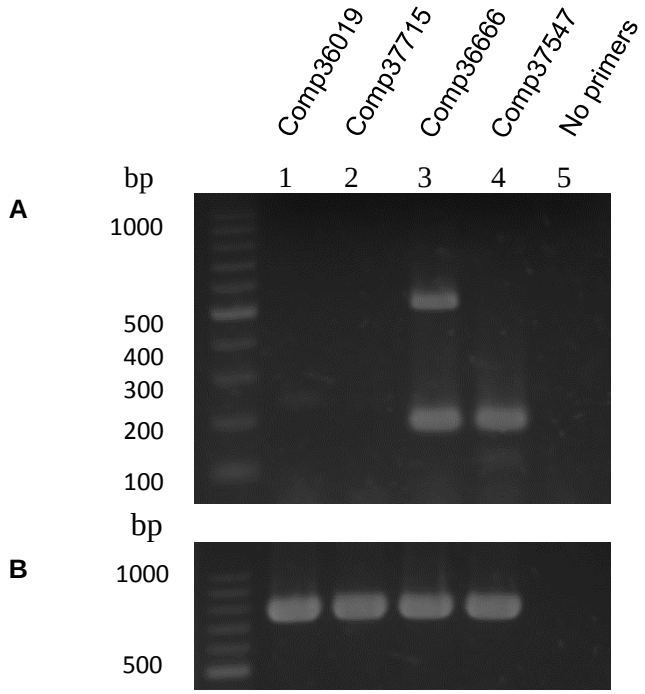


Figure 2.2. Results of PCR using cDNA prepared from the midgut of *O. furnacalis* and four primer pairs designed to amplify partial sequences of comp36019 (233 bp), comp37715 (234 bp), comp36666 (189 bp), and comp37547 (191 bp) (**A**). Numbers in parentheses indicate expected size of the PCR products. Actin was used as a control (784 bp) (**B**)

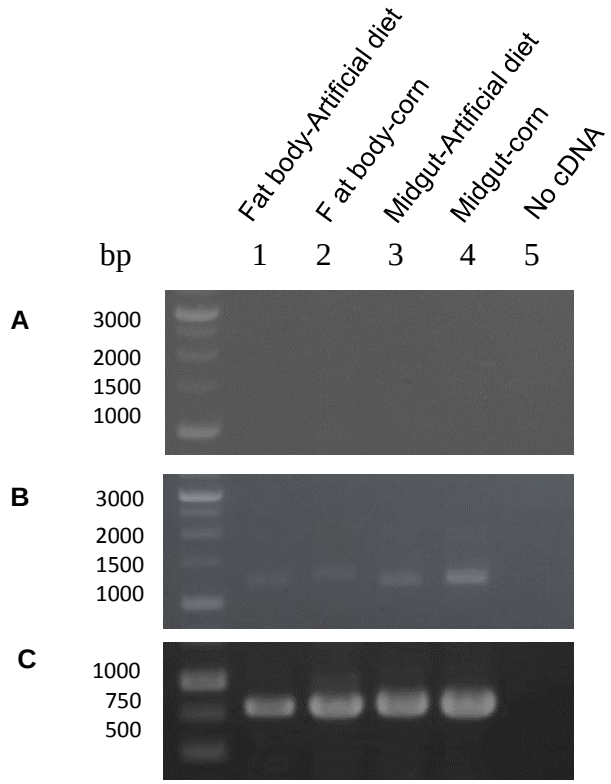


Figure 2.3. Expression of comp36666 "CDS" (1624bp) (**A**) and comp37547 "CDS" (1203 bp) (**B**) in the fat body and midgut tissues of *O. furnacalis* larvae that had fed on artificial diet and corn. Actin used as a control (784 bp) (**C**).

```
1    GAAAATTAAAGGATTGTGACTAATGTTTGTTGGGTGTCGATTGTGTTGAAGTTTGAAGTG
61   ATGATATTTTAGGATTATTTATGTAATAACAAAGTATTCTCATCAACGCGTAGTGTTTTG
121  TATTTAAATATAATGAATCTTTTAGGAAAATTCCTGCTAAGTGCAGCTCTATGCTTGAGT
                M  N  L  L  G  K  F  L  L  S  A  A  L  C  L  S    16
181  ATCAGCGAGGCGTATAAGATTCTGGTGGTGTTCCCTCTACCAGGCCCGAGCCACGGCATC
     I  S  E  A  Y  K  I  L  V  V  F  P  L  P  G  P  S  H  G  I    36
241  CTGGGAGATGGCGTGGTGCGGCATCTGCTGAATGCTGGACATGAGGTCACTTACGTCACT
     L  G  D  G  V  V  R  H  L  L  N  A  G  H  E  V  T  Y  V  T    56
301  CCGTTCCCGAAAGACAGCAAGAATCCGAAGTTGAAGCAGATAGATGTGTCAGTCGACGAT
     P  F  P  K  D  S  K  N  P  K  L  K  Q  I  D  V  S  V  D  D    76
361  GCAGCCATGCCTAAGATGAACCTCAAGGACATATTGAACAAGGAGCAGAGCGCGTTTGAT
     A  A  M  P  K  M  N  L  K  D  I  L  N  K  E  Q  S  A  F  D    96
421  CCGAACAAATTCTTCGATTTCACCATTGGGACGCACCAAAGGGCTATACAGAATGAGAAC
     P  N  K  F  F  D  F  T  I  G  T  H  Q  R  A  I  Q  N  E  N   116
481  ATGCAGAAGATACTGAATGATCCTCAACAGACCTTCGACGTGGTGGTGGCTGAGTGGATG
     M  Q  K  I  L  N  D  P  Q  Q  T  F  D  V  V  V  A  E  W  M   136
541  GTGTGCGAACTCTACACTGGGCTCGCGGCTTTCTACGGCTGTCCCTTCATCTGGGTATCA
     V  C  E  L  Y  T  G  L  A  A  F  Y  G  C  P  F  I  W  V  S   156
601  ACCGTTGAGCCTCACTCTACAATCCTGTCATTGATCGATGACAGCTTGAACCCAGCTTAC
     T  V  E  P  H  Sw T  I  L  S  L  I  D  D  S  L  N  P  A  Y   176
661  AACCCTGGCCTATTCTCCACTACTATTCCTCCATACAACTTTGTGGAGCGCGCGAAGGAA
     N  P  G  L  F  S  T  T  I  P  P  Y  N  F  V  E  R  A  K  E   196
721  TTGTTACTGTCCGTCGCAAATGTTGTGTTGAAAGATGTGGTCTTAGTCAGATATTACGAG
     L  L  L  S  V  A  N  V  V  L  K  D  V  V  L  V  R  Y  Y  E   216
781  CAAGCAGCGTACGACGAATTGTACGTACCACTTTTGAAGAAGAAGGGCCGTCCAGTCCTC
     Q  A  A  Y  D  E  L  Y  V  P  L  L  K  K  K  G  R  P  V  L   236
841  ACTTATGAAGAAGTGAGGTACAACGTGTCGCTGGTTTTGGGCAACTCGCACGTATCCTTG
     T  Y  E  E  V  R  Y  N  V  S  L  V  L  G  N  S  H  V  S  L   256
901  GGCCAGGCCACCAGGCTGCCGCAGAACTACAAACCCATTGGTGGATATCATATTGACACT
     G  Q  A  T  R  L  P  Q  N  Y  K  P  I  G  G  Y  H  I  D  T   276
961  AATTTCAAACCGCTACCCGAGGATCTAAAAAATCTGCTAGATAATGCTAAAAATGGCGTA
     N  F  K  P  L  P  E  D  L  K  N  L  L  D  N  A  K  N  G  V   296
1021 ATATACTTCAGCATGGGATCCAATATAAAGAGTAAGGACATGCCAGAGGAACTCAAGAGG
     I  Y  F  S  M  G  S  N  I  K  S  K  D  M  P  E  E  L  K  R   316
1081 AGCCTCCTCAAAATGTTTTCTGGACTCAAGCAGACGGTCTTGTGGAAGTTCGAAGAAGTC
     S  L  L  K  M  F  S  G  L  K  Q  T  V  L  W  K  F  E  E  V   336
1141 CTGACAGATTTGCCCAAAAATGTGCACATAGTGAAATGGGCGCCGCAACCTGCTATCCTT
     L  T  D  L  P  K  N  V  H  I  V  K  W  A  P  Q  P  A  I  L   356
1201 TCGCATCCTAACTGCATCCTGTTTATAACGCACGGCGGTCTCCTTTCGTACACTGAAGCA
     S  H  P  N  C  I  L  F  I  T  H  G  G  L  L  S  Y  T  E  A   376
1261 GTCCATTTCGGGAAGCCCACAGTTGGGATCCCAGTGTTCGCCGATCAGTTCCTTAACGTG
     V  H  F  G  K  P  T  V  G  I  P  V  F  A  D  Q  F  L  N  V   396
1321 GAGAGGATTGGAAAGAAGGGCTTGGGGAAGAGAGTAGACCTTTCGTACACAATGGCTGAT
     E  R  I  G  K  K  G  L  G  K  R  V  D  L  S  Y  T  M  A  D   416
1381 GATTTGAAGATCGCTATTAACGACGTCCTTTCCAATCCAAGCTACATGACCAAAGCGAAG
     D  L  K  I  A  I  N  D  V  L  S  N  P  S  Y  M  T  K  A  K   436
1441 GAACTCTCCCTGATCTACCACGACCGGCCAACGCCCCCTGGTGGAGAGTTAGTACACTGG
     E  L  S  L  I  Y  H  D  R  P  T  P  P  G  G  E  L  V  H  W   456
1501 GTGGAGCACGTCATCAAGACTGGTGGCGCCCCCCACCTGCGGTCTCCCGCTTTAAACGTG
     V  E  H  V  I  K  T  G  G  A  P  H  L  R  S  P  A  L  N  V   476
1561 CCCTTCTACCAGAAGATGTACCTGGACTTAGCAGCCTTAGTAGTTGTAGTTATTATTGCC
     P  F  Y  Q  K  M  Y  L  D  L  A  A  L  V  V  V  V  I  I  A   496
1621 CTTAAATTAATTGTGAAGCGTGTGTGCAACAGTTGTAGGAAAAAGAAAGTAAGCAGTGAG
     L  K  L  I  V  K  R  V  C  N  S  C  R  K  K  K  V  S  S  E   516
1681 AAGAAGAATAAGTGAATAGTTATAATATGTTGGTGATACTCGTATCATGGTGA
     K  K  N  K  *                                                 520
```

Figure 2.4. Nucleotide sequence of OfurUGT1 (1733bp) and its predicted amino acid sequence (520 aa). Underlines indicate the positions of primers (UGT-"CDS": Red; UGT-RACE- 5' and UGT-RACE-5' nested: Purple; UGT-RACE- 3' and UGT-RACE-3' nested: Green), which were used for sequencing the full length of *OfurUGT1*.

```
OfurUGT1        MNLLGKFLLSAALCLSISEAYKILVVFPLPGPSHGILGDGVVRHLLNAGHEVTYVTPFPK 60
comp37547       ------------------------------------------------------------

OfurUGT1        DSKNPKLKQIDVSVDDAAMPKMNLKDILNKEQSAFDPNKFFDFTIGTHQRAIQNENMQKI 120
comp37547       ------------------------------------------------------------

OfurUGT1        LNDPQQTFDVVVAEWMVCELYTGLAAFYGCPFIWVSTVEPHSTILSLIDDSLNPAYNPGL 180
comp37547       ---------------MVCELYTGLAAFYGCPFIWVSTIEPHSTILSLIDDSLNPAYNPGL 45
                              **********************:**********************

OfurUGT1        FSTTIPPYNFVERAKELLLSVANVVLKDVVLVRYYEQAAYDELYVPLLKKKGRPVLTYEE 240
comp37547       FSNTIPPYNFVERAKELLMSVANVVLKDVVLVTYYEQAAYDELYVPLLKKKGRPVLTYEE 105
                **.***************:************* **************************

OfurUGT1        VRYNVSLVLGNSHVSLGQATRLPQNYKPIGGYHIDTNFKPLPEDLKNLLDNAKNGVIYFS 300
comp37547       VRYNVSLVLGNSHVSLGQATRLPQNYKPIGGYHIDTNFKPLPEDLKNLLDNAKNGVIYFS 165
                ************************************************************

OfurUGT1        MGSNIKSKDMPEELKRSLLKMFSGLKQTVLWKFEEVLTDLPKNVHIVKWAPQPAILSHPN 360
comp37547       MGSNIKSKDMPEELKRSLLKMFSGLKQTVLWKFEEVLTDLPENVHIVKWAPQPAILSHPN 225
                ****************************************:*******************

OfurUGT1        CILFITHGGLLSYTEAVHFGKPTVGIPVFADQFLNVERIGKKGLGKRVDLSYTMADDLKI 420
comp37547       CILFITHGGLLSYTEAVHFGKPTVGIPVFADQFLNVERIGKKGLGKRVDLSYTMADDLKI 285
                ************************************************************

OfurUGT1        AINDVLSNPSYMTKAKELSLIYHDRPTPPGGELVHWVEHVIKTGGAPHLRSPALNVPFYQ 480
comp37547       AINEVLSNPSYMTKAKELSLIYHDRPTPPGGELVHWVEHVIKTAGAPHLRSPALNVPFYQ 345
                ***:****************************************.****************

OfurUGT1        KMYLDLAALVVVVIIALKLIVKRVCNSCRKKKVSSEKKNK 520
comp37547       KMYLDLAALVVVVIITLRLIVKRLCNSCRKKKISSEKKNK 385
                ***************:*:*****:********:*******
```

Figure 2.5. Comparison of the predicted amino acid sequences of OfurUGT1 and comp37547. The N terminal of the amino acid sequence of comp37547 is truncated as compared with that of OfurUGT1.

Figure 2.6. Comparison of the nucleotide sequences of OfurUGT1 and comp37547. An erroneous frame shift, which had occurred due to inaccurate *de novo* assembling of comp37547, was found to have caused erroneous estimation of its CDS.


comp37547 CCTCAAAATGTTTTCTGGACTCAAGCAGACGGTCTTGTGGAAGTTCGAAGAAGTCCTGAC 1254
OfurUGT1 CCTCAAAATGTTTTCTGGACTCAAGCAGACGGTCTTGTGGAAGTTCGAAGAAGTCCTGAC 1145
comp37547 AGATTTGCCCGAAAATGTGCACATAGTGAAATGGGCGCCGCAGCCTGCCATCCTTTCGCA 1314
OfurUGT1 AGATTTGCCCAAAAATGTGCACATAGTGAAATGGGCGCCGCAACCTGCTATCCTTTCGCA 1205
comp37547 TCCAAACTGCATCCTCTTTATAACGCACGGTGGTCTCCTTTCGTACACTGAAGCAGTCCA 1374
OfurUGT1 TCCTAACTGCATCCTGTTTATAACGCACGGCGGTCTCCTTTCGTACACTGAAGCAGTCCA 1265
comp37547 TTTCGGGAAGCCCACAGTTGGGATTCCAGTATTCGCCGATCAGTTCCTCAACGTGGAGCG 1434
OfurUGT1 TTTCGGGAAGCCCACAGTTGGGATCCCAGTGTTCGCCGATCAGTTCCTTAACGTGGAGAG 1325
comp37547 AATTGGGAAGAAAGGCTTGGGGAAGAGAGTAGACCTTTCTTATACAATGGCTGATGATTT 1494
OfurUGT1 GATTGGAAAGAAGGGCTTGGGGAAGAGAGTAGACCTTTCGTACACAATGGCTGATGATTT 1385
comp37547 GAAGATCGCTATTAACGAAGTCCTTTCCAATCCAAGCTACATGACCAAAGCGAAGGAACT 1554
OfurUGT1 GAAGATCGCTATTAACGACGTCCTTTCCAATCCAAGCTACATGACCAAAGCGAAGGAACT 1445
comp37547 CTCCCTGATCTACCACGACCGGCCAACGCCCCCTGGTGGAGAGTTAGTACACTGGGTGGA 1614
OfurUGT1 CTCCCTGATCTACCACGACCGGCCAACGCCCCCTGGTGGAGAGTTAGTACACTGGGTGGA 1505
comp37547 GCACGTCATCAAGACTGCTGGCGCCCCCCACCTGAGGTCACCTGCTTTAAACGTGCCCTT 1674
OfurUGT1 GCACGTCATCAAGACTGGTGGCGCCCCCCACCTGCGGTCTCCCGCTTTAAACGTGCCCTT 1565
comp37547 CTACCAGAAAATGTACCTGGACCTAGCAGCCTTAGTAGTTGTAGTTATTATTACCCTTAG 1734
OfurUGT1 CTACCAGAAGATGTACCTGGACTTAGCAGCCTTAGTAGTTGTAGTTATTATTGCCCTTAA 1625
comp37547 ATTAATTGTGAAACGTCTGTGCAATAGTTGTAGGAAAAAGAAAATAAGCAGCGAAAAGAA 1794
OfurUGT1 ATTAATTGTGAAGCGTGTGTGCAACAGTTGTAGGAAAAAGAAAGTAAGCAGTGAGAAGAA 1685
True termination site
comp37547 AAATAAGTGAATAGTTA----ATGTTGGTGATACTCGTATCATGGTGATATTGTGATATG 1850
OfurUGT1 GAATAAGTGAATAGTTATAATATGTTGGTGATACTCGTATCATGGTGA---------- 1733
comp37547 ATTTTGTACAATAAAATTAAATAATGTAGGATATTGTTGTTTAAAAATAAAC 1902
OfurUGT1 ----------

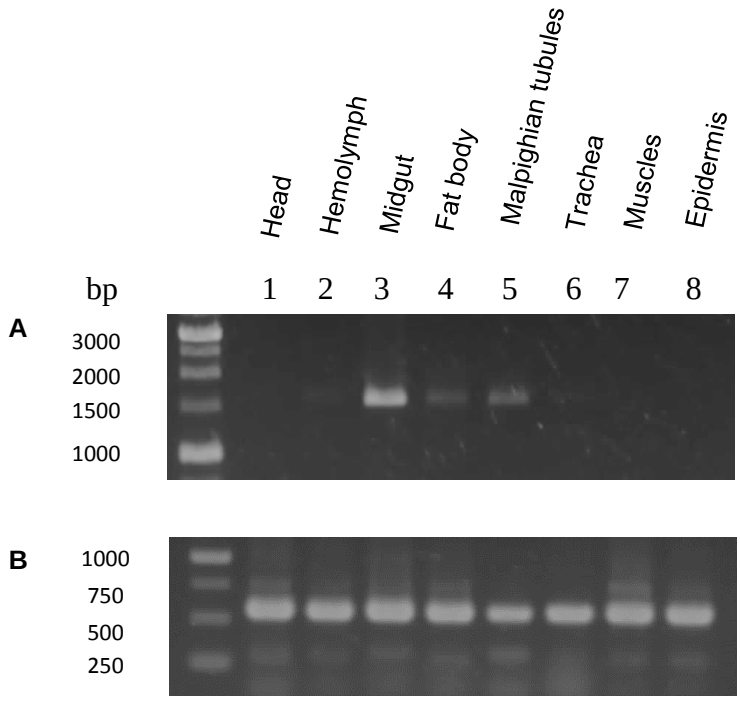


Figure 2.7. A) Semi-quantitative RT-PCR analysis of the expression of *OfurUGT1* (1575 bp) in various tissues of *O. furnacalis* larvae. **B**) Actin was used as a control (565 bp).

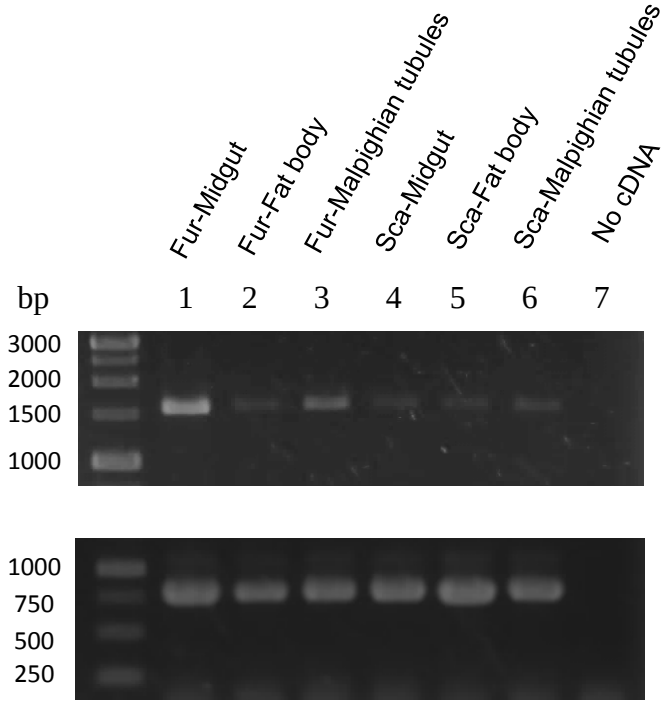


Figure 2.8. (**A**) Comparison the expression levels of *OfurUGT1* (1575 bp) in the midgut, fat body, and Malpighian tubules of *O. furnacalis* (Fur) and *O. scapulalis* (Sca) using semi-quantitative RT-PCR. **B**) Actin was used as a control (784 bp).

2.3.5. Phylogenetic analysis of OfurUGT1

Maximum-likelihood tree constructed using amino acid sequences of OfurUGT1 and representative lepidopteran UGTs belonging to the family UGT40 has shown that OfurUGT1 is relatively closely related to UGT40R or UGT40Q (**Fig. 2.9**). However, belonging of OfurUGT1 to either UGT40R or UGT40Q subclasses cannot be concluded only from this result. Actually, it appears that OfurUGT1 does not form compact clade with neither UGT40R nor UGT40Q. Therefore, OfurUGT1 may form another clade together with unreported UGTs in other lepidopteran species. Further studies are required to clarify the phylogenetic origin of OfurUGT1.

2.3.6. Primary structure of *O. furnacalis* UGT protein

To characterize the primary structure of OfurUGT1 protein, amino acid sequences of lepidopteran (*B. mori*, *H. armigera*, and *S. littoralis*) UGTs belonging to UGT40 family and human UGT2B7, which were retrieved from public databases, were aligned with OfurUGT1 by CLUSTAL W (**Fig. 2.10)**. Human UGT2B7 was included as a reference because crystal structure and functional analyses of all regions of UGT was determined using this UGT (Miley et al., 2007; Radominska-Pandya et al., 2010).

OfurUGT1 protein had both N-terminal substrate binding domain and the C-terminal sugar-donor binding domain. The C-terminal UDP-glucose binding domains of insect UGTs were more highly conserved than the N-terminal substrate binding domain. In N-terminal substrate binding domain, the signal peptide cleavage sites and catalytic residue were detected (**Fig. 2.10**). The catalytic residue

of OfurUGT1 was the same with that of UGTs from *B. mori*, *H. armigera*, *S. littoralis*, and human UGT2B7. In the C-terminal sugar-donor binding domain, the UGT signature motif and donor binding region 1 (DBR1) and donor binding region 2 (DBR2) were identified in the OfurUGT1. These regions in UGTs were highly conserved in different insect species. The catalytic residues in DBR1 and DBR2 of insect UGT were the same as human UGT2B7 (**Fig. 2.10**).

2.3.7. Heterologous expression of OfurUGT1

UGT gene was amplified by RT-PCR using cDNA isolated from midgut of *O. furnacalis* and a pair of primers for pFastBact1 transformation, which are listed in **Table 2.2**. UGT gene was isolated from electrophoresed gel (**Fig. 2.11A**). After UGT gene was cloned successfully into pFastBac1 (**Fig. 2.11B**), recombinant pFastBac-UGT plasmids were continuous transformed into DH10Bac competent *E. coli* to obtain recombinant Bac-UGT (**Fig. 2.11C**). Recombinant Bac-UGT was applied for transfection into insect cells to obtain recombinant baculovirus stock P1 and then P2. Recombinant baculovirus stock P2 was applied for infecting insect cells to determine UGT protein expression with His-tag. The recombinant UGT protein was analyzed by SDS/PAGE and immunoblot (**Fig. 2.11D**). The molecular mass of expressed UGT protein was estimated to be 58.33 kDa, which is in good agreement with the value estimated from the predicted amino acid sequence.

2.3.8. Proteins differentially expressed in the midgut of *O. furnacalis* larvae that had fed on an artificial diet with and without addition of DIMBOA

Total protein extracted from midgut of *O. furnacalis* that had fed on a normal artificial diet and a diet containing 0.5 mg/g of DIMBOA for 24 hours were

separated by SDS/PAGE on 10% gel and stained by Coomassie Blue (**Fig. 2.11E**). A band with a size of approximately 58.7 kDa, which coincides with the molecular mass of OfurUGT1, was observed when the larvae of *O. furnacalis* were fed on diet containing 0.5 mg/g of DIMBOA while it was not observed in the larvae fed on diet only. This finding is consistent with our results of RT-PCR experiments, i.e., transcription of *OfurUGT1* is stimulated by the ingestion of DIMBOA (Fig. 2.3).

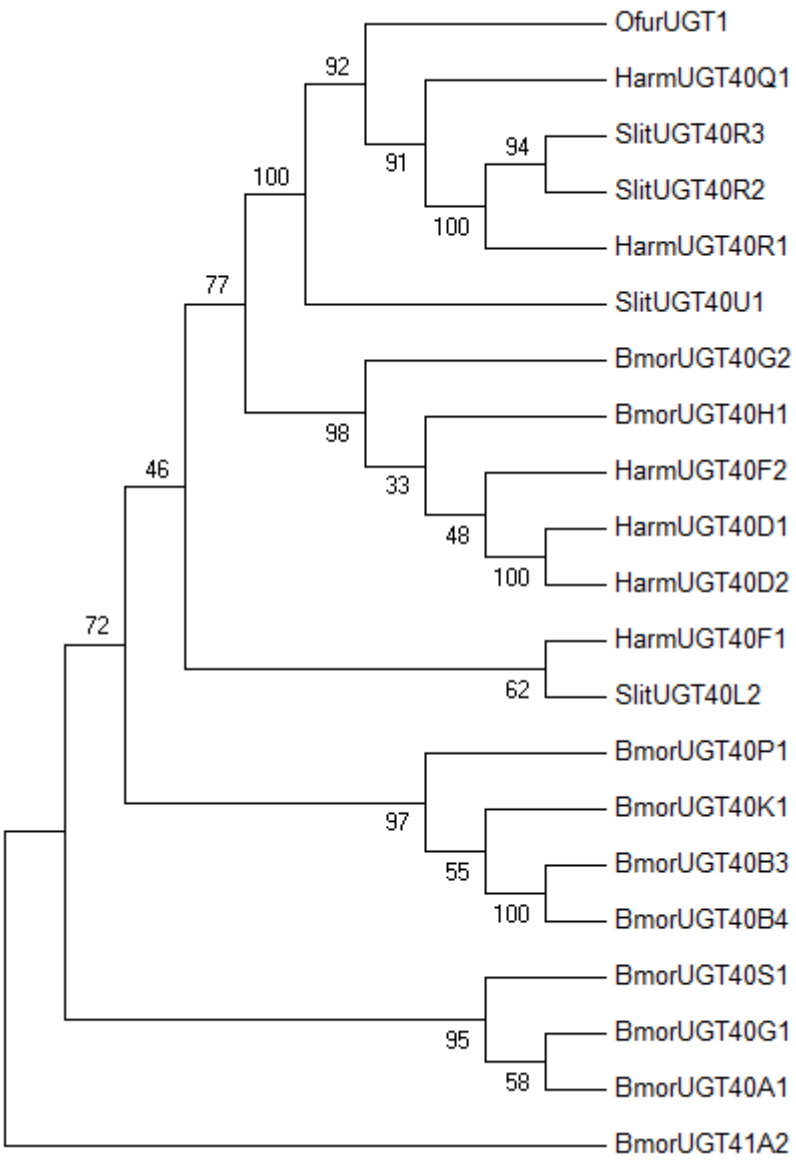
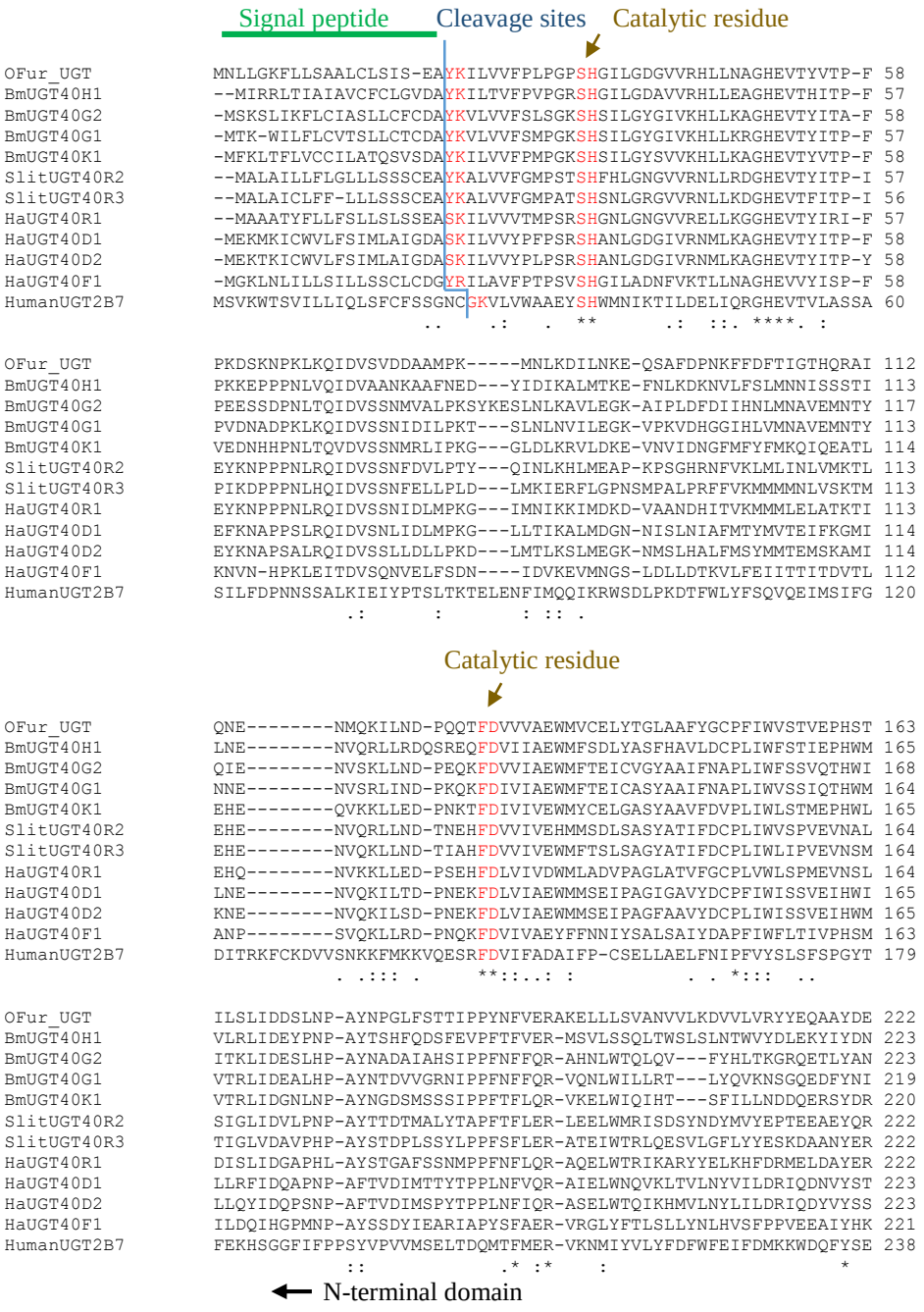


Figure 2.9. Maximum-likelihood tree of amino acid sequences of OfurUGT1, UGTs of UGT40 family, and UGT41A2 (outgroup) proteins from *B. mori*, *H. armigera*, and *S. littoralis* obtained from public databases. Refer to the APPENDIX for the sequences and GenBank Accession Numbers of the referenced genes.


Signal peptide
Cleavage sites
Catalytic residue
OFur_UGT MNLLGKFLLSAALCLSIS-EAYKILVVFPLPGPSHGILGDGVVRHLLNAGHEVTYVTP-F 58
BmUGT40H1 --MIRRLTIAIAVCFCLGVDAYKILTVFPVPGRSHGILGDAVVRHLLEAGHEVTHITP-F 57
BmUGT40G2 -MSKSLIKFLCIASLLCFCDAYKVLVVFSLSGKSHSILGYGIVKHLLKAGHEVTYITA-F 58
BmUGT40G1 -MTK-WILFLCVTSLLCTCDAYKVLVVFSMPGKSHSILGYGIVKHLLKRGHEVTYITP-F 57
BmUGT40K1 -MFKLTFLVCCILATQSVSDAYKILVVFPMPGKSHSILGYSVVKHLLKAGHEVTYVTP-F 58
SlitUGT40R2 --MALAILLFLGLLLSSSCEAYKALVVFGMPSTSHFHLGNGVVRNLLRDGHEVTYITP-I 57
SlitUGT40R3 --MALAICLFF-LLLSSSCEAYKALVVFGMPATSHSNLGRGVVRNLLKDGHEVTFITP-I 56
HaUGT40R1 --MAAATYFLLFSLLSLSSEASKILVVVTMPSRSHGNLGNGVVRELLKGGHEVTYIRI-F 57
HaUGT40D1 -MEKMKICWVLFSIMLAIGDASKILVVYPFPSRSHANLGDGIVRNMLKAGHEVTYITP-F 58
HaUGT40D2 -MEKTKICWVLFSIMLAIGDASKILVVYPLPSRSHANLGDGIVRNMLKAGHEVTYITP-Y 58
HaUGT40F1 -MGKLNLILLSILLSSCLCDGYRILAVFPTPSVSHGILADNFVKTLLNAGHEVVYISP-F 58
HumanUGT2B7 MSVKWTSVILLIQLSFCFSSGNCGKVLVWAAEYSHWMNIKTILDELIQRGHEVTVLASSA 60
PKDSKNPKLKQIDVSVDDAAMPK-----MNLKDILNKE-QSAFDPNKFFDFTIGTHQRAI 112
BmUGT40H1 PKKEPPPNLVQIDVAANKAAFNED---YIDIKALMTKE-FNLKDKNVLFSLMNNISSSTI 113
BmUGT40G2 PEESSDPNLTQIDVSSNMVALPKSYKESLNLKAVLEGK-AIPLDFDIIHNLMNAVEMNTY 117
BmUGT40G1 PVDNADPKLKQIDVSSNIDILPKT---SLNLNVILEGK-VPKVDHGGIHLVMNAVEMNTY 113
BmUGT40K1 VEDNHHPNLTQVDVSSNMRLIPKG---GLDLKRVLDKE-VNVIDNGFMFYFMKQIQEATL 114
SlitUGT40R2 EYKNPPPNLRQIDVSSNFDVLPTY---QINLKHLMEAP-KPSGHRNFVKLMLINLVMKTL 113
SlitUGT40R3 PIKDPPPNLHQIDVSSNFELLPLD---LMKIERFLGPNSMPALPRFFVKMMMMNLVSKTM 113
HaUGT40R1 EYKNPPPNLRQIDVSSNIDLMPKG---IMNIKKIMDKD-VAANDHITVKMMMLELATKTI 113
HaUGT40D1 EFKNAPPSLRQIDVSNLIDLMPKG---LLTIKALMDGN-NISLNIAFMTYMVTEIFKGMI 114
HaUGT40D2 EYKNAPSALRQIDVSSLLDLLPKD---LMTLKSLMEGK-NMSLHALFMSYMMTEMSKAMI 114
HaUGT40F1 KNVN-HPKLEITDVSQNVELFSDN----IDVKEVMNGS-LDLLDTKVLFEIITTITDVTL 112
HumanUGT2B7 SILFDPNNSSALKIEIYPTSLTKTELENFIMQQIKRWSDLPKDTFWLYFSQVQEIMSIFG 120
Catalytic residue
QNE--------NMQKILND-PQQTFDVVVAEWMVCELYTGLAAFYGCPFIWVSTVEPHST 163
BmUGT40H1 LNE--------NVQRLLRDQSREQFDVIIAEWMFSDLYASFHAVLDCPLIWFSTIEPHWM 165
BmUGT40G2 QIE--------NVSKLLND-PEQKFDVVIAEWMFTEICVGYAAIFNAPLIWFSSVQTHWI 168
BmUGT40G1 NNE--------NVSRLIND-PKQKFDIVIAEWMFTEICASYAAIFNAPLIWVSSIQTHWM 164
BmUGT40K1 EHE--------QVKKLLED-PNKTFDIVIVEWMYCELGASYAAVFDVPLIWLSTMEPHWL 165
SlitUGT40R2 EHE--------NVQRLLND-TNEHFDVVIVEHMMSDLSASYATIFDCPLIWVSPVEVNAL 164
SlitUGT40R3 EHE--------NVQKLLND-TIAHFDVVIVEWMFTSLSAGYATIFDCPLIWLIPVEVNSM 164
HaUGT40R1 EHQ--------NVKKLLED-PSEHFDLVIVDWMLADVPAGLATVFGCPLVWLSPMEVNSL 164
HaUGT40D1 LNE--------NVQKILTD-PNEKFDLVIAEWMMSEIPAGIGAVYDCPFIWISSVEIHWI 165
HaUGT40D2 KNE--------NVQKILSD-PNEKFDLVIAEWMMSEIPAGFAAVYDCPLIWISSVEIHWM 165
HaUGT40F1 ANP--------SVQKLLRD-PNQKFDVIVAEYFFNNIYSALSAIYDAPFIWFLTIVPHSM 163
HumanUGT2B7 DITRKFCKDVVSNKKFMKKVQESRFDVIFADAIFP-CSELLAELFNIPFVYSLSFSPGYT 179
ILSLIDDSLNP-AYNPGLFSTTIPPYNFVERAKELLLSVANVVLKDVVLVRYYEQAAYDE 222
BmUGT40H1 VLRLIDEYPNP-AYTSHFQDSFEVPFTFVER-MSVLSSQLTWSLSLNTWVYDLEKYIYDN 223
BmUGT40G2 ITKLIDESLHP-AYNADAIAHSIPPFNFFQR-AHNLWTQLQV---FYHLTKGRQETLYAN 223
BmUGT40G1 VTRLIDEALHP-AYNTDVVGRNIPPFNFFQR-VQNLWILLRT---LYQVKNSGQEDFYNI 219
BmUGT40K1 VTRLIDGNLNP-AYNGDSMSSSIPPFTFLQR-VKELWIQIHT---SFILLNDDQERSYDR 220
SlitUGT40R2 SIGLIDVLPNP-AYTTDTMALYTAPFTFLER-LEELWMRISDSYNDYMVYEPTEEAEYQR 222
SlitUGT40R3 TIGLVDAVPHP-AYSTDPLSSYLPPFSFLER-ATEIWTRLQESVLGFLYYESKDAANYER 222
HaUGT40R1 DISLIDGAPHL-AYSTGAFSSNMPPFNFLQR-AQELWTRIKARYYELKHFDRMELDAYER 222
HaUGT40D1 LLRFIDQAPNP-AFTVDIMTTYTPPLNFVQR-AIELWNQVKLTVLNYVILDRIQDNVYST 223
HaUGT40D2 LLQYIDQPSNP-AFTVDIMSPYTPPLNFIQR-ASELWTQIKHMVLNYLILDRIQDYVYSS 223
HaUGT40F1 ILDQIHGPMNP-AYSSDYIEARIAPYSFAER-VRGLYFTLSLLYNLHVSFPPVEEAIYHK 221
HumanUGT2B7 FEKHSGGFIFPPSYVPVVMSELTDQMTFMER-VKNMIYVLYFDFWFEIFDMKKWDQFYSE 238
N-terminal domain
LYVPLLKKKGRPVLTYEEVRYNVSLVLGNSHVSLGQATRLPQNYKPIGGYHIDTNFKPLP 282
BmUGT40H1 NIAPIIKKNGKPVPNYDEVRYNGSLLLGNSHVSLGDAIKVPINYKAIGGYHIDGKVKELP 283
BmUGT40G2 EIVPIIKKRGLVPPSFNDLLYNSSLVLSNTHVSYAAATRLPQNYKPIGGFHIDEEVKPLP 283
BmUGT40G1 AVVPVIEKRGLVPPTFEDVQFNGSLVLSNSHLSYAPAVRLPQNYKTVGGFHVEEKVEPLP 279
BmUGT40K1 LVRPLIEKKGRKAPSFEDLKFNASLVLGNSHVSLGEATGTPQSYKPIAGYHIEEVVKPLP 280
SlitUGT40R2 LIVPQLQKRGRQVPPYSEVRYNATLVLGNSHVSTGIPLGFPQNYKSMGGYHIEEEVKPLP 282
SlitUGT40R3 IVVPQVQKRGRQAPPLSEVQYNASLVLGNSHVSMGLPLSLPQNYKPVGGYHIEEEVKPLP 282
HaUGT40R1 LIVPYVEKRGRQAPSFYDVRYNASLILGNSHVSMGQALALPQNYKPIGGYHIDEDVKPLP 282
HaUGT40D1 YLAPIVEKRGRKAPTLDELRYNVSMIFSNAYVDTSSALSLPQSHKYIGGYHIDEKVKPLP 283
HaUGT40D2 YLAPFVEQRGRKAPTLHELRYNVSMIFSNAYVDTSSALSLPQNHKYIGGYHIDEKVKPLP 283
HaUGT40F1 HIPTILKSLGKPIADYKVLTYNVSMVLGNSQVAIESAVPLPPNFKHIGGYHIDDDVKPLP 281
HumanUGT2B7 VLG--------RPTTLSETMGKADVWLIRNSWNFQFPYPLLPNVDFVGGLHCKP-AKPLP 289

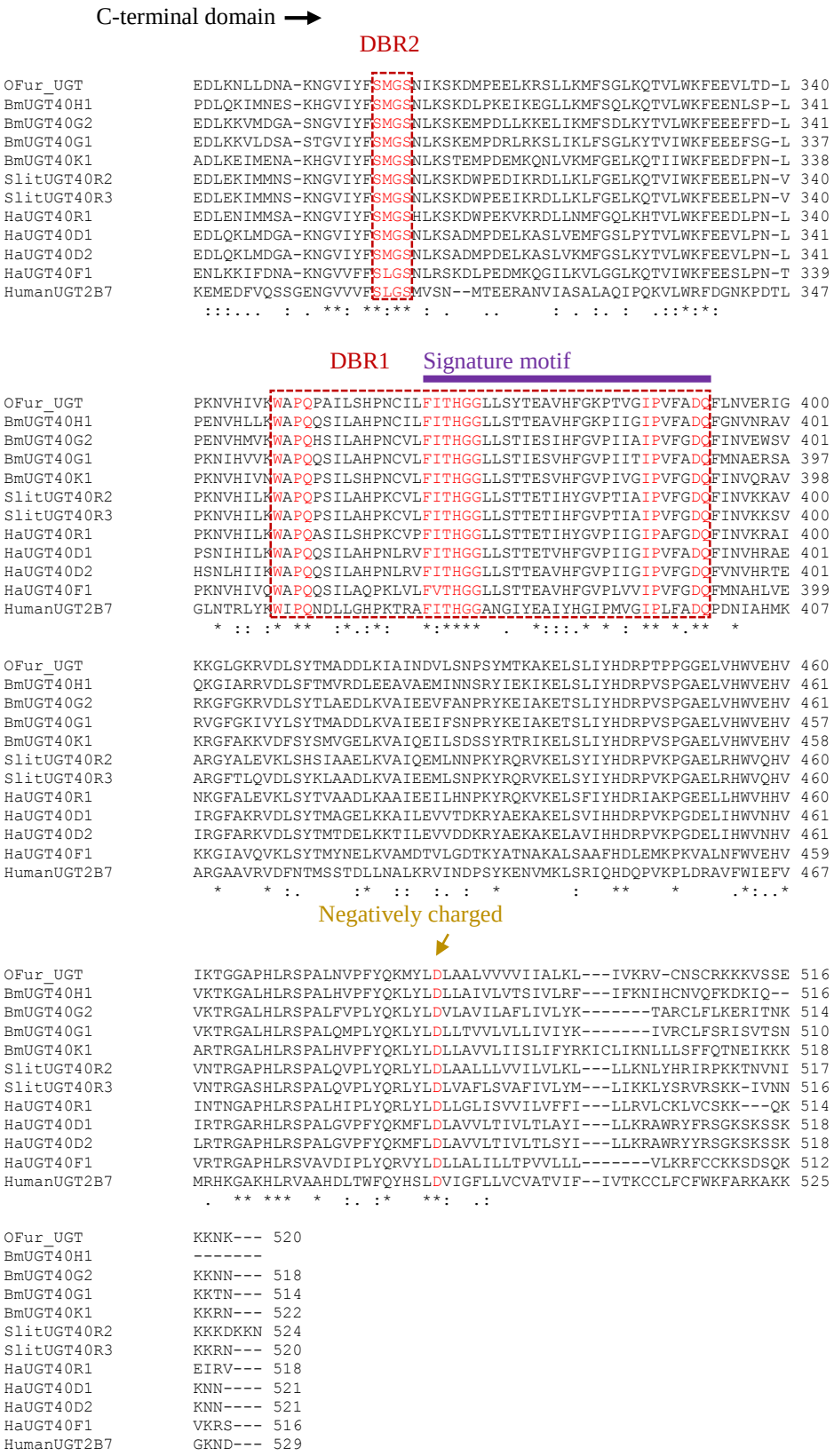


Figure 2.10. Multiple alignment of OfurUGT1 protein and ten UGT proteins from *B. mori*, *H. armigera*, *S. littoralis*, and human UGT2B7.

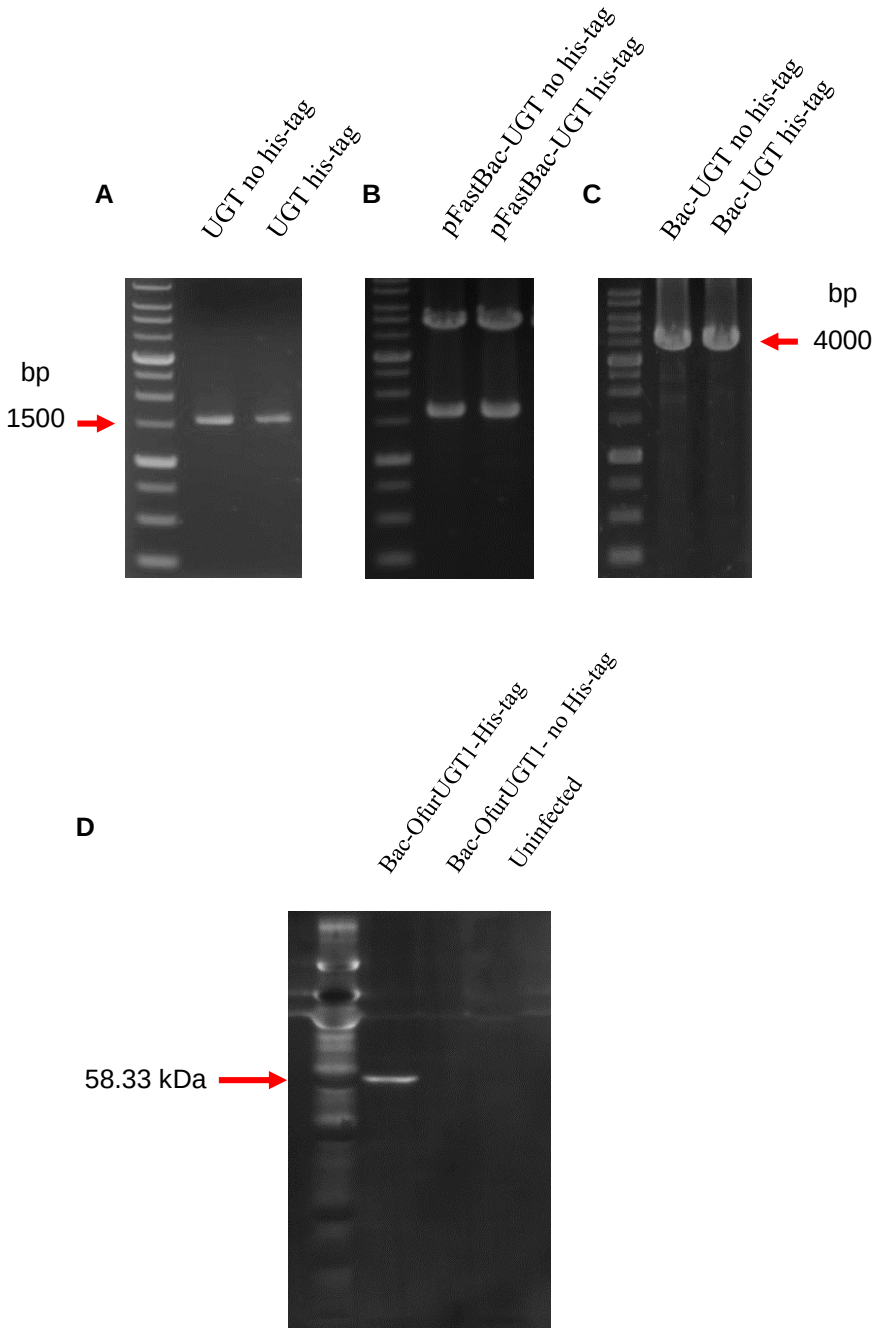


Figure 2.11. Expression of OfurUGT1 protein. **A**) CDS of *OfurUGT1* gene amplified by RT-PCR. **B**) Recombinant pFastBac1-UGT. **C**) Recombinant Bacmid-UGT. **D**) Result of Western blot analysis of total proteins extracted from Sf9 infected with Bac-OfurUGT1for 72 hours. The molecular size of recombinant protein OfurUGT1 is estimated as 58.33 kDa.

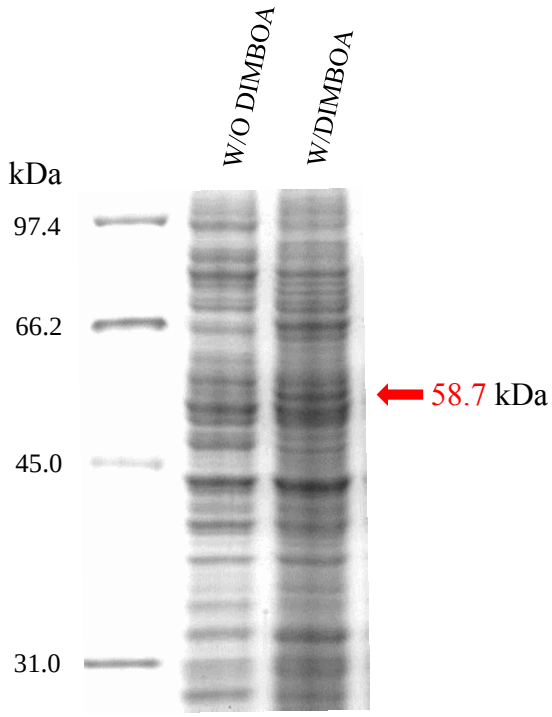


Figure 2.12. SDS/PAGE (10% separating gel) analysis of total protein extracted from midgut of *O. furnacalis* that had fed on an artificial diet with and without 0.5 mg/g of DIMBOA for 24 hours.

2.4. Discussion

<Is OfurUGT1 responsible for the catabolism of DIMBOA?>

At present, I have not yet succeeded to observe any activities of OfurUGT1 toward DIMBOA. Addition of DIMBOA to the homogenate of Sf9 cells expressing OfurUGT1 did not decrease the amount of DIMBOA and no formation of DIMBOA-2-*O*-glucoside was observed (data not shown). Many factors, for example, optimization of the production of recombinant OfurOGT1, localization and solubility of the expressed OfurUGT1, have to be considered before drawing any conclusion about the activity of OfurUGT1. Although the catalytic activity of OfurUGT1 is yet to be proven, it has many characteristics that a UGT responsible for the catabolism of DIMBOA or other maize allelochemicals should possess. 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 an artificial diet containing DIMBOA, and 3) higher expression level in *O. furnacalis* as compared with the non-maize feeder *O. scapulalis*. Therefore, at present, OfurUGT1 remains to be the first UGT whose function has to be investigated.

Induction of a 58.7 kDa protein, whose molecular mass coincided with that of UGTs in general, was clearly observed in the midgut of *O. furnacalis* larvae that had fed on an artificial diet containing DIMBOA (**Fig. 2.12**). This finding suggests the induction of UGT protein(s), although it may not be OfurUGT1 itself, by the ingestion of DIMBOA. Further studies are required to characterize all UGTs involved in the catabolism of maize allelochemicals.

<Utilization of RNA-seq data>

I utilized RNA-seq data obtained for a tissue and species different from the ones I am interested in. As I expected, since species in the genus *Ostrinia* are extremely closely related, RNA-seq data for another species provided ample information useful for my study. However, probably because many UGT genes, similar in sequences, are expressed in a single species, inaccurate *de novo* assembling of short reads appeared to have occurred frequently. Actually, the sequences of two contigs I focused on, comp36666 and comp37547, were both not accurate.

In this thesis, due to limitation of the time, I disregarded comp36666 at the very early stage of the investigation. Since partial sequence of comp36666 was also amplified by PCR when cDNA prepared from the midgut of *O. furnacalis* was used as template, sequencing and characterization of comp36666 should be conducted in the future. Moreover, at least all UGT genes in the UGT40 family, which are known to be highly expressed in the midgut and Malpighian tubules, are worth examining for their activity.

<**Primary structure of UGTs**>

OfurUGT1 protein comprises 520 amino acids and the molecular mass is approximately 58 kDa, which is similar to UGT37a1 of *D. melanogaster*, and UGT40A1 and UGT40K1 of *B. mori* (Luque and O'Reilly, 2002; Luque et al., 2002; Xu et al., 2013). OfurUGT1 belongs to family UGT40, the second largest family of insect UGTs identified to date. UGTs belonging to this family were reported to have 7 introns and highly expressed in the midgut and fat body (Ahn et

al., 2012). The amino acid sequences of insect UGT proteins are highly variable in the N-terminal substrate binding domain while conserved in the C-terminal UDP-glucose binding domain. Actually, the UGT signature motif was identified in the C-terminal. These characteristics in the primary structure of UGT are considered to explain the diversity of substrates of UGT while the sugar donor is fixed to UDP-glucose.

GENERAL DISCUSSION

Catabolism of DIMBOA in *Ostrinia furnacalis*

Increased tolerance of herbivorous insects against ingested toxic plant secondary compounds can be achieved not only by enhanced detoxification but also by several other mechanisms such as non-absorption of toxins from the digestive tract, degradation of toxins within the digestive tract, enhanced excretion from the body, and insensitivity of target molecules with which toxins interact (Després et al., 2007; Pentzold et al., 2014). In the case of tolerance against DIMBOA, maize feeders such as *Spodoptera* and *Mythimna* are known to tolerate DIMBOA by enhancing its excretion by re-glucosylation of it via the function of UGT (Sasai et al., 2009; Wouters et al., 2014). Actually, in these species, DIMBOA-glucoside is found in their frass. Although involvement of UGT is also indicated in *O. furnacalis*, no DIMBOA-glucoside was found in the *in vitro* enzyme assay using the homogenate of *O. furnacalis* digestive tract (section 1.3.2). Using DIMBOA-glucoside in place of DIMBOA in the same enzyme assay system, I have shown that DIMBOA-glucoside can be rapidly broken down, probably into smaller compounds, by unknown enzymes in the assay system (Fig. 1.7). These results strongly suggested that *O. furnacalis* copes with DIMBOA not by excreting DIMBOA in the form of its glucoside in the frass but by degrading it into very small compounds. Actually, my preliminary HPLC-MS analyses of the frass of *O. furnacalis* consistently failed to detect DIMBOA and its direct derivatives (data not shown). The final forms of DIMBOA catabolites and the enzymes involved in the degradation are of great interest, and must be identified in future studies.

Furthermore, from the standpoint of evolution, it is very interesting to know why and how this unique strategy to tolerate DIMBOA, which is different from other maize feeders, was adopted in *O. furnacalis*.

Mechanism of increased UGT activity in *O. furnacalis*

An increase in the UGT activity was found in *O. furnacalis* in comparison with its congener *O. scapulalis*. The increase can be achieved, in general, via the following three mechanisms.

a. Increased transcription of the UGT gene
b. Multiplication of the UGT gene
c. Mutation of the UGT gene

The data obtained in the present study are consistent with the hypothesis that the increased DIMBOA catabolizing activity in *O. furnacalis* is achieved through increased transcription of the relevant UGT gene, *UGT1*. Both *O. furnacalis* and *O. scapulalis* have *UGT1* genes, *OfurUGT1* in *O. furnacalis* and *OscaUGT1* in *O. scapulalis*, but *UGT1* is expressed at a greatly higher level in *O. furnacalis* than in *O. scapulalis*. An increase in the transcription of *OfurUGT1* in the *O. furnacalis* larvae that had fed on artificial diet containing DIMBOA further supports this hypothesis. However, it must be noted that the possibility of the involvement of mechanism **b** or **c** cannot be completely excluded from the data obtained in the present study. It is necessary to clone *OfurUGT1* and *OscaUGT1* and compare their enzymatic activity by *in vitro* functional assays. The possibility of multiplication of UGT1 must be checked by quantitative PCR using genomic DNAs of *O. furnacalis* and *O. scapulalis*.

THESIS SUMMARY

Tran Thi Thu Phuong
March 2016

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

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.

REFERENCES

Ahmad, S.A., Hopkins, T.L., 1992. Phenol β-glucosyltransferase and β-glucosidase activities in the tobacco hornworm larva Manduca sexta (L.): Properties and tissue localization. Arch. Insect Biochem. Physiol. 224, 207–224.

Ahmad, S.A., Hopkins, T.L., 1993a. Phenol β-glucosyltransferases in six species of insects: properties and tissue localization. Comp. Biochem. Physiol. Part B Comp. Biochem. 104B, 515–519. doi:10.1016/0305-0491(93)90276-B

Ahmad, S.A., Hopkins, T.L., 1993b. β-Glucosylation of plant phenolics by phenol β-glucosyltransferase in larval tissues of the tobacco hornworm, Manduca sexta (L.). Insect Biochem. Mol. Biol. 23, 581–589. doi:10.1016/0965-1748(93)90031-M

Ahn, S.-J., Vogel, H., Heckel, D.G., 2012. Comparative analysis of the UDP-glycosyltransferase multigene family in insects. Insect Biochem. Mol. Biol. 42, 133–147. doi:10.1016/j.ibmb.2011.11.006

Berenbaum, M.R., 1995. The chemistry of defense: theory and practice. Proc. Natl. Acad. Sci. U. S. A. 92, 2–8. doi:10.1073/pnas.92.1.2

Cambier, V., Hance, T., de Hoffmann, E., 1999. Non-injured maize contains several 1, 4-benzoxazin-3-one related compounds but only as glucoconjugates. Phytochem. Anal. 10, 119–126.

Cambier, V., Hance, T., de Hoffmann, E., 2000. Variation of DIMBOA and related compounds content in relation to the age and plant organ in maize. Phytochemistry 53, 223–229.

Campos, F., Atkinson, J., Arnson, J.T., Philogène, B.J.R., Morand, P., Werstiuk, N.H., Timmins, G., 1989. Toxicokinetics of 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) in the European corn borer, Ostrinia nubilalis (Hübner). J. Chem. Ecol. 15, 1989–2001.

Daimon, T., Hirayama, C., Kanai, M., Ruike, Y., Meng, Y., Kosegawa, E., Nakamura, M., Tsujimoto, G., Katsuma, S., Shimada, T., 2010. The silkworm Green b locus encodes a quercetin 5-O-glucosyltransferase that

produces green cocoons with UV-shielding properties. Proc. Natl. Acad. Sci. U. S. A. 107, 11471–6. doi:10.1073/pnas.1000479107

Després, L., David, J.P., Gallet, C., 2007. The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol. Evol. 22, 298–307. doi:10.1016/j.tree.2007.02.010

Ehrlich, P.R.., Raven, P.H.., 1964. Butterflies and Plants : A Study in Coevolution. Evolution (N. Y). 18, 586–608.

Feng, R., Houseman, J.G., Downe, a. E.R., Atkinson, J., Arnason, J. t., 1992. Effects of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxybenzoxazolinone (MBOA) on the detoxification processes in the larval midgut of the European corn borer. Pestic. Biochem. Physiol. 44, 147–154. doi:10.1016/0048-3575(92)90112-D

Fürstenberg-Hägg, J., Zagrobelny, M., Bak, S., 2013. Plant defense against insect herbivores, International Journal of Molecular Sciences. doi:10.3390/ijms140510242

Hartmann, T., 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: A case study in chemical ecology. Planta 219, 1–4. doi:10.1007/s00425-004-1249-y

Hofman, J., Hofmanová, O., 1969. 1,4-Benzoxazine derivatives in plants. Eur. J. Biochem. 8, 109–112. doi:10.1016/S0040-4039(01)88868-7

Howe, G. a, Jander, G., 2008. Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59, 41–66. doi:10.1146/annurev.arplant.59.032607.092825

Huang, F.-F., Chai, C.-L., Zhang, Z., Liu, Z.-H., Dai, F.-Y., Lu, C., Xiang, Z.-H., 2008. The UDP-glucosyltransferase multigene family in Bombyx mori. BMC Genomics 9, 563. doi:10.1186/1471-2164-9-563

Ishikawa, Y., Takanashi, T., Kim, C.G., Hoshizaki, S., Tatsuki, S., Huang, Y., 1999. Ostrinia spp. in Japan: Their host plants and sex pheromones. Entomol. Exp. Appl. 91, 237–244. doi:10.1023/A:1003601407578

Kojima, W., Fujii, T., Suwa, M., Miyazawa, M., Ishikawa, Y., 2010.

Physiological adaptation of the Asian corn borer Ostrinia furnacalis to chemical defenses of its host plant, maize. J. Insect Physiol. 56, 1349–55. doi:10.1016/j.jinsphys.2010.04.021

Larsen, E., Christensen, L.P., 2000. Simple method for large scale isolation of the cyclic arylhydroxamic acid DIMBOA from maize (Zea mays L.). J. Agric. Food Chem. 48, 2556–2558.

Luque, T., O'Reilly, D.R., 2002. Functional and phylogenetic analyses of a putative Drosophila melanogaster UDP-glycosyltransferase gene. Insect Biochem. Mol. Biol. 32, 1597–1604. doi:10.1016/S0965-1748(02)00080-2

Luque, T., Okano, K., O'Reilly, D.R., 2002. Characterization of a novel silkworm (Bombyx mori) phenol UDP-glucosyltransferase. Eur. J. Biochem. 269, 819–825.

Maag, D., Dalvit, C., Thevenet, D., Köhler, A., Wouters, F.C., Vassão, D.G., Gershenzon, J., Wolfender, J.L., Turlings, T.C.J., Erb, M., Glauser, G., 2014. 3-β-d-Glucopyranosyl-6-methoxy-2-benzoxazolinone (MBOA-N-Glc) is an insect detoxification product of maize 1,4-benzoxazin-3-ones. Phytochemistry 102, 97–105. doi:10.1016/j.phytochem.2014.03.018

Meihls, L.N., Kaur, H., Jander, G., 2012. Natural variation in maize defense against insect herbivores. Cold Spring Harb. Symp. Quant. Biol. 77, 269–83. doi:10.1101/sqb.2012.77.014662

Miley, M.J., Zielinska, A.K., Keenan, J.E., Bratton, S.M., Radominska-Pandya, A., Redinbo, M.R., 2007. Crystal structure of the cofactor-binding domain of the human phase II drug-metabolism enzyme UDP-glucuronosyltransferase 2B7. J. Mol. Biol. 369, 498–511. doi:10.1016/j.jmb.2007.03.066

Mukanganyama, S., Figueroa, C.., Hasler, J.., Niemeyer, H.., 2003. Effects of DIMBOA on detoxification enzymes of the aphid Rhopalosiphum padi (Homoptera: aphididae). J. Insect Physiol. 49, 223–229. doi:10.1016/S0022-1910(02)00269-X

Mutuura, A., Munroe, E., 1970. Taxonomy and distribution of the European corn borer and allied species: genus Ostrinia (Lepidoptera: Pyralidae). Mem.

Entomol. soc. Can. 1–112.

Niemeyer, H., 1988. Hydroxamic acids (4-hydroxy-1, 4-benzoxazin-3-ones), defence chemicals in the Gramineae. Phytochemistry 27, 3349–3358.

Ortego, F., Ruíz, M., Castañera, P., 1998. Effect of DIMBOA on growth and digestive physiology of Sesamia nonagrioides (Lepidoptera: noctuidae) larvae. J. Insect Physiol. 44, 95–101. doi:10.1016/S0022-1910(97)00103-0

Pentzold, S., Zagrobelny, M., Rook, F., Bak, S., 2014. How insects overcome two-component plant chemical defence: plant β -glucosidases as the main target for herbivore adaptation. Biol. Rev. 89, 531–551. doi:10.1111/brv.12066

Radominska-Pandya, A., Bratton, S.M., Redinbo, M.R., Miley, M.J., 2010. The crystal structure of human UDP-glucuronosyltransferase 2B7 C-terminal end is the first mammalian UGT target to be revealed: the significance for human UGTs from both the 1A and 2B families. Drug Metab. Rev. 42, 133–44. doi:10.3109/03602530903209049

Sakai, R., Fukuzawa, M., Nakano, R., Tatsuki, S., Ishikawa, Y., 2009. Alternative suppression of transcription from two desaturase genes is the key for species-specific sex pheromone biosynthesis in two Ostrinia moths. Insect Biochem. Mol. Biol. 39, 62–7. doi:10.1016/j.ibmb.2008.10.001

Sasai, H., Ishida, M., Murakami, K., Tadokoro, N., Ishihara, A., Nishida, R., Mori, N., 2009. Species-specific glucosylation of DIMBOA in larvae of the rice Armyworm. Biosci. Biotechnol. Biochem. 73, 1333–8. doi:10.1271/bbb.80903

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30, 2725–2729. doi:10.1093/molbev/mst197

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. doi:10.1093/nar/22.22.4673

Tipton, C.L., Klun, J.A., Husted, R.R., Pierson, M.D., 1967. Cyclic hydroxamic acids and related compounds from Maize. Isolation and characterization. Biochemistry 6, 2866–2870.

Woodward, M.D., Corcuera, L.J., Helgeson, J.P., Upper, C.D., 1978. Decomposition of 2, 4-dihydroxy-7-methoxy-2H-1, 4-benzoxazin-3 (4H)-one in aqueous solutions. Plant Physiol. 61, 796–802.

Wouters, F.C., Reichelt, M., Glauser, G., Bauer, E., Erb, M., Gershenzon, J., Vassão, D.G., 2014. Reglucosylation of the Benzoxazinoid DIMBOA with Inversion of Stereochemical Configuration is a Detoxification Strategy in Lepidopteran Herbivores. Angew. Chemie Int. Ed. 53, 11320–11324. doi:10.1002/anie.201406643

Xu, X., Wang, M., Wang, Y., Sima, Y., Zhang, D., Li, J., Yin, W., Xu, S., 2013. Green cocoons in silkworm Bombyx mori resulting from the quercetin 5-O-glucosyltransferase of UGT86, is an evolved response to dietary toxins. Mol. Biol. Rep. 40, 3631–9. doi:10.1007/s11033-012-2437-7

Yan, F., Liang, X., Zhu, X., 1999. The role of DIMBOA on the feeding of Asian corn borer, Ostrinia furnacalis (Guenee)(Lep., Pyralidae). J. Appl. Entomol. 123, 49–53.

Yan, F., Xu, C., Li, S., Lin, C., Li, J., 1995. Effects of DIMBOA on several enzymatic systems in Asian corn borer, Ostrinia furnacalis (Guenee). J. Chem. Ecol. 21, 2047–2056.

APPENDIX. Amino acid sequences used for construction of the phylogenetic tree of *Ostrinia zaguliaevi* UGTs (comp15776–comp38172) and representative lepidopteran UGTs retrieved from public databases (UGT33D1–UGT340C1, *Numbers in parentheses indicate GenBank Accession Numbers).

>comp15776
```
    1 MQSSTILVLI SMILMNSVNC ARILGIFPMP SRSHQIVFQS YTKELAKRGH
   51 ELVVVSPDPF PPDTRPENLT DIDVSFSYQV MKNLFTANLL DLKQGVIMDI
  101 DAIIAGNLYE KIVYAYVDQM NHPPVRKLIN DKNQRFDLVV VEGFLDYHLM
  151 FTEIFKAPVI MFPSFMGFAE QYEMLGGIGR HPILYPHLHR NKFDDLNLFE
  201 WAKELYYEYR MYAMFERLEH KQNELLKENF GANAPTVNEL RENIDLLLLN
  251 SYADFANNRP VPPNIIYLGA VQLQPVKEIP KDLKDYLDGS SRGVIYVSFG
  301 SNIMPSRMSK ELLGAILEAF EKLPYDILWK FDGDNLENVP KNVKYMKWFP
  351 QRDLLFHPNI KAFVTQCGLQ STDEAIDAAV PLVGIPMMAE QAYNAKKYKD
  401 FGIGVKLDPM ALTADDFVNG VNTVVEDISY KNNILRLKKI HQDQPQSPLE
  451 RAVWWTEYVI RHSGSRHMRS PAANMPWHKY YMLDILLPLL GLFITVLIVV
  501 ATLLRFVFNI IGVFGGKEKV KQK
```

>comp16953
```
    1 MLWYCMGVIF LSICSECANI LYVVPFTSKS HYIMLKPIGL ELAKRGHNVT
   51 VITGHKTDVN LTNYHQVMVD DKEIWELTGM KRPNVFTMVN ISAEEFHDII
  101 LWRGGLGHTE VTLQSPQVKH FLANDNKFDL VISEQFFQEA MFTLAHKYNA
  151 PLVLITTYGN CMRHNIVSRN PLQLATVVSE FLDVKDPTSF WGRLRNLYFT
  201 VYEYVWWKYW YLEKQEEFVR KYLLNLPQPV PSLYELQKNA ALILINSHFS
  251 FDGPVAYLPN IVEVGGLHLT RSTSKLPQDL QKLLDESKHG VVYVNFGSNV
  301 RSSEMPPEKK LAFVKIFSEL KQTVFWKWED DNFDIETNNV VIRKWFPQKD
  351 VLSHPNVKVF ISHGGLIGTQ EAIFHGVPII GVPIYADQYN NLLQAQKLGF
  401 GKILQYRDIN EDTIRKNLHE VLKDDSYKNK AQEMSKRFKD RPMPALDTAM
  451 YWIEYVIRNK GADFIKNPAH ELSWFANNML DVFAFLLLSF IVSAYVVFIV
  501 VRALIIIAQS SSTNKSKKIK TK
```

>comp26748
```
    1 MAQNIALVFY FVAAIYTTTS YRILGIFPSL DRNNYLTYKS LFFELANRNH
   51 DVTLVSHFSQ PDAPATYKEV LLSENQLVYK GLSYESVIVN EVSRVPFETL
  101 VATKAGNDDC KTLMNNHYVL HMIRTRPRFD VIVVESYNSD CALALAANLS
  151 APYIAFSPQP IQPWQYNRLG IGFNSAYVTQ AGLPYGKEPW FFDRLKSYVL
  201 YHVTNWVYYV GSQVTDHVYL YKYLGDSLPS LESIASNASL MFVNTHPSIF
  251 GGVARPDNVI DIGGIHVRPP KVIPTEIERF INEAEHGVIY VNLGSTVKDS
  301 TLPKDKLQEM LSAFSKLPLR VLWKWDGGSL ELPRNVMTMR WFPQYDILKH
  351 DNVKVFISHT GILSTIEAVD AGIPVVAIPL FGDQYGNAAV LQDAGIASIV
  401 SYQDLKKNYL LDAINEVLDP TFQQRAKQVS RIWHDRTISP LENAIYWTEY
  451 VARYRGAPNL RTPSADLPLY QQLQLDVLAF IALVLYILCY VFYKILSVLC
  501 CCCCQNEQEI QTSSEERRSK RVKFE
```

>comp27021 (5' partial)
```
    1 QAARLLVVLP TNTRSHYAMY GRLVEALARK NHHLTVISHF PMKIRPPNVE
   51 EISLAGTIPD IYNNLTEQHY SLKPDFVHNL EQIMAECVHA CDMVSRMPAV
  101 KALLNSTVTY DLVIVEVFGT ECFLPLGERF KAPVVGLLSS VPLPWFNEQL
  151 GNPEATAYVP AYMTGFGQHM NLIERLSNTI SVLWAKILYR YKSQIPSQAI
  201 ADRLFGYGTK LDKLAQNYSL VLSNSHFTIN EVRPLVPALV EVGGLHLDES
  251 QKLSGELKTL LDASTDGIIY WSFGSMSKIE TIPSEKLAQI FAVISELSQT
  301 VLVKMNRMRL STNLTVPDNI YTMDWIPQYA TLCHPNVKVF ISHGGLLGTQ
  351 EAVACGVPML TVPLYADQAL NARAMADRGV SKTITLKNTN KHTWKQALHE
  401 LLTDARYKDN MLKLRNVFLD RPMPPLDTGI YWIEYVLRHK GAPHLRSPAL
```

```
  451 DLSVVQYLLL DVVVLSIAIA ITTIYILHIL FRYLCTRCIK WWPKEKRVFE
  501 KRLFRNFSVF LCLLWRYKAK AN

>comp32482
    1 MAKAQIILCV LGVICCADAY KILVVFPFPI RSLNNLGEGY VRHLLKAGHE
   51 VTFITAFPLK NEKNEKLRQI DISSNQQLMV SGDTTFSIKS VMDGQIPMND
  101 VKMMQEFGLY SSIMMFEHEN VKKFLEDPSQ QFDAVIVDLY ETEIYSGLSA
  151 LYNCPMIWSY SMGAHWLVLR LIDEPTNPAY SADYLSSNVL PFTFKQRLEE
  201 LWAQILWTWT KWTSTMPKEK EAFAKYFKPL LEKGGRTSPD YDQLIYNASL
  251 IFGNEHHAFG NIPRTPQNFK FIGGFHIETP AKPLPKDLQT LMDDSKDGVI
  301 YFSMGSAWNS KDLPESVVDG LVKMFGELKQ TVIWKFEADL PNLPKNLHIT
  351 KWAPQPSILA HPNCLFFITH GGHLSSTEAI HFGVPIIGVP IFFDQFININ
  401 KALSKGYALK VNLNYDLPRN LKAAIQTMLS DSKYRKQAEE LSAIYHHRPV
  451 PPGQEMVHWV THVIRTGGAP HLRSPALNMA FYQKMYLDFA ALVAAVVVAV
  501 VLVVKKLCGG RKAGMKEKKN

>comp33154
    1 MSTPMLTALL LLLFASQAWS YNVLCIFPTP SKSHNHLGKG IVDALLDAGH
   51 QVTWGTPFPS KTLNNNLRMI DLSGTVALSE AIDMTQPRFR NAGPDFVRSF
  101 ARNISISALS SQELRDALTK QQFDAVVTEW FFSDYDCGYA AVQQAPWILL
  151 SGMTMHPHLE MLVDEVRSIQ THPLLFNDFP VPMTLWQRMI NSFIFGMMTI
  201 SNWRDQSDND AYYQSAYGPL AKARGLSLPA YQDALYNVSI LFVNTDPAVD
  251 RPRSVPANVI SIAGYHIDAN PGPLPKDLQT ILDSSSKGVI YFSMGSVLKS
  301 SAFPENLIAD LLKLFGELPY TVLWKFEKEL TGAPKNVHVR AWFPQASILA
  351 HPNVKLFITH GGLLSTLEAA NAGVPILAVP MFGDQPSNAA RSVRAGVARK
  401 VDFSHHMADE LRKELNEMLS NDNYLKTAQS VSKLFRKRPV APSKLISHYV
  451 EVAIESKGAY HLRSPSKLYA WYERYMLDQL AIVGAILYLI VKLIMTAVNV
  501 IKRKVSGGKK QKRS

>comp33913
    1 MRSSTSQVCL KKTPVPNLTE IDLSSIEEAF KQDKESNEHF KLKNLVGQKN
   51 FGDSIFFLYL SYEINKVSME HEAVQTFLAD PKQKFDAVIL EWFFSDFIAG
  101 IAPLFNAPLI WMGSTEAHWQ VLKLVDEIPN PAYSVDLFSV KRPPLTFWER
  151 MVELWTLAKR YVIINAVVVP FEKRLYNIIF PELAAKRGVT MPGYDDAVYN
  201 ASLMYLYSHP SIGTPFRLSQ NAKYVGGYHV DTEVRALPKD LQKIMDEAKD
  251 GVIYFSMGSN LKSVDMTENM RNSLLKMFSK LKQKVIWKFE EDLQNVPKNI
  301 HLVKWAPQQS ILAHPNLRMF ITHGGQLSTT EAIHFGVPVV GIPVFGDQYV
  351 NTKSAVDKGF CISVTLAEDM ADDIYAAVQE ILRNPAYKTK AKELSAIFHD
  401 RPMKPGEELV YWLEYVVRTH GAKHLRSPAV NVPMYQKLFL DLLLIVVVGC
  451 YVLCKIKQKV FGRRKADKPA KSGKKTKTN

>comp34920
    1 MLRPASARPA GKLCRRAARA HLFRTMRYHF LTTLCLLAYT TNAIKILGIF
   51 PYDGKSHFIV IKVLLEELAR RGHDVTVISH FPDDNPPKNY HDVSLFIPKL
  101 NNDSVEDAVK IERSYFGVFE VGVYLALSGK NDCEVMLANK DVQKLVNRKD
  151 KYDLVLTEQF NSDCSLGIAY KLGAPVVGIT THILMPWHYK RLGIPNNPSY
  201 VSFHFLEGGT KPTLFQRVER VFFDAYFKTL YYLISQRSNQ NELAKYYDDI
  251 PPLEDLAGQI KFLLLNHHYV LTGSTLYPAN VVEIGGFHVG KPNPLSGELK
  301 IFVEQAEHGV IFLSFGTTVS LSLTSVEKIQ AILDTIEELP QRFIWRWDKK
  351 TTLDKKPFNQ LSKKHLDLLA NKKKIYIGNW LPQVDILGHP KVVAFISHGG
  401 MGGTTEAIHF AVPIVAMPIT GDQPANAAAI EESGFGVHQP INSLTKEDLV
  451 ASLRKVLDPK FREQVKLRSK AWHDRPVSPM NSAVYWIEYA ARNGNFTFRT
  501 PAATVPLYQY LYLDTMAVYA VFFTAVFLLF KAFCCTSSRK ETKTPMNNKK
  551 KKQN
```

>comp35471
```
    1 MEMLRRILCL FCVFSSIEGY KVLVAFPLPV RSLNLLGEGL VRHLLNAGHE
   51 VTYITAYPFK DPPKKNFRQI DLSSVKSVFA NQNKLNTGYI MMNNRHTNNI
  101 YYVQELALNC AKATFADQNL QKLLQDTSES FDVIIADLLE TEIYAGLAAV
  151 YDCPMVWLYS MGAHSVALRL VDQPANPAYA SDYLTGHIPP LTFIQRVEQL
  201 WAHVEWYFLK WFFIQPEEKT LYQHTFGPLL SKRGRSLPDY QELIYNVSLM
  251 FSNEHNALGN VPAIPQNFKF VGGFHIDDPP KALPKDLQAI MDSSKHGVIY
  301 FSMGSTWQSK DIPESVTRGL LNMFGELKET VLWKYEENLQ NLPPNVKIVH
  351 WAPQHSILAH PNLRMFISHG GLLSSTEALH FGVPTIGIPV MFDQYINVNK
  401 AVSSGYALSA ELSDDLPNTL RPLIREMLDN PKYRQKAKQS SMIYHDRPAT
  451 AGQELVHWTE HVIKTKGAPH LRSPALRMPV YQKLYLDFVA CCCSFAIYL
```

>comp36019
```
    1 MDLTKLLFLL LFGFSSAYKI LVVFPYPGKS HTILGEGFVK HLVRAGHEVT
   51 YITPIPINNP PKGLRQIDVS SNIKTFESMS SSLSFKTVLN KEADLKDTRA
  101 WVGVINNIAN QTIWHHNVQK LMYDDNEEFD LVIAEWLYTE LYCGFAAVFN
  151 CPFIWSSSID PHGLVLGLID EEPNPAYTAN HMSSFEAPFT FSQRLEELWE
  201 VIYLKYMKWA IYDHENRIFQ EGYGPAVAKR GRTIPSLYEV SHNASLMFGN
  251 SHFSSGRPVR LPQNYIPIAG YHIDEEVDKP LPTDIQKIMN NAQHGVIYFS
  301 MGSMIRSSSM PDGIKQGFLK MFGSLKQTVI WKFEEVLPNL PKNVHILKWA
  351 PQQSILAHPN CLVFISHGGL LSTSEALHYG VPIIGIPMFA DQFINVDRAM
  401 KKGFALKVDI AEDMTVHLKA AIEEILGNPR YHERMKELSF IYHHRTTTPG
  451 QEILHWVDHV VKTRGALHLR SPALDVPFYQ KIYLDLITLI AVATIVLFRI
  501 AKRLVCKSAV TKKVKKN
```

>comp36231
```
    1 MLRGRVKHLL HCVSFLSFIF FFLVFAGAIN VNDEQKEIVD PWEAYGIYGT
   51 IILYVLRLLT LLTIPQVLCN FAGLIFFNAF PGKVKLKGSP LLAPFICIRV
  101 VTRGDFPKLV KENVTKNMNL CLDAGMENFM VEVVTDKAIN LPKHRRVREV
  151 VVPSEYKTKT GALFKSRALQ YCLEDSVNIL AGTDWIVHLD EETLLTENSI
  201 RGILNFVLDG QHQFGQGLIT YANENIINWV TTLADSFRVA DDMGKLRFQF
  251 YLFHKPLFSW KGSYVVTQVS AERKVSFDNG LDGSVAEDCY FAMKAYMEGY
  301 SFNFVEGEMW EKSPFTLWDF IQQRKRWIQG ILLVVHSKEI PLVNKIFLAI
  351 SCYSWVTLPL STSNVLLAAL CPIPCPTLLD IVCGFIGAVN IYMYIFGVIK
  401 SFPIYRFGPL KFFLFIGGAL ATIPFNIVIE NIAVVWGVLG KKHKFYIVNK
  451 EVKIPVTV
```

>comp36263
```
    1 MRALLTVFSL ATVLTLDDAN AARVLGLFPH TGKSHQMVFD PLLRTLAERG
   51 HHVTVVSFFP VKNPPENYTD VSLEGIAGLG LEVIDLGMYE NGNVLLKMLG
  101 LDNIARQLLD FEPLAEMALD VCSKLVSFPP LAEVLRKDYD VILVENFNSD
  151 CMLGLSHVYG KKVPVIGLLT SSLMQWSADR IGVTDNPAFV PVLSAHYTSR
  201 MNFYERLENT FLNVYFKVWF RYNIQLKEQE IIERHFGRRI PDLRDLAKNT
  251 TLLLANVFHS LNGVRPLIPG LVEVGGMHLN HKRTVVPPYI ERFMNESDHG
  301 VVLLSFGSLI KTSTMPEYKE RMIISALSRL KQRVIWKFEE SEEEGTLEGN
  351 VMKVRWIPQY DLLRHKKVLA FIGHGGLLGM TEAISAGKPM VVVPFFGDQP
  401 YNAAMAEEVG LGVQLPYEQL TEESLLKAVQ TVLSAEMRLS ARRISKIWHD
  451 REAKPLDTAV YWTERVIRWG YHDKLYSAAR DLNFIEHNLL DVAAAFVLAI
  501 IVLVLIAKLL LTAVLKIFKA SISGKDKEKL H
```

>comp36666
```
    1 MSPPISSSCK LKKFSIVCLL LASLQVGFAY KILVVFPMPG KSHTILGEGV
   51 VRHLANAQHD VTYITPILLK SPPKNVRQID VTSNFDFMKS NDMLNLKTHM
  101 DNNGEMDLTM VFNMMMQIHN MTYHNPNVQK LLSDTSEQFD VVVAEWMFSE
  151 LYSGFSAIFN VPLIWVSTIE PHWLVLRLMD EVCNPAYTSD TLSANIPPFS
  201 FITRLQQLGS QIFGFGLKKF LIEGFEEKAY AELTPYFKMR GREAPAFKEL
  251 AFNASLMLGN SHVSLGQPMS LPQSYINVGG YHIETNLAPL PKDLQILMDN
  301 AKHGVIYFSL GSNIQSKDLP DELKQSLLKM FGELKHTVIW KFEETLPGLP
  351 SNVHILKWAP QPSILAHPNC ILFITHGGLL STTETIHFGK PIIGIPVFAD
  401 QFVNVNRAVA KGFAKRVDLS YGMAPELGAA IKDIIGDPKY SNNVKQLSLI
  451 YHDRPVPPGK ELVHWVEHVV KTNGAPHLRS PALSVPFYQK MYLDLLALIV
  501 VILLGIRAIF RRIFKKKSSK VKKE
```

>comp36903
```
    1 MRLFLICLLL ASAVNLEAYK VLLCFPFPAR SMNSLGDGYA RHLIDAGHEV
   51 TFITAIPKKQ NIPNLREIDV SDNYEIIANE NFNNISFILE NILDLSSDVE
  101 FLQRLTLDIA LKTLENKDVK ALMGNPKETF DVFIADLLET ELYAGFAALY
  151 NCPLVWAYSM GAHWVAMRLI DDPTNPAYSS DYFTTPIAPF SFTDRFRVLW
  201 ENVKWRYAKI FITQPKEEAA YISIFFPEFK KRGMIMPDYD DLIYNASLVL
  251 SNDHHASGNT PKTPQNWKFV GGFHIEEPVK LLPETLKTTM DNAVHGVIYF
  301 SMGSVWNSEL IPKQITDGLL KTFGELKATV IWKYEGNLPN VPKNVHLIKW
  351 VPQQSILAHP NCKLFITHGG LLSSTEALHF GVPIIGVPIS YDQFLNIEKA
  401 VTRGYALQVA LSYNLPDELR SAIDVVFDNP KYRDQVKKLS KIYHDRPIAP
  451 GKELVHWIEH VIRTQGAPHL RSPANLVPFY QKAYLDILVI AIAVVALVIY
  501 LKNLMFGESN KRQSKKKYKR N
```

>comp37547
```
    1 MVCELYTGLA AFYGCPFIWV STIEPHSTIL SLIDDSLNPA YNPGLFSNTI
   51 PPYNFVERAK ELLMSVANVV LKDVVLVTYY EQAAYDELYV PLLKKKGRPV
  101 LTYEEVRYNV SLVLGNSHVS LGQATRLPQN YKPIGGYHID TNFKPLPEDL
  151 KNLLDNAKNG VIYFSMGSNI KSKDMPEELK RSLLKMFSGL KQTVLWKFEE
  201 VLTDLPENVH IVKWAPQPAI LSHPNCILFI THGGLLSYTE AVHFGKPTVG
  251 IPVFADQFLN VERIGKKGLG KRVDLSYTMA DDLKIAINEV LSNPSYMTKA
  301 KELSLIYHDR PTPPGGELVH WVEHVIKTAG APHLRSPALN VPFYQKMYLD
  351 LAALVVVVII TLRLIVKRLC NSCRKKKISS EKKNK
```

>comp37715
```
    1 MLARAVVLYL VCAGASALRL LLVFPVPGPS HAILAGGLSK HLIGAGHEIT
   51 CITPLPSKNA SKNLRQVDIS ANFQLVPLGD VLQLEKIMSK EINMKDLAFI
  101 KSLMISLANA TLTNPNVKRL MEDPAERFDA VIAEWMYTEL FAGISAVFNC
  151 PLIWFSSMDP QALVLRLIDG TPSPAYFADP MSAEHPPFDF WQRIKGLWLL
  201 FRRMKLEWST RSIEDSIYNS EYGPVAAVRG ITLPPLTVMR YNASLMLGNS
  251 HISMGQSISL PQNYKEILGY HIADKVQPLP DNIKKIMDEA KHGVIYFSMG
  301 SMLKSTTFPE ALKRELLDMF RGLKQTVLWK FEDVPPKLPA NVHVVKWAPQ
  351 QDVLAHPNCV LFITHGGLLS ITEAIHHAVP IIGIPMFADQ FLNINRAVRK
  401 GFGIKVSLDW DLTKNLKSAI EEIFRNFSYQ EKVKEVSFVY HHRPAPPGAE
  451 LVHWIEHVVK TRGALHLRSP ALNVAFYQKM YLDLAAVVVV VLVVVVKVVK
  501 SILKSKKGSE KSKEKQR
```

>comp37971
```
    1 MKLPVTIITI LTTIFINEVT PLNILGVFPY QGRSHFFVFE PYLRELAARG
   51 HNVTVITHFP QKTPVKNYQD ISLAGTSIQV EGLIPVEKSY FTLIMIGVYL
  101 IGTGTDNCKA LLADDNVQKL WKSEAKFDVV LVEQFNSDCS LGLAHKLGAP
  151 VIGLTSHTLM PWQYNRFGVE FNPSYVSTQF LSGGTKPSLF ERVERVIVYN
  201 IFNTAFKYAC QRTDESTLRE YFDVVPPLEE LAQNIRVQLV YTHHTLSGVY
  251 LYPPKIVEVN GYHVAKPKPL PEKLKKFIDE AEHGVIYVSF GSMLKAASTP
  301 RDKLEAITKA LSQLPQRVIF KWEEKTLPGD YKNIYISDWL PQNDILAHPN
  351 VVAFYSHCGL LGTTEAIYHG VPIVGMPIFG DQPSNAAAME EGGMGVQIQT
  401 TELTTEKLLE KFKIVLDPQF RANVKRLSKV WHDRPSSPMD TAIYWTEYVA
  451 RNPNFTFVPP TVHVPFYQFW CLDVLAVCIL ITLISFYVLK FLCCLVCRRK
  501 SKEVVKIANT EKKSKKDN
```

>comp38172
```
    1 MPDVTFLLIA LCLSCAGSEA ARILAYFPTP SISHQVVFRS LMQELAKRGH
   51 EVTVLTTDPV FTKTPAPPNL KEVDLHDLSY KTWREEFIQR SSANKDDIVS
  101 QMKILLKLLN DIMEKQLLSA EVKNVIDVKK NKYDLVIVEA YARQLMVLSH
  151 LFKTPLIQFS SLGGTFDTFS TVGAPIQELL YPSNVRQKLY NLTMWDKVTE
  201 LAKFYQMKYY YDTQVEEENA MLERVFGDVP SINELSNNVD LLFLNIHPIW
  251 EGSRPVPPNV IHIHGIHEKP QRDLPNDLKT YLDSSKHGVI YISFGTNVKP
  301 SLLPPEKIKI MVNVMSKLKF DVLWKWDKEV MEGKSENIKL AKWLPQSDLL
  351 RHPNIKLFIT QGGLQSTDEA INAGVPLIGI PMLGDQWYNV ENYVHHKIGL
  401 RINMDTMSEE SLREAVKKVT EDQSYRQNIV RLRSLMKDQR DTPLERAVWW
  451 TEYVLRHSGA RHLRSPSANM PWHQYFELEL ISTVLGVIFV CLIVVVIALV
  501 KLVKGLKIVL GLQVKVKRS
```

>UGT33D1(JQ070229)
```
MLLITILQGTKAARILGVFPTPSISHQVVFRRLTLELHKRGHELVIVTTDPMYQKGEAPQNYTEIDVHDISYTTWRNDFMKLSRGSSD
                        DLFEQSAVILELTTNLFEMQLKSKEVQALIKVKDAKKFDLLLLEACIRPAIILTHVFDAPAIL
                        VSSFGGVEYVFRILGVPTHPVLYPPPLHQRIFNLTFWEKTHEIFTHYYLEYLFWKAEYKVDEM
                        VKRIFGPSTPTVRDTYKNVEMILLNAYAVWENNTPVPPNVIYVGGLHQKPEKDLPGDLKEYLD
                        SSKHGVVYISFGTNVEPSLLPPERIQLLIKVFSELPYDVLWKWDQDELPGKSENIKIAKWLPQ
                        SDLLRHPKIKVFITQGGLQSTEEAITAGVPLIGIPMLMDQWYNVEKYVQLNIGLKLDLGSITE
                        DSFRNAINTVTGDESYRQNVARLRSQVFDQPQGPLERAVWWTEHVLRHGGATHLRAAGALKSW
                        TEYFELNLIAVLLVSFLIAIAFIVTLISSLMTSLKMYFNYDDKIKKH
```

>UGT34A2(JQ070244)
```
MWLKFFYFFSLVLCPCYAAQGASILAVFSSLSYSDHLVFRGYVSLLAQRGHSVVVMTPYPGEFQYPEVENIIELDVSQESAPFWEEYK
                        RLMTNTDDYYPRLKALNELSLKIAIAQLKSKQMTALLINPNVKFDLVITEADVPLLYALADKY
                        QTPHITISTFNGKIHQYEAKGNPIHPILYPDVNSLNYGNLTRWQKIIEFYRHIQTKTEFYNNY
                        LPLCDVAAKKILGLKRDLQEVEYDIDMLFIASNPLLIGNRPVVPAIQFVDRMHIKPRMSLPQN
                        LQSLLDSQTKGVIYFSLGTLQEAEKLSVKTLQVFADAFRELPFTVLWKIGKMSTLKLSDNVIT
                        DVWFPQQQLLAHKNVRAFITHGGPRSLEEALFYEVPIIGFPLITSRKIFIRELTKYGAGEILD
                        PLHIDKQTLKQVISTVATDEKYKKAIIKLKGMVVDPLISGPDNAVWWTEYVLRNRGAQHLRSP
                        VVGVTFIKYYMLDILTYILAVVLFLLYLTFLVLKCIYRRLRARFVLRTGQGPEGKFKAL
```

>UGT39B1(JQ070245)
```
MFNSFIFLFVVVTGLCESANILYVMPFTSKSHHIMLKPIGLELARRGHNVTVITGFRDKNAPANYRQIQVDQKEIWDVIGTKRPNVFD
                        MVGVSTEEFHNMILWRGGLGFTNLTLNSAEVKSFLAEDNKFDLVICEQFFQEAMNILAHKYKA
                        PLVLVTTFGNCMRHNIMIRNPLQLATVISEFLEVRNPTSFFARLRNVYFTVYEYVWWRYWYLE
                        EQEKLVKKYIPNLEEPVPTLLEMQKNASLILINGHFSFDTPAAYLPNIIEIGGVHLSKSDTKL
                        PADLQNILDEAKHGVIYINFGSNVRSAELPLEKRNVFLNVIKKLKQTVVWKWEDDSLDKMDNL
                        VVRKWLPQKEILSHPNIKVFISHGGLIGTQEAIFHGVPIIGVPIYADQYNNLLQAEEIGFGKI
                        LEFKDIREQNLDNYLRELLTNNTYRDKAKEMSIRFKDRPTTALDTAMYWIEYIIRHNGASFMK
                        NPARKLHWIQYAMLDVYGFILAVVLTIFYTIYKLSSFILHKLKAPERLIRKKFD
```

>UGT40A1(JQ070247)
MLRAIILCLILVTCSAYKLLAVFPFPGKSHMILGEGYVRLLLEANHDVTYLTPAPLKIGHPKLRQIDVSSTEKGFDFDGLFNFKKLIN
KEVDLSDTNHKYIVMQYVTESLLMHPNVQKLLLDTDQTFDAVIVEWMYSDLYSGFSTVFNCPF
IWSSSLEPHPMILRLIDSLPNPAYFPDHTSSLEPPYTFLERVEQLWNIAKTLYNRWRLKEKEE
SIYENAFGPAVKKRNRVLPPYNEVKYNGSLILGNSHVSTGVAFSLPQNYKAISGYYIPKKIPQ
LPDKIKNIMDKAENGVIYFSMGTMVKSKTLPEELKRNLVDMFGNLKQTVIWKFEEDLDGLPNN
VHIVSWAPQQSILAHPNCVLFITHGGLLSTTEALHYGVPIIGIPVFADQFLNIKRATTKGFAL
EVDINYETPGNLKLAIDEILNSPKYRENIKQLSLVYHDRPVSPGAELVHWVEHVVKTKGALHL
RSPALHVPFYQKLYLDLFAVILMMPLVLCILLRYMKNKLFGVIKKKGKKTKKNQ
>UGT40B1(JQ070248)
NIFKSFIDTANDTVANAEVQQLVLDPQTHFDVVIAEWMVTEIFSGFSEIFNCPLIWASSMEPHSVILRLIDEXPHPAYSSNMLGIFEP
PYNFVQRAINTSLEIALKVIKWFISLIEERIYKEGFAAAFKAKGLVQPSLEELRYSVALVLGN
SHVSSGAPLKLPQNYKAIGGYHIAEQSKPLPKEFKNILDNSKHGVIYFSLGSVVSSKSMPAAI
KNGLFEMFRSLKYTVIWKFEDEFQNVPDNVHIVKWAPQQSILAHPNCILFITHGGLLSTTETL
HYGVPIIGIPLFGDQTMNIKKAVYKGIGLEVKLNFDTPKNLKAAINEVLSNQKYRDRVKELSM
IYHDRPVSPGAELVHWVEHVVKTKGALHLRSQALHVPLYQKLLLDLIFVSLLLFLGFVFFVKF
MVTRCLKKKTDIRKKTL
>UGT40B2P(JQ070249)
KAGHELTYITPYPKTPAPNLRVIQIAEHAFEEKMNSMFTIEKLIDNTFALKEMFSILKSFIDTANDTVSNTEVQQLMLDPQTHFDVVI
AEWMLTEIFSGFSTIFNCPLIWSSSMEPHSPILQLIDEMVHPAYSSNMLGLFEPPYNFFQRAI
NTLIEIGLKVAKWFSALIEEQIYKEAFAAAFKAKDLVQPSLEELRYSAALVLGNSHVSSGAPL
KLPQNYKAIGGYHIDEQSKPLPKEFKNILDNSKHGVIYFSLGSIVPSKSMPAEIKNGLFEMFR
NLKYTVIWKFEDEFQNVPDNVHIVKWAPQQSILAHPNCILFITHGGLLSTTETLHHGVPIIGM
PIFGDQAMNVKKAVHKGIGLEVKFDSDTPKNLKAAINEVLSNQKYRDRVKELSLIYHDRPVSP
GAELVHWVEHVVQTKGALHLRSPALHVPLYQKLLLDIIFVSLLLFLGFVFFVKFMLTRYLKKK
TDIRKKTL

>UGT40B3(JQ070250)
MNFQTIHLLVLSALACDAYKILLVFPFPSKSHAILGEGYVRNLLKAGHEVTYITPYPRDPAPNLRIIQVSQHDFEEKINSTLTIEKLI
DYSFTVMEMFNITKSFIYTANDTVANTEVQQLMLDPQTHFDVVVAEWMITEIFSGFSVIFNCP
LIWSSSMEPHSWILPLIDEIPHPAYSSNILGLFEPPYNFVQRAINTSLEIALKVIKWLVTLIE
EQIYKEGFAAAFKAKGLIQPSLEELRYSAALVLGNSHISSGAPLKLPQNYKAIGGYHIDEQSK
PLPKDFKNILDNSKHGVIYFSLGSMAPSKSMPAAIKNGLFEMFRSLKYTVIWKFEDEFQNVPD
NVHIVKWAPQQSILAHPNCILFITHGGLLSTTETLHYGVPIIGMPMFGDQVMNIKKAVHKGFG
LEVKLNFDTPKNLKAAINEVLSNQKYRDRVKELSLIYHDRPVSPGAELVHWVEHVVKTKGALH
LRSQALHVPLYQKLLLDIIFVSLLLFLGFVFFIKYMVTRCLKKKIDIRKKTL
>UGT40B4(NM_001257036)
MNVQTIHLLVLSALACDAYKILLVFPYVSKSHAILGEGYVRNLLKAGHEVTYITPYPKDPAPNLRVIQIAQHALEEKMNSTFTIEKLM
HKTHAGMEMFKLVKSFINTANDTVSNTEVQQLMLDPQTHFDVVIAEWMVTEIFSGFGKIFNCP
FIWSSSMEAHSLILRLIDEIPHPAYSSNTLGLFEPPYNFFQRAINTLMEIGLKVAKWFSISIE
EHIYKEGFAAAFKAKGLVQPSLEELRYSAALVLGNSHVSSGAPLTLPQNYKAIGGYHIDEQSK
PLPKEFKNILDNSKHGVIYFSLGSVVSSKSMPAAIKTGLFEMFRSLKYTVIWKFEDDFQNIPD
NVHVVKWAPQQSILAHPNCILFITHGGLLSTTETLHYGVPIIGIPIFGDQVMNIKKAVHKGIG
LEVKLDLDTPKNLKAAINEVLSNQKYRDRVKELSLVYHDRPVTPGAELVHWVEHVVKTKGALH
LRSQGLLVPLYQKLLLDIIFVSLLLFLGFVFFVKFMVTRYLKKKIDIRKKTL
>UGT40D1(JQ070207)
MEKMKICWVLFSIMLAIGDASKILVVYPFPSRSHANLGDGIVRNMLKAGHEVTYITPFEFKNAPPSLRQIDVSNLIDLMPKGLLTIKA
LMDGNNISLNIAFMTYMVTEIFKGMILNENVQKILTDPNEKFDLVIAEWMMSEIPAGIGAVYD
CPFIWISSVEIHWILLRFIDQAPNPAFTVDIMTTYTPPLNFVQRAIELWNQVKLTVLNYVILD
RIQDNVYSTYLAPIVEKRGRKAPTLDELRYNVSMIFSNAYVDTSSALSLPQSHKYIGGYHIDE
KVKPLPEDLQKLMDGAKNGVIYFSMGSNLKSADMPDELKASLVEMFGSLPYTVLWKFEEVLPN
LPSNIHILKWAPQQSILAHPNLRVFITHGGLLSTTETVHFGVPIIGIPVFADQFINVHRAEIR
GFAKRVDLSYTMAGELKKAILEVVTDKRYAEKAKELSVIHHDRPVKPGDELIHWVNHVIRTRG
ARHLRSPALGVPFYQKMFLDLAVVLTIVLTLAYILLKRAWRYFRSGKSKSSKKNN

>UGT40D2(JQ070208)
MEKTKICWVLFSIMLAIGDASKILVVYPLPSRSHANLGDGIVRNMLKAGHEVTYITPYEYKNAPSALRQIDVSSLLDLLPKDLMTLKS
LMEGKNMSLHALFMSYMMTEMSKAMIKNENVQKILSDPNEKFDLVIAEWMMSEIPAGFAAVYD
CPLIWISSVEIHWMLLQYIDQPSNPAFTVDIMSPYTPPLNFIQRASELWTQIKHMVLNYLILD
RIQDYVYSSYLAPFVEQRGRKAPTLHELRYNVSMIFSNAYVDTSSALSLPQNHKYIGGYHIDE
KVKPLPEDLQKLMDGAKNGVIYFSMGSNLKSADMPDELKASLVKMFGSLKYTVLWKFEEVLPN
LHSNLHIIKWAPQQSILAHPNLRVFITHGGLLSTTEAVHFGVPIIGIPVFGDQFVNVHRTEIR
GFARKVDLSYTMTDELKKTILEVVDDKRYAEKAKELAVIHHDRPVKPGDELIHWVNHVLRTRG
APHLRSPALGVPFYQKMFLDLAVVLTIVLTLSYILLKRAWRYYRSGKSKSSKKNN
>UGT40F1(JQ070209)
MGKLNLILLSILLSSCLCDGYRILAVFPTPSVSHGILADNFVKTLLNAGHEVVYISPFKNVNHPKLEITDVSQNVELFSDNIDVKEVM
NGSLDLLDTKVLFEIITTITDVTLANPSVQKLLRDPNQKFDVIVAEYFFNNIYSALSAIYDAP
FIWFLTIVPHSMILDQIHGPMNPAYSSDYIEARIAPYSFAERVRGLYFTLSLLYNLHVSFPPV
EEAIYHKHIPTILKSLGKPIADYKVLTYNVSMVLGNSQVAIESAVPLPPNFKHIGGYHIDDDV
KPLPENLKKIFDNAKNGVVFFSLGSNLRSKDLPEDMKQGILKVLGGLKQTVIWKFEESLPNTP
KNVHIVQWAPQQSILAQPKLVLFVTHGGLLSTTEAVHFGVPLVVIPVFGDQFMNAHLVEKKGI
AVQVKLSYTMYNELKVAMDTVLGDTKYATNAKALSAAFHDLEMKPKVALNFWVEHVVRTRGAP
HLRSVAVDIPLYQRVYLDLLALILLTPVVLLLVLKRFCCKKSDSQKVKRS
>UGT40F2(JQ070210)
MEKLKMWLFGILLCSCLCDGLKILTVFPVPSPSHGILGDNMVKHLLNGGHEVTYITPFKNKNPHPKLHIIDVSANMKLFDPNMLDVKK
IMDGDKYMQDNALLFVLMNSIASSTLGNPNVQKMLKDPNTKFDVIIGEFMFSDLYSALPAVLQ
CPFIWFSTIEAHSMILNQVHGPLNPAYTADYLVARVPPFSFYGRVHELWTLLVGLYHHNFDYH
AKEVSDYETLIAPIAREQGKPVPDFNVLKYNASLLLGNTHVAISNAVPLPPCYKHIGGYHIDE
EVKPLPEDLQKIMDSAKHGVIYFSMGSNLKSKDLPDELKQGLLKVFGGLKQTVIWKFEENLPN
TPKNVHIVQWAPQQSILAHKNLVLFVTHGGLLSITEAVHFGVPLIAIPVFADQYLNANRIEKK
GFAIKVDLSRTMDKDLKVALQEVLGNKKYAETAKALSIAYHDRPQKPKDALNFWVEHVVRTRG
APHLRSVAVDIPLYQRVYLDLLALILLALVVLLVVVKKIVGLLTSKKSVKQKKN

>UGT40G1(JQ070252)
MTKWILFLCVTSLLCTCDAYKVLVVFSMPGKSHSILGYGIVKHLLKRGHEVTYITPFPVDNADPKLKQIDVSSNIDILPKTSLNLNVI
LEGKVPKVDHGGIHLVMNAVEMNTYNNENVSRLINDPKQKFDIVIAEWMFTEICASYAAIFNA
PLIWVSSIQTHWMVTRLIDEALHPAYNTDVVGRNIPPFNFFQRVQNLWILLRTLYQVKNSGQE
DFYNIAVVPVIEKRGLVPPTFEDVQFNGSLVLSNSHLSYAPAVRLPQNYKTVGGFHVEEKVEP
LPEDLKKVLDSASTGVIYFSMGSNLKSKEMPDRLRKSLIKLFSGLKYTVIWKFEEEFSGLPKN
IHVVKWAPQQSILAHPNCVLFITHGGLLSTIESVHFGVPIITIPVFADQFMNAERSARVGFGK
IVYLSYTMADDLKVAIEEIFSNPRYKEIAKETSLIYHDRPVSPGAELVHWVEHVVKTRGALHL
RSPALQMPLYQKLYLDLLTVVLVLLIVIYKIVRCLFSRISVTSNKKTN
>UGT40G2(JQ070253)
MSKSLIKFLCIASLLCFCDAYKVLVVFSLSGKSHSILGYGIVKHLLKAGHEVTYITAFPEESSDPNLTQIDVSSNMVALPKSYKESLN
LKAVLEGKAIPLDFDIIHNLMNAVEMNTYQIENVSKLLNDPEQKFDVVIAEWMFTEICVGYAA
IFNAPLIWFSSVQTHWIITKLIDESLHPAYNADAIAHSIPPFNFFQRAHNLWTQLQVFYHLTK
GRQETLYANEIVPIIKKRGLVPPSFNDLLYNSSLVLSNTHVSYAAATRLPQNYKPIGGFHIDE
EVKPLPEDLKKVMDGASNGVIYFSMGSNLKSKEMPDLLKKELIKMFSDLKYTVLWKFEEEFFD
LPENVHMVKWAPQHSILAHPNCVLFITHGGLLSTIESIHFGVPIIAIPVFGDQFINVEWSVRK
GFGKRVDLSYTLAEDLKVAIEEVFANPRYKEIAKETSLIYHDRPVSPGAELVHWVEHVVKTRG
ALHLRSPALFVPLYQKLYLDVLAVILAFLIVLYKTARCLFLKERITNKKKNN
>UGT40H1(JQ070254)
MIRRLTIAIAVCFCLGVDAYKILTVFPVPGRSHGILGDAVVRHLLEAGHEVTHITPFPKKEPPPNLVQIDVAANKAAFNEDYIDIKAL
MTKEFNLKDKNVLFSLMNNISSSTILNENVQRLLRDQSREQFDVIIAEWMFSDLYASFHAVLD
CPLIWFSTIEPHWMVLRLIDEYPNPAYTSHFQDSFEVPFTFVERMSVLSSQLTWSLSLNTWVY
DLEKYIYDNNIAPIIKKNGKPVPNYDEVRYNGSLLLGNSHVSLGDAIKVPINYKAIGGYHIDG
KVKELPPDLQKIMNESKHGVIYFSMGSNLKSKDLPKEIKEGLLKMFSQLKQTVLWKFEENLSP
LPENVHLLKWAPQQSILAHPNCILFITHGGLLSTTEAVHFGKPIIGIPVFADQFGNVNRAVQK
GIARRVDLSFTMVRDLEEAVAEMINNSRYIEKIKELSLIYHDRPVSPGAELVHWVEHVVKTKG
ALHLRSPALHVPFYQKLYLDLLAIVLVTSIVLRFIFKNIHCNVQFKDKIQ

>UGT40K1(JQ070255) = BmUGT10286
MFKLTFLVCCILATQSISDAYKILVVFPMPGKSHSILGYSVVKHLLKAGHEVTYVTPFVEDNHHPKLTQVDVSSNMRLIPKGGLDLKR
VLDKEVNVIDNGFMFYFMKQIQEATLEHEQVKKLLEDPNKTFDIVIVEWMYCELGASYAAVFD
VPLIWLSTMEPHWLVTRLIDGNLNPAYNGDSMSSSIPPFTFLQRVKELWIQIHTSFILLNDDQ
ERSYDRLVRPLIEKKGRKAPSFEDLKFNASLVLGNSHVSLGEATGTPQSYKPIAGYHIEEVVK
PLPADLKEIMENAKHGVIYFSMGSNLKSTEMPDEMKQNLVKIFGELKQTIIWKFEEDFPNLPK
NVHIVNWAPQPSILSHPNCVLFITHGGLLSTTESVHFGVPIVGIPVFGDQFINVQRAVKRGFA
KKVDFSYSMVGELKVAIQEILSDSSYRTRIKELSLIYHDRPVSPGAELVHWVEHVARTRGALH
LRSPALHVPFYQKLYLDLLAVVLIISLIFYRIICLIKNLLLSFFQTNEIKKKKKRN
>UGT40L1(JQ070211)
MSHKTAGLLLLSLLVSSEALRILVCFPMTSKSHSILGHGYANRLLEAGHEVVHITSYPSKRIVQNLTEIDISYLQDFFKEQTMNDDAF
KLKNMIGKKNFEESVFFFYFVFTMHKNFLTDPNVVKLLSDPKEKFDAVVLEWFFTEITAGIPA
LLECPLIWACSTEPHWQALRLIDEISNPAYTLDLFSHNRIPLTFWQRAEGLWKVVKRNVQLAI
YYPFEKWAYNSIYPEIAAKRGVTMPSYEEAMYNGSFMLLNAHPSIGGSMKLPQNAANIAGYHI
ETTKPLPKDLQKLMDEAKHGVIYFSMGSIVQSDGMSEEMKKSLLDMFSKYEQTVIWKFESDLT
DVPKNVHLVKWAPQPSILAHPNLKLFITHGGQLSTSEAIHYGVPLVGLPVMADQHYNMISVEA
KGFGIKVTLAEDMVPELDAAVRKILTDETYTNRAKELSALFHDREMPPGVALTHWVELVVRNR
GAPHLRSPAIAVPLYQKLYLDLGVVLAIIIGLILKVVKYVLNRRSNKQSKEKSS
>UGT40L2(KF777114)
MKYKIVTSIFLLSLLVSSEALRILVCYPMTSKSHSILGHGIVNRLLEAGHEVVHITSFPNGKVLPNLTEVNVSSIAEVFTKDVDGVEQ
FKLKNLIGKGNFGDSALFMYYVYIIHRNFLEEPSVVKLFSDPKEKFDAVVLEWFFTEMNAGIP
ALFNCPLIWVCSTEPHWQSMRVMDGITNPAYTLDIFTHNKLPLNFWQRAEGLWKVVKKAVQVL
ILNQFEKWSYYSIYPEIAAKRGVTMPSYEEAVYNGSFMLINAHPSIGGAIKLPQNSANIAGYH
IDKVKPLPKDLQKIMDEAKNGVIYFSMGSIVQSDGMSEQMQKSILNMFSKYKQTVIWKFESDM
KDNIPSNVHLVKWAPQQSILAHPNLKLFITHGGQLSTSEAIHYGIPLVGIPVMADQVLNMISV
ENKGFGIKVTLSEDMIPELNAAIKKVLTDDAYRKKAKEISALFHDRVMTPGAAVPYWIEYVVR
TRGAPHLRSPALDVPLYQKLYLDLAAFIAVVEIVLKKVVKYLRKREVIKRRRAKNL
>UGT40M1(JQ070212)
MKLLVLFSLFFVLCSVESLKVLVLFHMPVKSLSILGTGVVRHLLNAGHEVTYVTVYPLKNPPTKNFRQIDISKNVELVAYDETLTMGY
VLEHQIEKNGAYQIQVFSQENARQTFQNENLKKLIQDPNEHFDVVFSDLLESEVYAGLAVLYD
CPMIWLYSMGAHWQVLRLIDHGSNPAFTPDYLSPNKLPLSLFERVEELWARVRWQFLKTFITQ
PEERKIYEETFGPLLAQRGRTLPDYEEVMYNASLIFANEHHAIRDRPATPQNFKYVGGFHIED
PVQPLPKHFQELIENSKHGVIYFSMGSFLKSNSLPKKLVQELLNMFGQLKQTVIWKFETNLPD
VPKNVHIVHWAPQPSILAHPNVKIFITHGGLLSSMEAIHFGVPIIGVPVFFDQFTNINKAVIN
GYALRVNLNYDLPKGLSAAIDVMLNDDKYSKKVKEMSAIYHDSLTKPGDEIVHWVEHVVRTRG
ARHLRSPAFNVPLYQRLYLDVLAIILAVVYLVKYIIASFDTKKAKKQSQKKKN
>UGT40N1(JQ070256)
MRSVLGLCLIFLANQVQGYTVLVITALPFRSLNILGASVVSHLLNAGHEVTYITTSPLKEKPKKNYREIDVSANTEIFKGEEMIDIAC
LMDNKVEMNHIFDLQNITIANALMTFENEDVKKLIQNTNESFDVVIADYIDTEVYAAFSALYG
CPLIWLSSLRTNWQTLRLIDEPTNPAYTVSSISMNYPPLNFKQRIEELWAQWKWQIVKRLYIV
SQEEKIYDNHFVPFIRKRGIKPPNYEDLIYNASLVLANDHHSLGNLPKTPQNFKQVGGFHISS
VVKPLDKVLQNIMDSSKDGVVYFSMGSAWQSKDIPEHIVNELLKVFGNLKQTVIWKFEKNLND
LPKNVHIVQWAPQTSILAHPNCLLFISHGGLLSSTEAIHFGVPIIGIPIFYDQFVNIQKAVIS
GYGIQVKLNYELPKSLEKALGEMLSDKKYREKAKQLSLIFHDRPVSPGAELVHWVEHVVKTRG
ALHLRSPALHVPFYQKLYLDLLAAIAMTLLMIKLVIEKTLSSFYKKTLKRKED
>UGT40P1(JQ070257)
MPLWILVVLLTFSYSGAHKILVVFPLPEQSHGILGARFVRHLLNYGHEVTYITPFIEKYTHPNQQQVDVSRNLKLIPENPVNLSSLIS
KEVSAPGFTETMNFMNLVAVQTLENENVQKLLKNPNLEFDLVILEWNFSELLAGIAAVFDVPY
IWVSNLEPHWLIARLAGESFNPIFNSNILSPYIPPLNLYQRVEELWTQITFHFHMYWYNDRIQ
RNDYERFFGDIIRMKGRESPLFEELKRNGSFVLGNSHLALGHEMRFPNNYKNVGGYHVDEEVK
PLSPKLEKLMNNAADGVIYFSMGSKLKSEDLPVDIKKGLMKMFGELKQTVLWKLDDKSIDPPS
NVHIFKRVPQQSLLAHSKCVLFITHGGILSTIEAVHYGVPIIGIPAYGDQFLNIERLVRKDQA
KRVDLSHSLVADLKYAIDELLNTKRYNDTAKNNSFIFTHRTVNAGAEIVHWVEHVILTKGAKY
LRTENLDLHWYQKLYLDLGLLLISAFLFLTYTCKLFLIFISKTRKVDVKKKKK

>UGT40Q1(JQ070213)
MNNWTLFLLSSICLSHVCAYKILVVFPYPGTSHSILGEGYVRHLLRAGHQVTYLTAIPYKKPHPNLKQVVVASVVEKFEFFKTLDFEK
FISKEVDLTDMQVMYETMITVANRSLTHENIQKFLMDTNEKYDLVVAEWLYHHLYSGFAAIYN
CPYVWSSSMEPHTAVLSLIDEPGNPAYFPDHMSPVSPPLTFSQRAYELYYLFYLRRVLWSIRG
LEQKTYEEVFGPAAAKRGITLPTLEEVKYNSSLMFGNSHISSGDPQRLPINHIPIAGYHIQDV
VPALPENLQKIMDEAPYGVIYFSMGSMMKSSTMPTKLKRDFLDVFGTLKETVIWKLEEELTDV
PKNVIMVKWAPQPSILAHPNCKLFVTHGGLLSTTETIHYGVPIIGIPLFADQFINVMRAVRKG
FALQVDLGYDTPANLKVAIEEIVSNPKYTQKVKDLSFIYHHRETKPGHTLVHWIEHVIETNGA
PHLRSPALHMPFYQKMYLDLLGIVLLGLIVLIKAVQILLRLAKKSDVKKKRS
>UGT40R1(JQ070214)
MAAATYFLLFSLLSLSSEASKILVVVTMPSRSHGNLGNGVVRELLKGGHEVTYIRIFEYKNPPPNLRQIDVSSNIDLMPKGIMNIKKI
MDKDVAANDHITVKMMMLELATKTIEHQNVKKLLEDPSEHFDLVIVDWMLADVPAGLATVFGC
PLVWLSPMEVNSLDISLIDGAPHLAYSTGAFSSNMPPFNFLQRAQELWTRIKARYYELKHFDR
MELDAYERLIVPYVEKRGRQAPSFYDVRYNASLILGNSHVSMGQALALPQNYKPIGGYHIDED
VKPLPEDLENIMMSAKNGVIYFSMGSHLKSKDWPEKVKRDLLNMFGQLKHTVLWKFEEDLPNL
PKNVHILKWAPQASILSHPKCVPFITHGGLLSTTETIHYGVPIIGIPAFGDQFINVKRAINKG
FALEVKLSYTVAADLKAAIEEILHNPKYRQKVKELSFIYHDRIAKPGEELLHWVHHVINTNGA
PHLRSPALHIPLYQRLYLDLLGLISVVILVFFILLRVLCKLVCSKKQKEIRV
>UGT40R2(KF777116)
MALAILLFLGLLLSSSCEAYKALVVFGMPSTSHFHLGNGVVRNLLRDGHEVTYITPIEYKNPPPNLRQIDVSSNFDVLPTYQINLKHL
MEAPKPSGHRNFVKLMLINLVMKTLEHENVQRLLNDTNEHFDVVIVEHMMSDLSASYATIFDC
PLIWVSPVEVNALSIGLIDVLPNPAYTTDTMALYTAPFTFLERLEELWMRISDSYNDYMVYEP
TEEAEYQRLIVPQLQKRGRQVPPYSEVRYNATLVLGNSHVSTGIPLGFPQNYKSMGGYHIEEE
VKPLPEDLEKIMMNSKNGVIYFSMGSNLKSKDWPEDIKRDLLKLFGELKQTVIWKFEEELPNV
PKNVHILKWAPQPSILAHPKCVLFITHGGLLSTTETIHYGVPTIAIPVFGDQFINVKKAVARG
YALEVKLSHSIAAELKVAIQEMLNNPKYRQRVKELSYIYHDRPVKPGAELRHWVQHVVNTRGA
PHLRSPALQVPLYQRLYLDLAALLLVVILVLKLLLKNLYHRIRPKKTNVNIKKKDKKN
>UGT40R3(KF777115)
MALAICLFFLLLSSSCEAYKALVVFGMPATSHSNLGRGVVRNLLKDGHEVTFITPIPIKDPPPNLHQIDVSSNFELLPLDLMKIERFL
GPNSMPALPRFFVKMMMMNLVSKTMEHENVQKLLNDTIAHFDVVIVEWMFTSLSAGYATIFDC
PLIWLIPVEVNSMTIGLVDAVPHPAYSTDPLSSYLPPFSFLERATEIWTRLQESVLGFLYYES
KDAANYERIVVPQVQKRGRQAPPLSEVQYNASLVLGNSHVSMGLPLSLPQNYKPVGGYHIEEE
VKPLPEDLEKIMMNSKNGVIYFSMGSNLKSKDWPEEIKRDLLKLFGELKQTVLWKFEEELPNV
PKNVHILKWAPQPSILAHPKCVLFITHGGLLSTTETIHFGVPTIAIPVFGDQFINVKKSVARG
FTLQVDLSYKLAADLKVAIEEMLSNPKYRQRVKELSYIYHDRPVKPGAELRHWVQHVVNTRGA
SHLRSPALQVPLYQRLYLDLVAFLSVAFIVLYMLIKKLYSRVRSKKIVNNKKRN
>UGT40S1(JQ070258)
MNIKLLISLFSFVLTCDCYKILIVFTTPMKSHNILGEAAAELLLNAGHEVTYVTPFPKESVPDKMRQVDVTYIGKIGLFDLKGYLKND
TVKPMSLRKISYFMHDVNVKAIQNENLQQLLNDPSQRFDAVIVDWLFSEIFVGLASLYDCPLI
WMSTMDPHWQILRLVDEMPNPVFLGRCFLDRIVPFRFWERTQELLYQISSLFFKDIEFFSEED
AAFKRLLGPVFARKKKPLPSFNAVRYNASLVLSNSHHSIGYPVKLPPNFISIGGFFIDDKKQR
LSLDLQTIMDNAKHGVILFSLGSNLKSKDMPEHLVRSLLNVFSELKQIVIWKVEEQIADLPQN
VHVLKWLPQQSILAHSNCILFITHGGLLSITEAYHHGVPLIGIPVFADQFKNVNLVSKKGFAK
KVDLTYNLPGDLKHAINEILHNKRYLEQAKLWSEIFHHRSVNPRKELVHWVEHVIHTRGATYL
RSPALDVPLYQKMYLDLLGLVMLVLMALTFLIKNVIKLFMVRGIVHEKME
>UGT40U1(KF777113)
MERIQTFWLALSVLLVCAEASKVLVVFPLPSRSHANLGDGIVRHLLNAGHEVTYITPFVYKNAPPNLRTIDVSSNFDVWPAHLITIKS
IIEDPDAFANMNMMAFLVTTIMNHTYENEAVAALLNDSKEHFDAVIVEWIFNEAIGGIATIFD
CPLIWMSSVEVHWKLLSLIDQPSNPAYSVDMTSSNQPPLSFTERVSELWTQIQISILSYFIFD
KMQDETYQKYVVPAITKRGRDAPSFYEMKYNASLILANAYVSTAIPQTMPQSHKYIGGYHVDE
VVKPLLEDKKLIESSKDGVIYFSLGSNLKSKDLPEEIRVSLLKMFGTLKQTVLWKFEANMTDL
PPNVHILEWAPQQAILSHPKLAVFITHGGLLSTIESVHFGIPIIGIPVLADQHMNIKKAVRNG
FALKVDLSYTMADQLKKAIIEVTSNSKYAQKAKELSFIHHDRPVKPGVELVHWVNHVINTHGA
PHLRSPALHVPFYQKMYLDLAAVLIILFLAGRLLLKKAYAAVFSKSKSNKKKTH

>UGT41A1(JQ070259)
MRCLGLLFFLVCVVTSARAYHVLCVFPIPSRSHNSLGKGIVDALLEAGHEVTWVTPYPPSELAKGLKIVDVSATVSISKTVDMHEQRN
SNTGVSFVKALAENITRVSLATPALQQAIVQGKYDAVITETFFNDAEAGYGAVLQVPWILMSS
IAMMPQLEAIVDEVRSVTTIPLLFNNAPTPMGFWDRLKNVFLHSVMVISDWLDRPKTVAFYES
LFAPLATARGVALPPFEEALYNVSVLLVNSHPAFAPPLSLPPNVVEIAGYHIDPKTPPLPKDL
QSILDSSPQGVVYFSMGSVLKSSKLSEQTRRELLDVFGSIPQTVLWKFEEDLQDLPKNVHIRS
WMPQSSILAHPNMKVFITHGGLLSILETLHYGVPILAVPVFGDQPSNANSAVRNGFAKSIEYK
PDMAKDMKVALNEMLSDDSYYKRARYLSKIFGDKLVPPAKVISHYVKVAIETNGAYHLRSKSL
LYPWYQRWLVDIIAALLLACLAVYVVARRVLCYLYTSVTGGGCNRSVKVKKN
>UGT41A2(JQ070260)
MRCLQLLLFLVCVVTSARAYHVLCVFPIPSRSHNSLGKGIVEALLGAGHEVTWATPFPPKESTKGLKIIDVSATASVSEMIDMNDQRN
ADAGIALIRTFAANITRLSLSVPALQQAIVSGKYDAVVTESFFNDAEAGYGAVLQVPWILLSS
VSIMPHLEAIIDEVRSITTIPLLFNNAPTPMGFWDRLTNIFIYSAMTISNWLERPNTVAFYES
LFAPLAAARGIALPPFEEALYNVSVLLVNSHPAFAPPMSLPPNVVEIGGYHINPETPPLPKDL
QHILDSSPQGVVYFSMGSVLKSSRLSERTRREILEVFGSLSQTVLWKFEEELKDLPKNVIVRP
WMPQSSILAHPNVKVFITHGGLLSTLETLHYGVPILAVPVFGDQPSNADRAVRHGFAKSIQYK
PDMANDMKVALNEMLSNDSYYTRARYLSKIFGDKLVPPAKLISHYVKVAIETNGAYHLRSKSL
LYPWYQRWLVDIIAALLLACLAVYVAARRVLCYLWSSVNGGDCNRIKVKKN
>UGT42A1(JQ070262)
MAKQTKIKLLLLTFIMSGVHTLNILGVFPYQGRSHFFVFQPYLEELARRGHSVTVISHFPQTKALKNYRDISLANTTKIMENAFSVER
SYKSLIEVSFYLMNTGVENCKIMLANKEVQDLWKNKIHFDVAVVEQFNSDCALGLAYKLGIPV
VGTNSHVLMPYQYERFGIHYNPSYMTFQFLEGGTKPTLFQRIERTIFHHYYNFIFEYLSQRTN
QNTLAQYFDDIPPLNELAREIKIMLFYHNFVLSGPNILPSNVKEVGGYHVAQPKELRPDVKKF
IEESEHGIIYISFGSMLKAAATSLDKIEAILGAVAELPQRVIWKWEEGTLPGNPKNIFISNWL
PQNDILAHPKVLAFYSHCGQLGTTEAIYHGVPVVGMPVFGDQPANAAAVEESGLGVQIQIEDL
TKENLLGKLRTVLNPEFRKRVKFISKAWNDRPVKAMDSAIFWTEFAAKYSNITFRSRSVDVPL
YQYLVLDVIAVLGSISVISVFVVFKLLGRLCTSKRENDKKNKLKRK
>UGT43B1(JQ070265)
MNFSLLGLFVFVNQCVSYKILAVFPYNGRSHHNLFSTLVEELALRDHSVTVVNYFPMKNISKLRQIPLEYKVSGSDVVDIDDTLKNLP
GILVNFHKALDTARAFKNLANSNCNKLMSNKEIQGIISSKTKFDLVIVEQFVTDCGLAVAFKL
NAPIVGITAHILMPWTYSRLGALNHPAYVPNHFIGSGTKPGFWDKIQSALINIAFNIYFKYVI
QKSDQMIINSVFEDVPDLDEIGKNISLILLNQYFPLTGSRLYGANVIEVGGLHIKENTTIDDE
EIKSFIDKAESDVIYISFGTVASNFPDRIIKEIINFITKSSVKVLWKIDNVGNLNLPKNVLIR
KWFPQTAVLCHPKVKAFITHSGMLSSIEAMHCGVPVISVPLFGDQFANAAAATEIGLGVTIDV
STMNERKINQALKTVMQDSYQIRAQNLSALWRDRPVSPLNLAIFWIEYVIRHKGNVELRPPTV
DLGFYELLMLDVCGMAIGILISFCLFFSIIISLIRFIRRRHINPNKTKTQ
>UGT44A1(JQ070266)
MTKRTIVCIFVTIILTTDCYKILGIFPSLDRTNYLTYRDLFKELANRNNDVTLISHFPMSDAPASYRDILLSDRHVYKGLSFESVIAS
EVSRVPFETLVATKAGNDDCKTLMNNNQVLHLIRTRPQYDVVLVESFNSDCGIALAANLSAPY
IALNPKPLQPWHYNRLGINFNAAYVTQTGLSYGKNPWFLDRVRGYILYHITNWVYYVGSQITD
HVYLYKYLGDNLPSLETLASNASLVFVNTHQSVFGGISRPDNVIDIGGIHVRPPKIIPTEIER
FINEAQHGVVYVNLGSTVKDSTLPAEKLAELLLTFRKLPHRVLWKWDGAAIQNLPRNVMTMKW
LPQYDILKHKNVKALITHAGILSTIEAIDAGIPVVAIPLFGDQYGNAAAMQDAGMATIVHYQD
LNKEHLLGAVNEVLDAKRQQQAKLTSRLWHDRSLSPLENAIYWTEYVARYQGAPNLQPLSSQA
PLYQQLQLDVLLFVAIVVYILFYALYKILRTLCCCCRADSGNGDDVGDTRKRKRVKFE
>UGT46A1(JQ070267)
MRAVPFHYILLVFIKDVLPARILGLFPHIGKSHQMAYDPLLRRLAERGHDVTAVTFFPLKDPPEHYRAVSLEGLTEIRVESINMSIYE
GHNVFLRLTGLDRIRSHISEIHPLADFALDTCSKLVSFKPLSELLRKEYDVILTENFNSDCML
GLANVYGQKAPIVYLSSCTAMYWALDRFGVTDNPSYVPLVSSIFTTPMTFLQRLENAVLNVYF
KVWFRYAIQLKEQKIIEEHFGRKIPDLQEMAKNVSLMLVNAHHSLNGVRPLIPGIVEVGGMHL
DKTRRPISQFFERFLNDSEHGVVLFSFGSLIKTSTLPKYKEDIIMKTLSQLKQRVIWKYEDSA
EEGTLVGNVLKVKWIPQYDLLQHSKIIAFVGHGGLLGMTESISAGKPMLVIPFFGDQHLNGAQ
AEKIGFGKVVSYADLSEKTFLDGLQSVLSPEMRLSARRASNIWSDRQADPLDTAVYWTERVIR
WGHRAPLHSPARDLPLHQYLLLDVAAAILVAILVLIAILRLIVVLIIRFFSGSVTAKEKLH

>UGT47A1(JQ070270)
MRVAWLLWLATVARAARLLVVLPTNTKSHYAMYSRLIEALAKRDHQLTVITHFPVDAPQPNINQISLAGTIPEITNNLTRKYDSLKPN
FIRNLEQIISECVNACETVVQQDSFKELLNSTASFDLIIVEVFGSDCFLPLGHRFRAPVVGLL
SSVPLPWVNDYLGNPETASYIPSYMMGYGQHMSLWERLSNTIAIILAKILYTYKSRIPSQVIV
DRVFGHGNNLQKLAKNYSVILSNSHFSINEVRPLVPGLVEVGGLHLDHSQTLPKNMKKLLDAS
TDGVIYWSFGSMSRIETIPSEKLSGIFEAISELPQLVFVKMDRRRLTKNITVPDNVYTMDWIP
QYATLCHPNVKLFISHGGLLGTQEAVACGVPMLMVPLYADQALNSQSMFDKNVARILDLHEAD
KYEWKRALHDLLSNKKYRENSKTLKEIFLDRPINPLDMGVYWIEYVLRYRGAPHMRSPALDLS
LSQYLLFDVIIINITITIVSVFILHALFKYLCTKCIKWCPKEKIVIEKRLFKNNVTLFVCLLR
KCKVKIN
>UGT48C1(JQ070271)
MSYRMMFGTVLFFCAVCLCWGSRVLVVSPVPSRSHQQLTDSIVKTLLDAGHEVTYINRLTEMKRKRLKVITVRPEDGQELDVNDLIER
HHTSHKTIELGVVLAKQVIKNEKVRELLQDNQETFDTVIAEWYYTRLLAPLAAVFECPLIWYT
ACDASWMTSQLMLEQTSPVYSTDLLSSEAALPPYGFRERVMRLARQVYLSGWITYMIHYVESP
AYYELYQSVLQHRGLSPSQYERVLYKASLLLINSDPEIGQILPLPPNTKYVGGHHIELPSKAL
PQNLQDLLDNAKHGAILFSADSKILPWYVKRTLLHVFSQFDQITMWETGEQLTDIPDNVYVFK
QLPRLRILNHNNTVLLITNGGTTSLLEAAYFGVPVIGIPLYQDQFVTMDLAHARRRGIKVKFS
EHIAHKIKDSVNKILSNNSYHKNAEKVSIYLQSTLEPQRQILHWIELVIKTGGAPQLRSPALL
QLTTLQMLNIDVLLLLFMFVWFLSKVLKVIQVHWRADVLDNKKND
>UGT50A1(JQ070272)
MWAGTRWPIIGLPILLVASVCGSDILMITMGGTKSHKIPFWSLAGGLTRRGHNITFISAFPPDFHIAGLEEIAPEGLVSYVKSYMSWD
LVGARMRGEEPLPAFDILRYGYEACDALLKDYEMRSFLQSGRTYDLIIIDGTYPECALGITYK
MKVPFMYINTVGFYSMPLSNAGNPAPYSVTPFFGRAFTDNMGIIERAMNSAWQIGAMALHGVS
MTILQGVLRRHFGSQMPHVYDMSKNVSFILQNAHYTVSYPRPYLPNVAEIACIHCIEPKRLDP
EIEEWISGAGDTGFVYVSMGSSVKTSKMPLTAHRMLINALGRLPQRVLWKQDAVQNMTDIPSN
VKLLKWSPQQDLLGHPKIKAFITHGGLLSMFETVYHGVPIVTIPVFCDHDANAAKAEVDGYAK
KLEFQYLTSDKLHEAIQEVINNPKYRREVKYRQNLLRDQKESPLDRAVYWTEYVIRHKGAYHL
QSPAKDLTFIQYYLLDVAMLFVISALAFYALISFAIRSSFQRLAVFIQNRQMKMLFDNSTGLI
GNSLMEQKKKL
>UGT340C1(JQ070242)
MNIVVFSLLCVATVEPARILGVFPMPSISHQVVFRALTLELAKRGHELVVITTNPVTKGNPIANLTEIDISESYDLIKTLFELCKDMN
QLKRGVISDIETMVHSRSTEGMFFLMAHMFKNDEVKKLMHDKTQKFDLVIAEAILHTHLVFGK
IFNAPIILFSSLSGFPEVFDIMGAATRHPFIYPSIFRNKFSNLTLLEKLREIYYEYKLTSLYW
HMEQLENQMLQEMLGDGAPTVNDLKQHISMLFLNTFPIFDNNRPVPPSIVYLGALHLQPVKEL
PVDLKQYLDNSKRGVIFVSLGTNVIPALMEKDLLDAFRKAFEILPYDILWKLNGVKLENVSSN
VRIQEWFPQRDLLFHPNIKLFVTQGGLQSTDEAIDAGVPLVGIPMLGDQWYNVNKYVELGVGV
QVDSLTMKAEDLVEAVKTVLSNDRYRENIMKLKAVMYDQPQKPMDRAVWWTEHVLRHGGAKHL
TSPAANMPWTKYFMLDVLGLVLTALVAILVTAIFAIYLIHRIFKTLSKVNKLKMQ