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Response of Three Kinds of Detoxifying Enzymes from *Odontotermes formosanus* (Shiraki) to the Stress Caused by *Serratia marcescens* Bizio (SM1)

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Abstract

Subterranean termite *Odontotermes formosanus* (Shiraki) (Blattodea: Isoptera: Termitidae), is a pest species found in forests and dams. *Serratia marcescens* Bizio (SM1) has a potential pathogenic effect on *O. formosanus.* However, the response of detoxifying enzymes to exposure by *S. marcescens* in *O. formosanus* has not been studied. In the present work, 20 detoxifying enzyme genes, including 6 glutathione S-transferases (GSTs), 5 UDP glycosyltransferases (UGTs) and 9 Cytochrome P450s (CYPs), were identified from the *O. formosanus* transcriptome dataset by bioinformatics analysis. Furthermore, the effects of SM1 infection on the transcription levels of detoxifying enzyme genes (GSTs, UGTs and CYPs) in *O. formosanus* were determined. The results showed that the expression of all detoxifying enzyme gene, except one GST, in *O. formosanus* were altered in response to the infection by SM1. The response of GSTs, UGTs and CYPs to SM1 in *O. formosanus* suggested that they may play an important role in the defense against bacterial infection such as SM1, and implies that termites have evolved a complex immune response to potential pathogens.

Introduction

The subterranean termite *Odontotermes formosanus* (Shiraki) (Blattodea: Isoptera: Termitidae) is a serious insect pest of trees and dams in China (Huang et al., 2012). At the present, the use of chemical pesticides is the main measure to control *O. formosanus*, but the potential residues and the harm to humanity have led researchers to focus more on other effective methods. Besides pesticides, a number of control methods have been used to alleviate the damage caused by *O. formosanus*, such as natural predation, microbial intervention, plant extracts and soil pH effect (Li et al., 2017; Xiong et al., 2018; Afzal et al., 2019; Ashraf et al., 2020). Despite a large body of literature about attempts in the laboratory to implement biological control to termite pests, no field trial or commercial success were reported (Chouvenc et al., 2011), as termites have evolved a complex immune defense against such infections (Chouvenc & Su, 2010, Rosengaus et al., 2010, Bulmer et al., 2010, Chouvenc et al., 2013).

Insects have to resist the influence of various environments and the invasion of pathogenic microorganisms. Therefore, insects possess a complex innate immune system during the long-term evolution (Rus et al., 2013; Ezzati-Tabrizi et al., 2013). Detoxifying enzymes, such as Cytochrome P450s (CYPs, EC 1.14.14.1), UDP glycosyltransferases (UGTs, EC 2.4.1.x) and glutathione S-transferases (GSTs, EC2.5.1.18), are important parts of their immune mechanisms (Schuler, 2011; Pan et al., 2019). Insects are able to metabolize foreign toxic substances through detoxification enzymes, including insecticides and plant secondary metabolites (Enayati et al., 2005; Zhou et al., 2017; Kim et al., 2017). GSTs are the

superfamily of enzymes that widely distributed in prokaryote and eukaryote, such as animals, plants, and microorganisms, which play a vital role in detoxification of xenobiotics, natural and synthetic compounds (Guo et al., 2008).

GSTs are generally classified into three types: mitochondrial, microsomal and cytosolic GSTs, and cytosolic GSTs can be categorized into six classes, such as omega, theta, delta, sigma, zeta and epsilon (Feng et al., 2001; Hayes et al., 2005), in which the delta and epsilon families are only found in insects and considered to be involved in insect detoxification reactions (Che-Mendoza et al., 2009). UGTs, are multifunctional superfamily of enzymes found in all organisms in nature, including viruses, bacteria, fungi, plants and animals (Li et al., 2018). Insect UGTs are major phase II detoxification metabolic enzymes, which are distributed in the fat body, midgut and antennae in insects (Ahmad & Hopkins, 1993; Bozzolan et al., 2015). Insect CYPs are significant metabolic enzymes involved in decomposition of endogenous and exogenous compounds (Feyereisen, 1999), including pesticides and plant allelochemicals (Schuler, 2011), and generally classified into 48 types: CYP4, CYP6, CYP9, CYP12, CYP18, CYP28, CYP49 and CYP301 to 341 (Amenya et al., 2008), of which the CYP6, CYP9, CYP12, CYP18 and CYP28 are unique to insects (Berge et al., 1998). Among the insect CYP genes, the families 4, 6 ,9 and 12 were considered to be associated with detoxification processes, making insects resistant to insecticides or host plant allelochemicals (Li et al., 2007). There is some evidence to suggest that plant secondary metabolites can inhibit the activities of detoxification enzymes such as GST, in *Reticulitermes chinensis* Snyder (Xie, 2013). And according to Zhou et al. (2006), P450s potentially involved in regulating caste differentiation in *Reticulitermes flavipes* (Kollar). However, not much is known about these detoxification enzymes in *O. formosanus*.

Serratia marcescens is a gram-negative bacteria and belongs to the family Enterobacteriaceae (Kumar et al., 2004), which can be generally isolated from environmental niches, such as soil, water, and air (Castelli et al., 2008), and had the potential of biological control (O'Callaghan et al., 1995; Wang et al., 2013). Moreover, it is also found that *S. marcescens* can increases plant resistance (Lavania et al., 2006; Chakraborty et al., 2010; Ting et al., 2010). Previously, we isolated a strain of *S. marcescens* from *O. formosanus*. After separation and purification, a pure strain was obtained (Fu et al., 2019). Then, the bioassay showed that the of *S. marcescens* have a high lethal effect on *O. formosanus* (Fu et al., 2020a). At the same time, we found that the insecticidal effect of *Metarhizium anisopliae* and *S. marcescens* was better than *M. anisopliae* or *S. marcescens* individually (Fu et al., 2020a). In addition, we reported that the death of *O. formosanus* due to the insecticidal protease in the supernatant (Fu et al., 2020b). These studies showed that *S. marcescens* had insecticidal effect on *O. formosanus* small groups in the laboratory, although field implementation on large populations remains to be tested in a safe and cost-effective formulation.

Although much effort has been invested in probing insecticidal effect of microbial origin to *O. formosanus*, a classical biological control approach is unlikely to succeed, as large healthy termite colonies can prevent epizootics (Chouvenc et al., 2011). However, investigating the corresponding responses from entomic immune system can provide valuable information on the defense pathways involved in disease resistances in termites, in hope to determine potential avenues for synergists that could inhibit such defense mechanisms. Up to present, the responds of detoxifying enzymes to infection of *S. marcescens* in *O. formosanus* have not been researched. In the present work, we used the *O. formosanus* workers infected by *S. marcescens* as the research materials, and confirmed the pathogen-specific changes in GST, UGT and CYP expression.

Materials and methods

Insects

Six colonies of *O. formosanus* were collected from Jurong County in Zhenjiang, Jiangsu Province and each colony was placed in a plexiglass feeding container (diameters of nest area and food area are 40 cm, 30 cm). The colonies were fed on pine wood chips and wet cotton without exposure to any insecticides under dark at 25 ± 1 °C and 90 % ± 5 % relative humidity. The colonies were reared for two weeks and for testing.

Bacteria culture and treatment of *O. formosanus*

Serratia marcescens (SM1) strain was isolated and purified from dead individual of *O. formosanus* and stored at -80 °C in Nanjing Forestry University, China. It was inoculated in a triangular flask equipped with sterilized fermentation medium, at 30 ± 1 °C oscillation culture in shaker until the OD was measured up to 0.6 using the UV-Visible spectrophotometer (Eppendorf, China, Ltd.), reserved at 4 °C for testing. 25 workers that in same size and health condition were picked out and placed in a petri dish (12.5 cm diameter, 2.5 cm high) with a filter paper wetted with distilled water on the bottom pad, and a total of 12 petri dishes were set up from 6 different colonies. A 0.5 μL sample of SM1 medium was dropped on the pronotum of each worker, then they were placed in a petri dish, and same sample of medium without SM1 was applied to the control groups, rearing conditions as above, and SM1 enters the midgut through the mouthparts due to the grooming behavior of workers (Kramm et al., 1982; Yanagawa et al., 2010) At the 13th and 20th hour, ten surviving workers from each treatment and control were selected for RNA preparation, respectively. All experiments were repeated 3 times.

Extraction of total RNA and cDNA synthesis

According to the instruction, total RNA was extracted from ten workers using Trizol Universal reagent (Invitrogen,

Thermo Fisher Scientific, Inc., Waltham, MA, USA). To ensure the integrity and quality of the total RNA, 1 % agarose gel electrophoresis was performed and the absorbance was measured by a Thermo Scientific NanoDrop2000. The cDNA was obtained from 5 mg total RNA by reverse-transcribing according to the manufacturer instructions (PrimeScript RT reagent Kit with gDNA Eraser), the acquired cDNA was stored at -80 °C for use.

Sequence comparison and phylogenetic analysis

The amino acid sequences of the GSTs, UGTs and CYPs were obtained from the transcriptome sequencing data and compared with representative insect species using NCBI Protein BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and these CYP gene sequences will be named by CYP Committee (http://drnelson.uthsc.edu/CytochromeP450.html) standard nomenclature. The Neighbour-Joining method was selected to structure three phylogenetic trees by MEGA 5.10, the bootstrap value calculated for 1000 replicates shown below branches (Tamura et al., 2013).

Response of three kind of detoxifying enzymes to SM1 in *O. formosanus*

Quantitative real-time PCR (qPCR) was applied to compare the mRNA expression of *O. formosanus* exposed to SM1 and the control using a 7500 Fast Real-Time PCR system (Applied Biosystems, Foster, CA). Primer Premier 5.0 was applied to design the primers of 6 GSTs, 5 UGTs, 9 CYPs and reference genes according to *O. formosanus* transcriptome dataset (Table 1) (Lalitha, 2000), and they were synthesized by Nanjing TSINGKE Biotechnology Co., Ltd. The cDNA concentration was adjusted to 50 μg/mL and the whole reaction preparations was carried out on ice. Ribosomal protein S18 (*RPS18*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were used as reference genes. The melting curve was analysed to evaluate the specificity and sensitivity of these primers, and the standard curves were derived to calculate the amplification efficiencies. The 20 μL qPCR reaction system contained 0.4 μL of Rox reference dye (503), 6.8 μL of double-distilled water, 0.4 μL of both sense primer (10 mM) and antisense primer (10 mM) of GSTs, UGTs and CYPs, 10 μL of SYBR Premix Ex Taq, and 2 μL of cDNA. The qPCR was performed using two-step method as followed: 95 °C for 30 s, then 40 cycles of 95 °C for 5 s and 60 °C for 34 s. The melting-curve cycles was performed to ensure that the specific products were amplificated, and the parameters as followed: 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Each experiment was repeated three times. The computational method of relative expression levels referred to Giulietti et al. (2001).

Statistical method and analysis

InStat software (GraphPad, San Diego, CA, USA) was applied to analyze the variance of collected data. Unpaired Student's t-test was applied to the statistical significance of all two-sample. Statistically significance was represented with $p < 0.05$.

Table 1. Primers of GST, UGT, CYP and reference genes.

Gene ID	Primer Sequence (5'to3')	Base number
OdfoCYP6AM4	F: CTACAAGATCCCTGGCACAAA	21
	R: ATCTCTCAGGCTCAGGGTAATA	22
OdfoCYP6SR1	F: TGAGTGAGTTGCTGTCGATAC	21
	R: CAGCTGAGGGTTCCTTGATAA	21
OdfoCYP6AMf	F: TGCACCAAGGACTACAAGATAC	22
	R: ATCTCTCAGGGTCAGGGTAATA	22
OdfoCYP4f1	F: TCCTATCACCCACCTGAAGA	20
	R: CGAAGTTATTGCCCACCTTATTG	23
OdfoCYP6AM5	F: GATAGCAGAAGTGTGGTCTTTCT	23
	R: GGGCTACATCACGATCCTAAAT	22
OdfoCYP9EH1a	F: GTCAGAAGTGGAAGGACATGAG	22
	R: GTCAACTGCTTACTGGTCTCTG	22
OdfoCYP9EH1b	F: CTTCTTGTGGAAGCGTTCTTTG	22
	R:CCACATTGTTGGAGACAGATAGA	23
OdfoCYP15A1	F: AGTACGCAAACGGAAGAAGAA	21
	R: GGACTGTGTTGTCACTGTATCA	22
OdfoCYP4SL2	F: AGGATGTCTCTCACGAACTCT	21
	R: TACACTCACTTCACCGCAATC	21
OdfoUGT1	F: CGCGGCAACATCTAACAATAAA	22
	R: CGCTGTATGGTGGACAGAAT	20
OdfoUGT2	F: GGCTGCCTCAACAGGATATT	20
	R: ATAGGCACACCATGATACACAG	22
OdfoUGT3	F: GCAGCTCATGACCAACATTTC	21
	R: GCCTTCTGTGCAAGTAATTCAG	22
OdfoUGT4	F: GCAGGTCACTACCAGGAAATAG	22
	R: AGGCGTGTAATAATGGTGTAAGG	23
OdfoUGT5	F: GAGGTTGGGACTGATGACTTC	21
	R: CCCTTCCTTCCTTCCTTCTTTC	22
OdfoGST1	F: GTGGTGTCTTCACACAGTAGTC	22
	R: CATTGTGGTTGATGCCTGTAATC	23
OdfoGST2	F: TTGCCTCTAAGCTGTCAACTAC	22
	R: AAGTTTGAAGGTGGGAGAAGAG	22
OdfoGST3	F: GGAATACTTCGCTACCTGTGTC	22
	R: CAAGTGCTGCCATTCCAAATAC	22
OdfoGST4	F: ACATAGGAGATGGCGTGAAAG	21
	R: GTCCAGCATTCCCGGATTAT	20
OdfoGST5	F: CTGTCTGCCACCTCCTTATTT	21
	R: TCAGTCAGAAATGCCAGGTTAT	22
OdfoGST6	F: CTGTCAGAGTAGGCACAGTATG	22
	R: CGTGCAGTTGGTGTTGATATAAG	23
RPS18	F: ATGGCAAACCCCCGTCAGTA	20
	R: CATACCACGATGCGCACGAA	20
GAPDH	F: TCGTATTGGCCGTCTTGTGC	20
	R: AGCGACCATGGGTGGAATCAT	21

Results

GST genes response to SM1 in *O. formosanus*

Many representative insect GST sequences was searched to ensure the accuracy of phylogeny, and these protein sequence was Gapped Blast using BLOSUM 62 matrix with an E-value cut-off of 10(-8) to identify the closest homologous structure (Table S1). The phylogenetic trees were established using GST sequences that searched from NCBI Protein BLAST and derived from transcriptome sequencing data (Table S2). A phylogenetic analysis of the six GSTs of *O. formosanus* revealed that the GSTs were categorized into four diverse cytosolic classes, delta (*OdfoGST1*), theta (*OdfoGST2*, *OdfoGST3*), omega (*OdfoGST4*), and epsilon (*OdfoGST5*, *OdfoGST6*). Sequence similarity analysis clearly supported the allocation of the six GSTs to the four classes. Six GST genes showed obvious genetic relationships with the GST genes from *Zootermopsis nevadensis* (hagen), *Cryptotermes secundus* (Hill), *Blattella germanica* (Linnaeus) and *Xenocatantops brachycerus* (Willemse), etc, a total of 70 genes from 50 different insects were used for construction of phylogenetic tree (Fig 1).

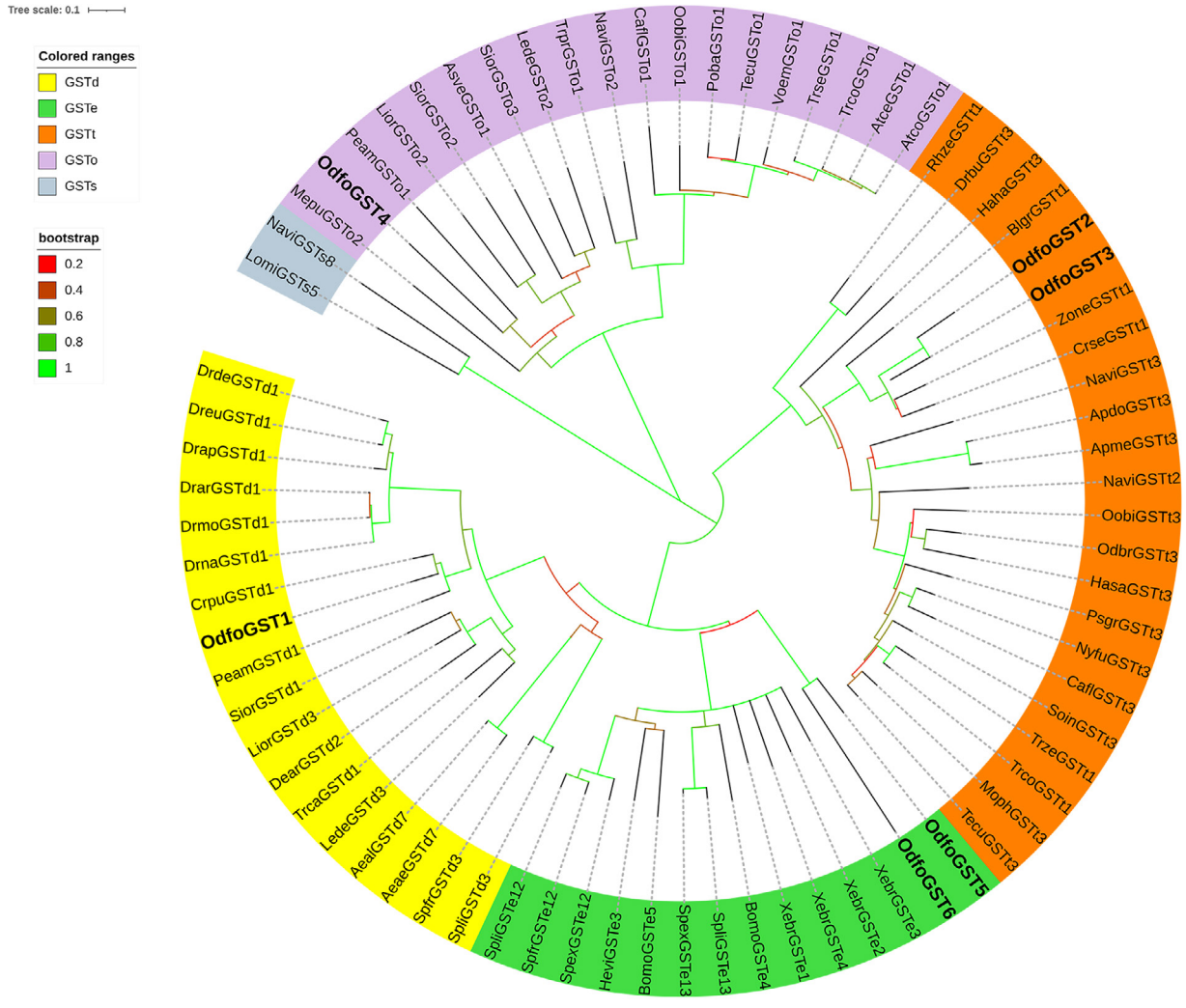

Fig 1. Phylogenetic analysis of GSTs in *O. formosanus* and representative insect species.
Footnote: The ClustalW alignments followed by MEGA 5.10 are applied to the generation of phylogenetic tree. Branch numbers in the tree represent bootstrap values (for 1000 replicates). The 6 GSTs of *O. formosanus* are marked with larger font sizes and bold. The sequences of representative insect species are listed in Table S1.
Lomi: *Locusta migratoria*; Navi: *Nasonia vitripennis*; Zone: *Zootermopsis nevadensis*; Crse: *Cryptotermes secundus*; Blge: *Blattella germanica*; Soin: *Solenopsis invicta*; Psgr: *Pseudomyrmex gracilis*; Trze: *Trachymyrmex zeteki*; Odbr: *Odontomachus brunneus*; Trco: *Trachymyrmex cornetzi*; Moph: *Monomorium pharaonis*; Tecu: *Temnothorax curvispinosus*; Hasa: *Harpegnathos saltator*; Rhze: *Rhagoletis zephyria*; Nyfu: *Nylanderia fulva*; Oobi: *Ooceraea biroi*; Drbu: *Drosophila busckii*; Cafl: *Camponotus floridanus*; Apdo: *Apis dorsata*; Apme: *Apis mellifera*; Haha: *Halyomorpha halys*; Xebr: *Xenocatantops brachycerus*; Bomo: *Bombyx mori*; Spli: *Spodoptera litura*; Spex: *Spodoptera exigua*; Hevi: *Heortia vitessoides*; Spfr: *Spodoptera frugiperda*; Aeae: *Aedes aegypti*; Aeal: *Aedes albopictus*; Crpu: *Cryptocercus punctulatus*; Peam: *Periplaneta Americana*; Lior: *Lissorhoptrus oryzophilus*; Drmo: *Drosophila mojavensis*; Drar: *Drosophila arizonae*; Sior: *Sitophilus oryzae*; Dear: *Dendroctonus armandi*; Drap: *Drosophila apicipuncta*; Drna: *Drosophila navojoa*; Lede: *Leptinotarsa decemlineata*; Trca: *Tribolium castaneum*; Dreu: *Drosophila eurypeza*; Drde: *Drosophila desallei*; Asve: *Asbolus verrucosus*; Trpr: *Trichogramma pretiosum*; Poba: *Pogonomyrmex barbatus*; Voem: *Vollenhovia emeryi*; Trse: *Trachymyrmex septentrionalis*; Atce: *Atta cephalotes*; Atco: *Atta colombica*; Mepu: *Meteorus pulchricornis*.

To analyze the GST genes responses to SM1 in *O. formosanus*, the relative expression of each gene was recorded at the13th and 20th hours after exposure to SM1 and normalized against the control. Our results indicated that except *OdfoGST2*, SM1 had a significant effect on the gene expression level of GSTs in *O. formosanus*. Specifically, three GST genes (*OdfoGST1*, *OdfoGST4*, *OdfoGST5*) were up-regulated by 1.70–2.12-fold at 13 hours, and four GST genes (*OdfoGST1*, *OdfoGST3*, *OdfoGST5*, *OdfoGST6*) were inhibited by 0.17–0.48-fold at 20 hours (Fig 2).

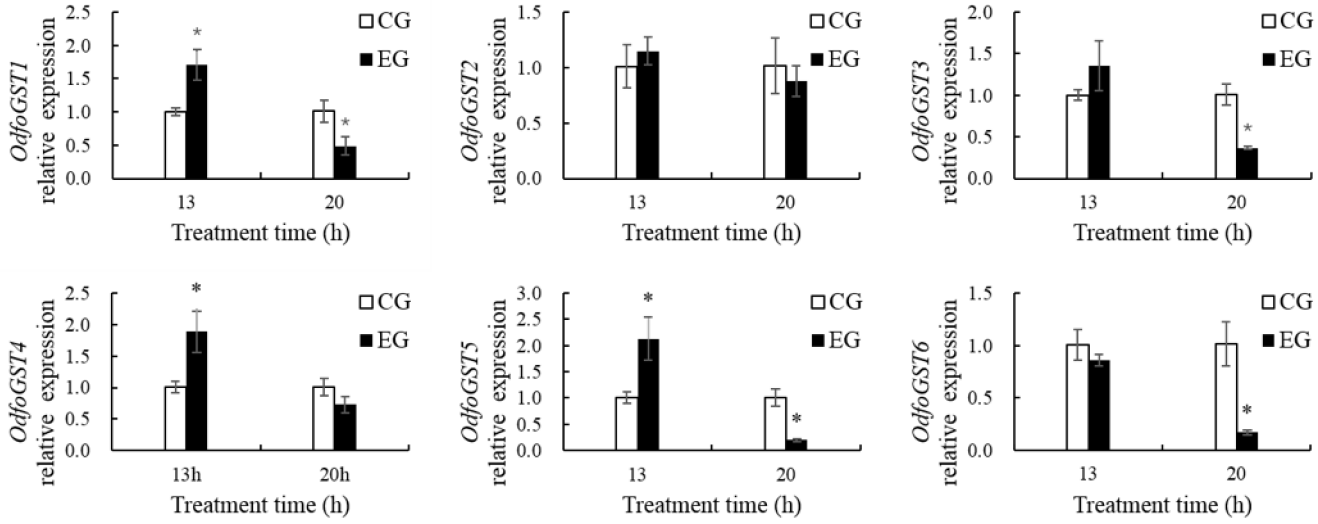

Fig 2. Relative expression levels of 6 GST genes in *O. formosanus* exposed to SM1.
Footnote: Averaged data from three independent experiments are given together with SD. The asterisk was used to indicate that significant difference between the control groups (CG) and experiment groups (EG) ($p < 0.05$).

UGT genes response to SM1 in *O. formosanus*

Many representative insect UGT sequences was searched to ensure the accuracy of phylogeny, and these protein sequence was Gapped Blast using BLOSUM 62 matrix with an E-value cut-off of 10(-6) to identify the closest homologous structure (Table S1). The phylogenetic trees were established using UGT sequences that searched from NCBI Protein BLAST and derived from transcriptome sequencing data (Table S2). The phylogenetic analysis of the five UGTs showed that all the UGTs belonged to UGT2 family. Sequence similarity analysis also clearly supported the allocation of all the five UGTs to UGT2. The different degrees of genetic relationships were revealed between five UGT genes and sixty-five UGT genes from 32 species, such as *Zootermopsis nevadensis* (hagen), *Cryptotermes secundus* (Hill), *Blattella germanica* (Linnaeus) and *Photinus pyralis* (Linnaeus) etc (Fig 3).

Our results suggested that SM1 infection caused alterations in the expression of 5 UGT genes in termite workers. Three UGT genes (*OdfoUGT2*, *OdfoUGT3*, *OdfoUGT5*) were induced by 1.36–2.52-fold at 13 hours compared with the control group, and three UGT genes (*OdfoUGT1*, *OdfoUGT2*, *OdfoUGT4*) were inhibited by 0.06–0.67-fold at 20 hours (Fig 4).

CYP genes response to SM1 in *O. formosanus*

Many representative insect CYP sequences was searched to ensure the accuracy of phylogeny, and these protein sequence was Gapped Blast using BLOSUM 62 matrix with an E-value cut-off of 10(-8) to identify the closest homologous structure (Table S1). The phylogenetic trees were established using CYP sequences that searched from NCBI Protein BLAST and derived from transcriptome sequencing data (Table S2). It was revealed that the CYPs belonged to four different classes, CYP4 (*OdfoCYP4f1*, *OdfoCYP4SL2*), CYP6 (*OdfoCYP6AM4*, *OdfoCYP6SR1*, *OdfoCYP6AMf*, *OdfoCYP6AM5*), CYP9 (*OdfoCYP9EH1a*, *OdfoCYP9EH1b*) and CYP15 (*OdfoCYP15A1*) according to the phylogenetic analysis of the nine CYPs. Different degrees of genetic relationships were showed between nine CYP genes and thirty-seven CYP genes from 26 species, such as *Zootermopsis nevadensis* (hagen), *Cryptotermes secundus* (Hill), *Blattella germanica* (Linnaeus) and *Tribolium castaneum* (Herbst) etc. (Fig 5).

Our studies showed that SM1 infection caused higher expression of partial CYP genes (*OdfoCYP9EH1a*, *OdfoCYP9EH1b*, *OdfoCYP4SL2* at 13 hours and *OdfoCYP6AM5*, *OdfoCYP4f1*, *OdfuCYP15A1*, *OdfoCYP9EH1a* at 20 hours), while it led to the down-regulation of CYP family genes (*OdfoCYP6AM5*, *OdfoCYP4f1*, *OdfuCYP15A1* at 13 hours and *OdfoCYP6AM4*, *OdfoCYP6SR1*, *OdfoCYP6AMf*, *OdfoCYP9EH1b* at 20 hours) The expression of CYP family genes is more complex after infected by SM1. Most CYP6 family genes were inhibited, while most CYP9 family genes were up-regulated (Fig 6).

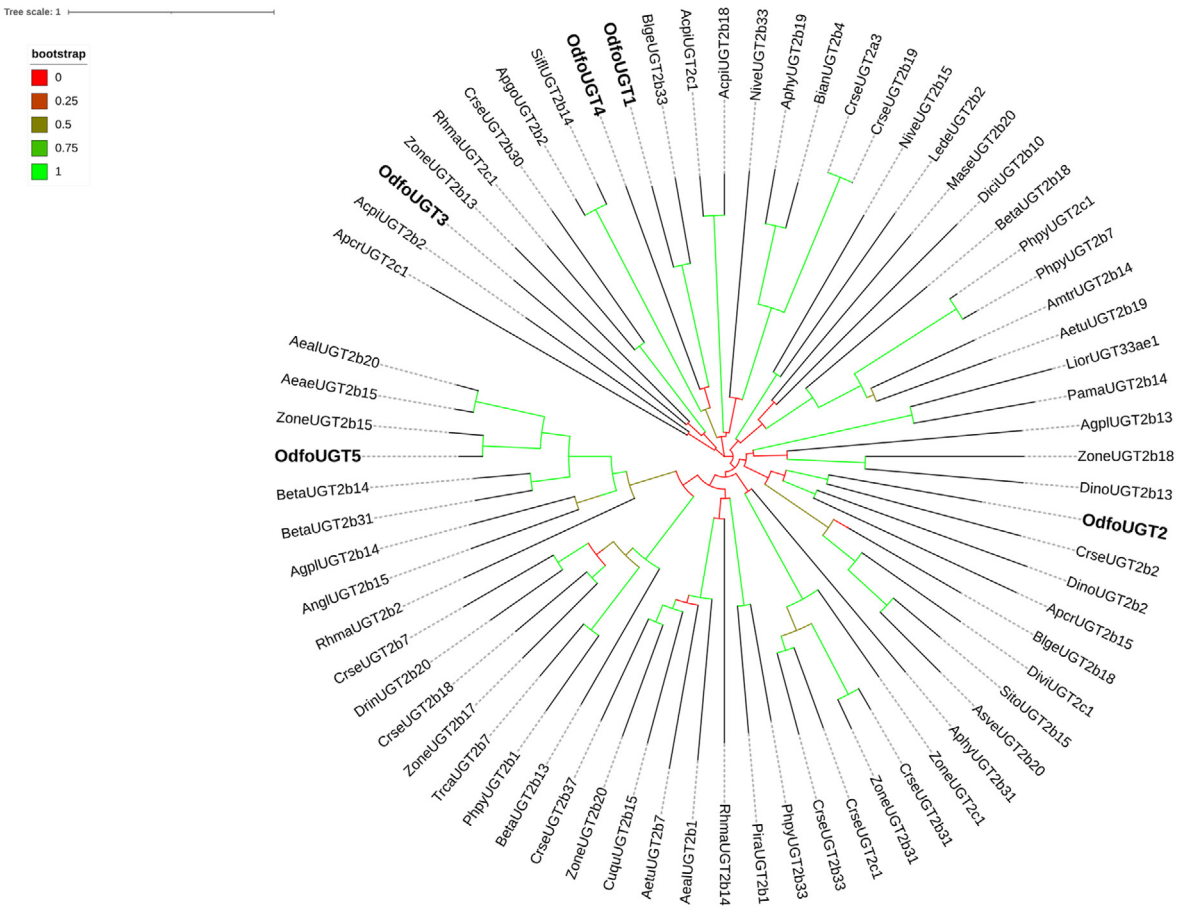

Fig 3. Phylogenetic analysis of UGTs in *O. formosanus* and representative insect species.
Footnote: The ClustalW alignments followed by MEGA 5.10 are applied to the generation of phylogenetic tree. Branch numbers in the tree represent bootstrap values (for 1000 replicates). The 5 UGTs of *O. formosanus* are marked with larger font sizes and bold. The sequences of representative insect species are listed in Table S1.
Aeal: *Aedes albopictus*; Aeae: *Aedes aegypti*; Zone: *Zootermopsis nevadensis*; Beta: *Bemisia tabaci*; Agpl: *Agrilus planipennis*; Angl: *Anoplophora glabripennis*; Rhma: *Rhopalosiphum maidis*; Crse: *Cryptotermes secundus*; Drin: *Drosophila innubila*; Trca: *Tribolium castaneum*; Phpy: *Photinus pyralis*; Cuqu: *Culex quinquefasciatus*; Aetu: *Aethina tumida*; Pira: *Pieris rapae*; Aphy: *Aphantopus hyperantus*; Asve: *Asbolus verrucosus*; Sior: *Sitophilus oryzae*; Divi: *Diabrotica virgifera virgifera*; Blge: *Blattella germanica*; Apcr: *Aphis craccivora*; Dino: *Diuraphis noxia*; Pama: *Papilio Machaon*; Lior: *Lissorhoptrus oryzophilus*; Amtr: *Amyelois transitella*; Dici: *Diaphorina citri*; Mase: *Manduca sexta*; Lede: *Leptinotarsa decemlineata*; Nive: *Nicrophorus vespilloides*; Bian: *Bicyclus anynana*; Acpi: *Acyrthosiphon pisum*; Sifl: *Sipha flava*; Apgo: *Aphis gossypii.*

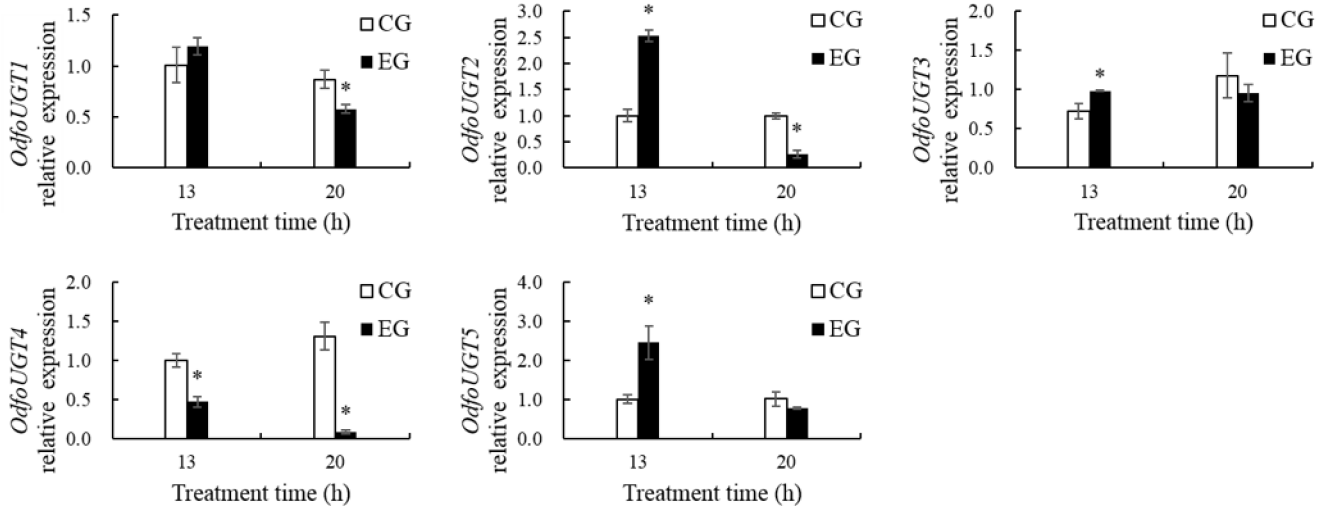

Fig 4. Relative expression levels of 5 UGT genes in *O. formosanus* exposed to SM1.
Footnote: Averaged data from three independent experiments are given together with SD. The asterisk was used to indicate that significant difference between the control groups (CG) and experiment groups (EG) ($p < 0.05$).

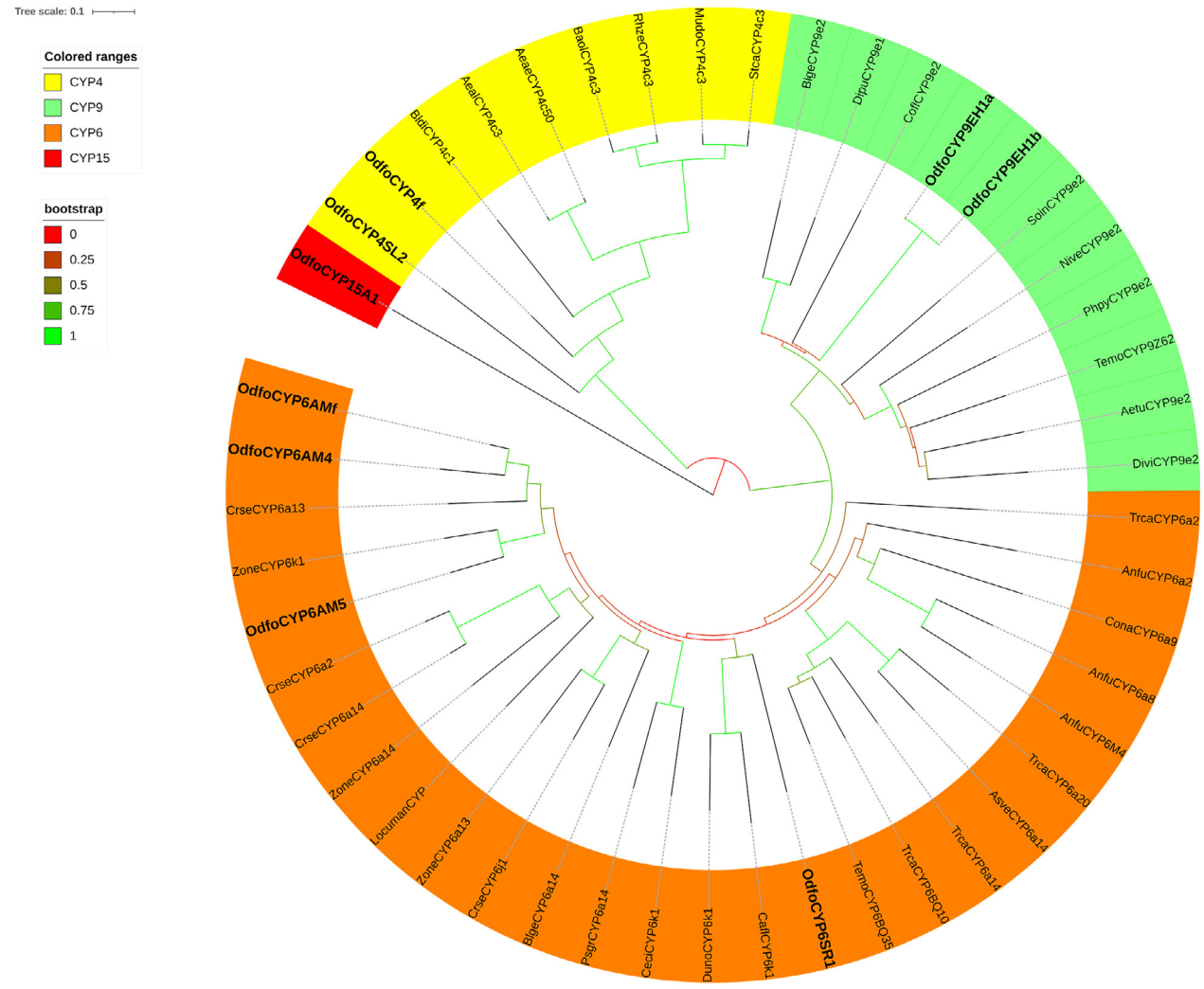

Fig 5. Phylogenetic analysis of CYPs in *O. formosanus* and representative insect species.
Footnote: The ClustalW alignments followed by MEGA 5.10 are applied to the generation of phylogenetic tree. Branch numbers in the tree represent bootstrap values (for 1000 replicates). The 9 CYPs of *O. formosanus* are marked with larger font sizes and bold. The sequences of representative insect species are listed in Table S1.
Crse: *Cryptotermes secundus*; Zone: *Zootermopsis nevadensis*; Blge: *Blattella germanica*; Psgr: *Pseudomyrmex gracilis*; Ceci: *Cephus cinctus*; Duno: *Dufourea novaeangliae*; Cafl: *Camponotus floridanus*; Temo: *Tenebrio molitor*; Trca: *Tribolium castaneum*; Asve: *Asbolus verrucosus*; Anfu: *Anopheles funestus*; Cona: *Contarinia nasturtii*; Divi: *Diabrotica virgifera virgifera*; Aetu: *Aethina tumida*; Phpy: *Photinus pyralis*; Nive: *Nicrophorus vespilloides*; Soin: *Solenopsis invicta*; Cofl: *Copidosoma floridanum*; Dipu: *Diploptera punctate*; Stca: *Stomoxys calcitrans*; Mudo: *Musca domestica*; Rhze: *Rhagoletis zephyria*; Baol: *Bactrocera oleae*; Aeae: *Aedes aegypti*; Aeal: *Aedes albopictus*; Bldi: *Blaberus discoidalis.*

Discussion

Odontotermes formosanus is termite pest species in some tropical and subtropical regions, and a variety of pathogenic microorganisms exist naturally in its living environment. In addition to relying on social immune behavior to resist external infection, there are also innate immune responses of insects, such as detoxification enzymes, to resist pathogens. In the process of SM1 infection, the changes of expression level in detoxification enzymes genes directly reflect the effect of bacteria on host cell responses.

GST, as an important detoxification enzyme system of insects, plays a role in insect anti-host exogenous toxin detoxification and resistance to insecticide (Mittapalli et al., 2007; Zhou et al., 2013).They bind to various harmful electrophilic substrates by catalyzing endogenous reductive glutathione (GSH) and increase the solubility of the deleterious electrophilic substrates to drain them from the cells, thereby protecting nucleic acids and proteins in organisms from electrophilic groups (Ketterman et al., 2011). Furthermore, GSTs act in the function of transporting kinds of hormones and exogenous metabolites in the cell (Listowsky et al., 1988). In more thoroughly studied Diptera and Lepidoptera insects, 3, 37, 32, 29 and 21 GST genes were identified in *Culex quinquefasciatus* (Say), *Drosophila melanogaster* (Meigen), *Anopheles gambiae* (Giles), *Aedes aegypti* (Linnaeus) and *Bombyx mori* (Linnaeus), respectively (Sun et al., 2020). Studies have shown that the mechanism by which GSTs participate in drug resistance is due to increased expression (David, 1991). GST activity and its gene expression levels in

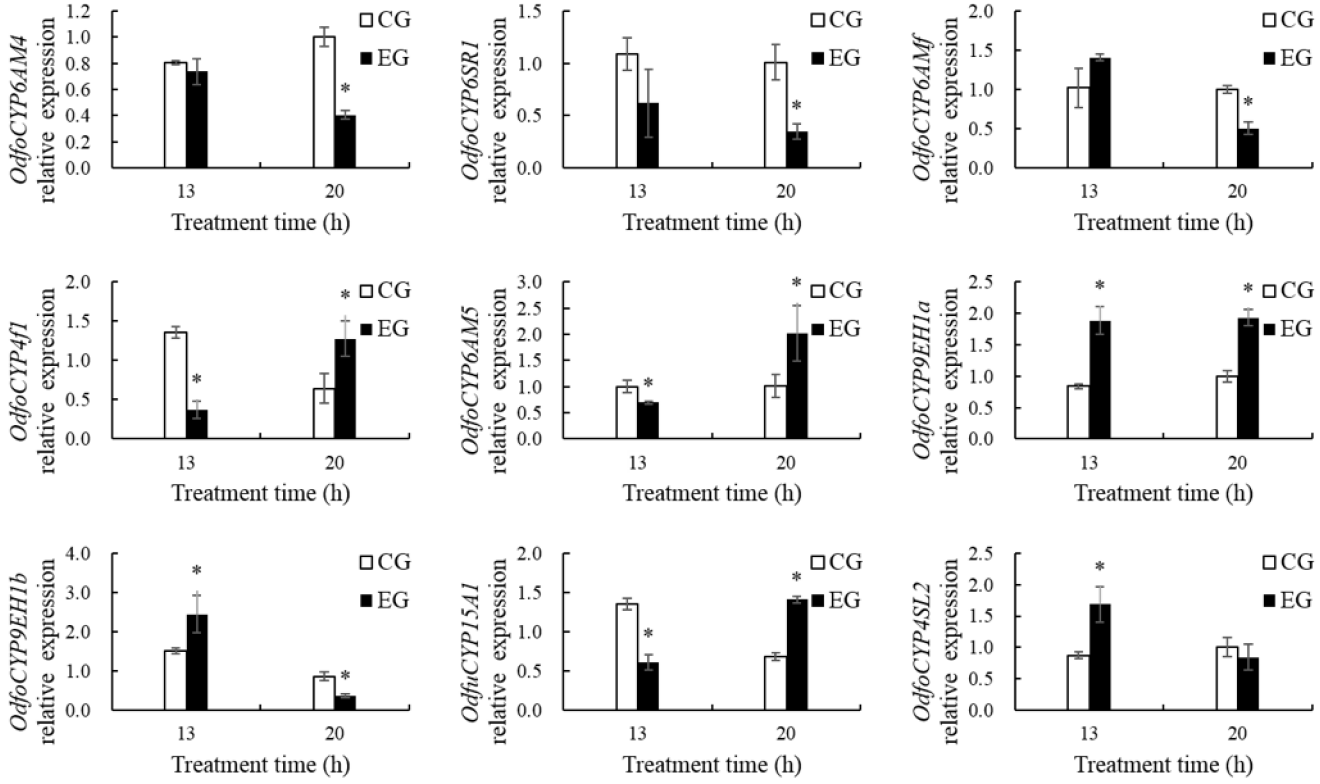

Fig 6. Relative expression levels of 9 CYP genes in *O. formosanus* exposed to SM1.
Footnote: Averaged data from three independent experiments are given together with SD. The asterisk was used to indicate that significant difference between the control groups (CG) and experiment groups (EG) ($p < 0.05$).

Helicoverpa armigera (Hüber) were up-regulated significantly at the early stage of nucleopoIyhedrovirus infection in *H. armigera* (Huang et al., 2015). The combined use of *Metarhizium anisopliae* var. acridum and aggregation pheromone phenylacetonitrile to control *Schistocerca gregaria* (Forskal) have been studied and found that the GST expression levels were up-regulated especially when nymphs exposed for 4 and 6 h (Abdellaoui et al., 2020). In another study, the qPCR results indicated the significant up-regulation of the GST genes in *Spodoptera exigua* (Hübner) following *S. marcescens* infection (De Mandal et al., 2020). When infected by *Heterorhabditis beicherriana*, the GST activity in *Galleria mellonella* (Linnaeus) larvae was strongly induced (Wu et al., 2013). Current studies on termites GST gene were still scarce. One GST gene was found in the genome of *Coptotermes formosanus* (Shiraki), which was considered as one part of caste-specific multi-layer defense mechanism (Hussain et al., 2017). In our work, 5 GST genes that found in transcriptome of *O. formosanus* showed significant changes on transcription levels when faced SM1 stress, which indicated that the detoxification function of partial GST genes was firstly activated and then destroyed under the stress of SM1.

UGTs is a phase II detoxification metabolic enzyme system in organisms that catalyzes the conjugation of small molecular lipophilic compounds with UDP-glycosides to form glycosides, thus changing the hydrophilicity of the substrate and helping it to be discharged from the body. For biotransformation of lipophilic endogenous and exogenous substances, glycosidic conjugation is an important metabolic pathway (Rowland et al., 2013; Bock, 2016). There were 33 UGT genes found in *D. melanogaster* (Luque et al., 2002); 42 UGT genes had been found in *B. mori*. Some specific UGT genes are expressed only in midgut and fat body, which may be related to the detoxification function of insects and relieve the toxicity of toxic phytochemicals in food (Huang et al., 2008). UGTs involved in the detoxification metabolism of pesticides has been reported in recent years, such as after the treatment of *Musca domestica* (Linnaeus) with pyrazolium and *H. armigera* with deltamethrin, the UGT activity in the insect is significantly increased to improve the resistance to insecticide stress. Transcriptome sequencing reveals up-regulation of many UGT genes in insect resistant strains, and the up-regulation may be related to resistance to DDT (Pedra et al., 2004), pyrethroid (Vontas et al., 2005), carbamate (Silva et al., 2012), and neonicotinoids (Yang et al., 2013). A number of literatures have shown that insects can enhance their resistance to insecticides by increasing the expression of UGT genes (Hu et al., 2017).

CYPs, also known as cytochrome monooxygenase, were reported in all aerobic organisms including insects, plants, birds, mammals, and bacteria, which are the terminal oxidase of microsomal multifunctional enzymes that act in metabolism of exogenous compounds including drugs, pesticides, phytotoxins, chemical carcinogens and mutants

(Nelson et al., 1993; Werck-Reichhart et al., 2000). More than 300 CYPs were found in insects, 90 CYP genes, belonging to 25 families, were identified from the genome of *D. melanogaster* and 111 CYP genes were identified from the genome of *A. gambiae*. Previous studies had found that many CYP genes are overexpressed or induced in resistant insects, confirming that CYPs is responsible for insect resistance, including pyrethroids (Liu et al., 2015; Seixas et al., 2017). Since the discovery of CYPs, the almost common characteristics of studied CYPs is that they can be induced. Typical insect CYPs inducers include drugs, plant secondary substances, insecticidal agents and other substances (Scott, 1999), but CYPs induction by bacteria has rarely been reported. When *Trichoplusia ni* (Hubner) infection by the Baculovirus *Autographa californica* Multiple Nucleopolyhedrovirus, it was showed that a number of cytochrome P450 genes were down-regulated following the infection (Shrestha et al., 2019). Furthermore, a cytochrome P450 (*CYP9Q1*) was up-regulated in response to honey bee larvae infected with the bacterial pathogen *Paenibacillus larvae* (Cornman et al., 2013).

A study indicated that toxic filamentous fungus *Aspergillus nidulans* (Eidam) has a lethal effect on *D. melanogaster* larvae, under the confrontation the expression of many genes involved in detoxification (e.g., CYP, GST and UGT genes) were markedly affected (Trienens et al., 2017). Similar to the results of this study, we found that the expression levels of GST, UGT and CYP genes were altered in response to the infection by SM1. Meanwhile, different trends of alteration in expression levels of these three types of genes suggested that the sensitivity of these genes to SM1 varied from each other. Fu et al. (2020b) reported that the death of *O. formosanus* due to the insecticidal protease of SM1, and these toxic proteins might disrupt termite metabolism. The up-regulation of detoxification enzymes may be due to the stress of these toxic proteins and metabolites, which also showed that they may have potential significance in the resistance mechanism of *O. formosanus* against SM1. Furthermore, these toxic proteins and metabolites also damaged *O. formosanus*, then the overall metabolic level of the host insect after infected by the SM1 was inhibited, therefore the expression levels of the detoxification enzymes were significantly inhibited. Our results suggested that transcription levels of GST, UGT and CYP genes in *O. formosanus* show an obvious up-regulation or down-regulation, most of which were inducible up-regulated and showed a same induction effect compared to virus, nematode, insecticide and plant secondary substances. A study suggested that GST and CYP enzymes were contributed to the resistance responses of *B. mori* against the *B. mori* densonucleosis virus (Chen et al., 2012). It is also confirmed that GST activity and transcriptional level were increased by infection with *Hyphantria cunea* (Drury) nucleopolyhedrovirus (HcNPV), especially when the hosts under the stress of sublethal concentrations (Sun et al., 2020). The emergence of down-regulation may be related to the physiological and biochemical mechanisms of the interaction between different types of insect microorganisms and host insects. Moreover, it was indicated that GST, UGT and CYP family genes may be related to the immune response of *O. formosanus* to SM1, each class of detoxification genes may exhibit different responses and effects in the defense of *O. formosanus* against SM1 due to the different ways and degrees of participation, each detoxification gene needs to be analyzed separately. A better understanding of the underlying molecular basis of termite defense mechanisms may provide approaches that may improve the development of putative biological control agents in termite. In addition, targeting these mechanisms and reducing termite's vigor would allow a variety of naturally occurring pathogens to spread within the weakened colony (Chouvenc and Su., 2010). Therefore, identification of these microbial-related specific reactions was able to further understand the interaction mechanism between *O. formosanus* and SM1, and contributed to promote the development of new insecticides or synergists.

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Authors' Contributions

FT: conceptualization, methodology, funding acquisition, project administration, supervision, writing (review and editing)
MX: methodology
XYL: investigation, formal analysis, wrting (draft)
MLN: investigation, formal analysis
KF: investigation

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