Comparative Biochemistry and Physiology, Part C 200 (2017) 1–8

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Functional characterization of BdB1, a well-conserved carboxylesterase among tephritid fruit flies associated with malathion resistance in *Bactrocera dorsalis* (Hendel)

Luo-Luo Wang[a,c], Xue-Ping Lu[a,b], Guy Smagghe[a,b,c], Li-Wei Meng[a,b], Jin-Jun Wang[a,b,*]

[a] Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400716, China
[b] Academy of Agricultural Sciences, Southwest University, Chongqing 400716, China
[c] Department of Crop Protection, Ghent University, B-9000 Ghent, Belgium

ARTICLE INFO

Keywords:
Bactrocera dorsalis
Carboxylesterase
Metabolic resistance
Malathion
RNAi
Heterologous expression

ABSTRACT

There are many evidences that insect carboxylesterase possess important physiological roles in xenobiotic metabolism and are implicated in the detoxification of organophosphate (OP) insecticides. Despite the ongoing resistance development in the oriental fruit fly, *Bactrocera dorsalis* (Hendel), the molecular basis of carboxylesterase and its ability to confer OP resistance remain largely obscure. This study was initiated to provide a better understanding of carboxylesterase-mediated resistance mechanism in a tephritid pest fly. Here, we narrow this research gap by demonstrating a well-conserved esterase B1 gene, *BdB1*, mediates malathion resistance development *via* gene upregulation with the use of a laboratory selected malathion-resistant strain (MR) of *B. dorsalis*. No sequence mutation of *BdB1* was detected between MR and the susceptible strain (MS) of *B. dorsalis*. *BdB1* is predominantly expressed in the midgut, a key insect tissue for detoxification. As compared with transcripts in MS, *BdB1* was significantly more abundant in multiple tissues in the MR. RNA interference (RNAi)-mediated knockdown of *BdB1* significantly increased malathion susceptibility. Furthermore, heterologous expression along with cytotoxicity assay revealed BdB1 could probably have the function of malathion detoxification.

1. Introduction

The tephritid fruit flies are a large and globally widespread family comprising > 4900 described species in over 500 genera (Schutze et al., 2017). Various species from this family are of major economic importance in agriculture for its destructive impact, especially in developing countries (Vargas et al., 2015). Nowadays, pest management techniques applied to tephritid fruit flies are still relying on cover sprays with conventional insecticides and toxic food baits. The oriental fruit fly, *Bactrocera dorsalis* (Hendel), as an important member of the Tephritidae family, is one of the most economically damaging and widely distributed pests in tropic and sub-tropic regions (Chou et al., 2010; Christenson and Foote, 1960; Hsu et al., 2015; Jin et al., 2011). Moreover, *B. dorsalis* is successfully established across the Hawaiian Islands and thus becomes a concern species in the United States (Stephens et al., 2007). In recent years, resistance monitoring of *B. dorsalis* demonstrated that this fly has quickly evolved resistance to many commonly used organophosphate (OP) insecticides in orchards which resulted in serious outbreaks in Guangdong and Taiwan of China as well as in Hawaii (Bateman, 1972; Chou et al., 2010; Hsu et al., 2011; Jin et al., 2011). Hence, given the increasing importance of OP resistance in *B. dorsalis*, a better understanding of the underlying resistance mechanisms is needed for effective resistance monitoring and management.

The development of OP resistance *via* the involvement of carboxylesterase (CarE) has been reported in many insect species, such as *Lucilia cuprina* (Claudianos et al., 1999; Jackson et al., 2013), *Drosophila melanogaster* (Birner-Gruenberger et al., 2012; Campbell et al., 2003), and *Musca domestica* (Claudianos et al., 1999). An important aspect of insect carboxylesterase that are involved in resistance is the constitutively transcriptional overexpression in insecticide resistant strains, causing enhanced metabolic detoxification of insecticides. Our previous study had also shed light on the critical role of elevated CarE during the development of malathion resistance in *B. dorsalis* (Wang et al., 2015b; Wang et al., 2016), however, much in contrast to the important roles of CarE, functional characterization of CarEs in *B. dorsalis* has not kept pace with that in other pest species. Moreover, the recent accessibility of the genome sequence of *B. dorsalis* has provided more significance

* Corresponding author at: College of Plant Protection, Southwest University, Chongqing 400716, China.
E-mail addresses: jjwang7008@yahoo.com, wangjinjun@swu.edu.cn (J.-J. Wang).

http://dx.doi.org/10.1016/j.cbpc.2017.07.001
Received 27 May 2017; Received in revised form 4 July 2017; Accepted 7 July 2017
Available online 08 July 2017
1532-0456/

and opportunity to develop new strategies with molecular tools for pest control. Meanwhile, as being an important member of the Tephritidae family, *B. dorsalis* can potentially serve as a promising research organism for resistance management.

Esterase B1 gene in *B. dorsalis* (*BdB1*) has been previously genomic annotated, however, the identification of this gene and the relationship between esterase B1 and malathion resistance in *B. dorsalis* has yet to be explored. In this study, the genomic/cDNA sequence, gene structure and spatial/temporal expression patterns of *BdB1* were analyzed. We further compared the transcriptional profiles and deduced amino acid sequences of BdB1 between the malathion-resistant (MR) and susceptible (MS) strains of *B. dorsalis*. To better evaluate its possible function, functional expression studies combined with cytotoxicity assays in Sf9 cells overexpressing *BdB1*, along with RNA interference (RNAi) bioassays down-knocking *BdB1* were performed. Taken together, this study presents detailed characterizations of esterase B1 gene in mediating malathion resistance in *B. dorsalis*. Moreover, we also suggest that this well-conserved carboxylesterase could serve as a potential molecular biomarker for OP resistance monitoring in tephritid fruit flies.

2. Materials and methods

2.1. Fruit fly strains and Sf9 cells

Two strains of *B. dorsalis*, MR and MS, were used in this study as previously described (Wang et al., 2016). The MR strain has developed 21-fold resistance to malathion after 37 generations of selection when this study was carried out. All the flies were maintained at 27 ± 0.5 °C, ~70% relative humidity and a photoperiod of 14 h light:10 h dark with artificial diet at a temperature-controlled incubator.

The Sf9 insect cells were routinely maintained in suspension under serum-free conditions (SF-900 II SFM, Invitrogen, Carlsbad, CA) at 27 °C and 120 rpm, which was used for baculovirus-mediated carboxylesterase protein expression.

2.2. RNA isolation, cDNA synthesis and reverse transcription quantitative PCR

Total RNA was extracted from MR and MS adults, from different developmental stages (eggs; 3rd instar larvae; late pupae; and adults on days 1, 3, 5 and 7), and from different body parts/tissues (head, thorax, abdomen, midgut, fat body, and Malpighian tubules) of adults on day 3 after emergence, respectively, using TRIzol reagent (Life Technologies, Carlsbad, CA) following the manufacturer's manual. The genomic DNA was eliminated with DNase I (Promega, Madison, WI) when isolating the RNA. The quality and quantity of RNA were ascertained with a Nanovue UV–Vis spectrophotometer (GE Healthcare, Fairfield, CT) and the integrity was checked by 1.0% agarose gel electrophoresis. An aliquot of ~1 μg of total RNA was used to synthesize first-strand cDNA using PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara, Dalian, China) with an oligo(dT) primer.

The reverse transcription quantitative PCR (RT-qPCR) assay was performed on a Stratagene Mx3000P System (Stratagene, La Jolla, CA) using iQ SYBR Green Supermix (Promega, Madison, WI) as previously described. Gene specific primers were shown in the Table 1.

2.3. Sequence, gene structure and phylogenetic analysis of BdB1

The predicted open reading frame (ORF) of *BdB1* was first obtained from the *B. dorsalis* midgut-specific transcriptome database (Shen et al., 2011). The complete cDNA sequences of *BdB1* were amplified from the MR and MS strains, respectively, using PrimeSTAR HS DNA Polymerase (TaKaRa Bio, Dalian, China). The purified PCR products were cloned into the pGEM-T Easy Vector (Promega, Madison, WI) according to the manufacturer's protocol. Positive clones were validated by DNA sequencing (Invitrogen, Shanghai, China). Validated *BdB1* cDNA sequence was used to BLAST in the *B. dorsalis* genome sequencing database (https://i5k.nal.usda.gov/Bactrocera_dorsalis) to obtain genomic DNA, mRNA and upstream DNA sequence. Multiple sequence alignments were performed with DNAMAN v.6.03 (Lynnon Biosoft, USA). Phylogenetic tree was constructed to classify this annotated esterase B1 gene based on classification scheme for insect CarE as previously described (Oakeshott et al., 2005). ClustalW (Thompson et al., 1994) and MEGA5.0 (Tamura et al., 2011) were used to construct the phylogenetic tree with the neighbor-joining method with 1000 bootstrap tests. All the other 59 CarE sequences were obtained from NCBI.

2.4. RNA interference of BdB1 and malathion bioassay

BdB1 was amplified by PCR using primers (Table 1) containing the T7 RNA polymerase promoter. The purified PCR products were used to synthesize dsRNA flowing the TranscriptAid T7 High Yield Transcription Kit (Thermo Scientific, Wilmington, DE). Approximately 1.5 μg dsRNA of BdB1 was injected into the abdomen between the first and second abdominal segments of each 3-day-old adult fly with a Nanoject II Auto-Nanoliter Injector (Drummond Scientific, Broomall, PA). Negative controls were injected with an equivalent volume of PBS. PBS/dsRNA-injected (MR) and non-injected (MR and MS) flies were reared under same conditions as described above, and the reductions in BdB1 transcription levels were determined using three randomly-collected adults at 12, 24, and 48 h post injection. Three replications were used for each treatment.

Insecticide bioassay was performed after *BdB1* was silenced by RNAi. Aliquots of 0.5 μl from the LD_{50} concentration of malathion (2550 μg/ml) was applied to individual insect in the dsRNA/PBS-injected groups (MR), non-injected MR/MS groups at 24 h after injection using a PB600-1 Repeating Dispenser (Hamilton Company, Reno, NV). Mortality was recorded at 0, 12, 24, 48, and 60 h after malathion treatment.

2.5. Functional expression of BdB1 and MTT cytotoxicity assay

The recombinant baculovirus DNA was constructed and transfected into Sf9 cells (Invitrogen, Carlsbad, CA) using the Bac-to-Bac baculovirus expression system (Invitrogen, Carlsbad, CA) as previously described. Briefly, full-length ORFs of *BdB1* and *eGFP* were amplified with primers containing *BamH I* and *Xho I* using PrimeSTAR HS DNA Polymerase. PCR products and pFastbac HT A expression vector (Invitrogen, Carlsbad, CA) were digested with *BamH I* and *Xho I* (Thermo Scientific) for 3 h and then PCR products were inserted into vectors with T4 DNA Ligase (Promega). The insert-pFastBacHT A ligation product was then transformed into *E.coli* DH5α competent cells. The recombinant transfer vector pFastBacHT-*BdB1* and pFastBacHT-*eGFP* were selected and verified by ampicillin resistance, double cleavage and sequencing, respectively. The recombinant transfer vector was then transformed into DH10Bac containing Bacmid vector for the transposition reaction. The positive clone was selected and verified as Bacmid-*BdB1* or Bacmid-*eGFP*. Sf9 cells were then co-transfected with the Bacmid-*BdB1* or Bacmid-*eGFP* and liposome. Baculovirus-infected cells were harvested 3 days later. CarE specific activity was evaluated using the spectrophotometric method as previously reported (Van Asperen, 1962). The aforesaid recombinant baculovirus were used to generate baculovirus-infected cells for further cytotoxicity assay.

Cytotoxicity effect of malathion was evaluated using the 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Cell Proliferation and Cytotoxicity Assay Kit (Solarbio, Shanghai, China) as previously reported. Cell viability was calculated as percentage of viable cells relative to cells treated with acetone. In enzyme inhibition study, cells were co-incubated with 100 μM malathion and triphenyl phosphate (TPP, the known esterase inhibitor) at concentrations of 0.1, 1 and 10 μM (in each case the final concentration of DMSO in media was 1%).