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Activities of four enzymes in *Galleria mellonella* larvae infected with entomopathogenic nematode *Heterorhabditis beicherriana* n. sp.

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To investigate the mechanism of *Galleria mellonella* infected with entomopathogenic nematodes (EPNs), we studied the effects of a new species of EPNs, *Heterorhabditis beicherriana* n.sp., on the activities of Tyrosinase (TYR), acetylcholinesterase (AChE), carboxylesterase (CarE) and glutathione S-transferase (GST) in whole body of *G. mellonella*. The last instar larvae of *G. mellonella* (0.18 to 0.20 g/larva) were used as host insects and were randomized into four groups (n = 60 in each group): 0 IJ/larva control group, 20 IJs/larva group, 40 IJs/larva group and 80 IJs/larva group. Infective juveniles (IJs) were injected into each selected *G. mellonella* larva. At 0, 8, 16, 24, 32 and 40 h post-infection, ten injected larvae were randomly collected from each group, enzymes were extracted, and the enzyme activities of TYR, AChE, CarE and GSTs were assessed following standard methods. We found that the activities of TYR, AChE, CarE and GST were significantly enhanced in a dose-dependent manner in *G. mellonella* larvae infected with *H. beicherriana,* which may be the overeactive stress response to the EPNs infection and the overreaction could lead to the death of host insects.

Key words: Entomopathogenic nematode, *Galleria mellonella,* tyrosinase, acetylcholinesterase, carboxylesterase, glutathione S-transferase.

INTRODUCTION

Entomopathogenic nematodes (EPNs) in the families of *Steinernematidae* and *Heterorhabditidae* are lethal endoparasites of insects, which could secrete insecticidal active substances, including toxins, proteases, and so on, contributing to the lethal effect on infected host insects (Brown et al., 2004, 2006; Ffrench-Constant et al., 2007; Toubarro et al., 2009). The lethal processes caused by insecticidal active substances are often related to the activity changes of some enzymes in the host insects (Grewal et al., 2005).

Tyrosinase (TYR) is an enzyme ubiquitously distributed in insects, and is responsible for some physiologically important processes. It can oxidize phenolic compound to produce melanin, which melanizes some larger invasive xenobiotics such as pathogenic microorganisms engulfed by the host cells, and then inhibites the growth and reproduction of the pathogens, reducing the damage to the host cells. Moreover, quinone produced in the process of melanization will kill the invasive microorganisms (Sugumaran et al., 2000). Acetylcholinesterase (AChE) is an important enzyme in the nervous system of insects, terminating nerve impulses by catalyzing the hydrolysis of neurotransmitter acetylcholine (ACh). Inhibition of AChE results in

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excessive accumulation of ACh, leading to hyperactivities and consequently paralysis and death. In addition, evidence showed that apart from the catalytic function in hydrolyzing ACh, AChEs are also expressed at other sites in insects, where they act as regulators affecting cell proliferation, differentiation, and responses to various stresses (Soreq and Seidman, 2001). AChE is a target of organophosphorus and carbamate compounds in insects, which remain to be widely used pesticides around the world (Fournier and Mutero, 1994). Carboxylesterase (CarE) and glutathione S-transferase (GST) are important detoxification enzymes for insects to degrade toxic substances. CarE, known as the primary metabolic enzyme, catalyses hydrolytic reactions for a broad range of substrates, including organophosphate, carbamate and pyrethroid insecticides. Studies have indicated that increased CarE activity is the major mechanism of insecticide resistance by increasing the hydrolysis and (or) sequestration of insecticides in insects (Herath et al., 1987). GSTs, a major family of detoxication enzymes, catalyse the conjugation of the tripeptide glutathione to electrophilic centres of lipophilic compounds, thereby making the resultant products more soluble and excretable from the cells. Thus GST plays a vital role in protecting tissues against oxidative damage and oxidative stress. Elevated GSTs activities have been reported to be associated with resistance to insecticides (Comoy et al. 1997; Hemingway, 2000).

However, up to now, there are few reports about the mechanism of *G. mellonella* infected with EPNs related to activity changes of the four enzymes. In this study, to determine the potential role of TYR, AChE, CarE and GST in *G. mellonella* infected with EPNs, we investigated the activity changes of the four enzymes in *G. mellonella* inoculated with a new species of EPNs *Heterorhabditis beicherriana* n.sp. (*H. beicherriana*), which belongs to the *Heterorhabditidae* genus and was isolated by our laboratory. We found it has strong lethal effect on *G. mellonella* larvae. Relative mortality rate reached 35-60% 40h post-infection, and after 48 h, almost all infected larvae died (Data not shown). This study aimed to acquire knowledge on the activity changes of the four enzymes of *G. mellonella* larvae infected with infection of *H. beicherriana.*

MATERIALS AND METHODS

Nematode and insect

Heterorhabditis beicherriana n.sp. (*H. beicherriana)* was originally collected and purified from a soil sample of rhizosphere of cherry tree in Shunyi District, Beijing, China. The nematode was propagated in the laboratory by passaging through last-instar larvae of *G. mellonella,* which were supplied by the Laboratory of Entomology and Nematology, China Agricultural University. Infective juveniles (IJs) were collected on a Petri dish trap as described previously (Mracek and Webster, 1993), then the collected IJs were surface sterilized with 0.2% thimerosal (Sigma), and suspended in phosphate-buffered saline (PBS, PH 7.4) prior to injection into the host insects (Maxwell et al., 1994).

For each experiment, last-instar larvae of *G. mellonella* (0.18-0.20 g / larva) from our laboratory were used as host insects in the experiments. The larvae were randomized into four groups (n = 60 in each group) for each experiment: 0 IJ/larva control group, 20 IJs/larva group, 40 IJs/larva group and 80 IJs/larva group.

To inoculate the insects with IJs, each selected *G. mellonella* larva was injected with 3.5 µl of PBS containing 0, 20, 40, or 80 surface-sterilized IJs of *H. beicherriana,* respectively, and then was incubated in the artificial climate chamber (28°C, 68% relative humidity, darkness). At 0, 8, 16, 24, 32 and 40 h after inoculation, ten injected larvae were randomly collected from each group and were temporarily stored at -20°C for further examination within one week. The experiment was carried out in triplicate, and ten larvae injected with PBS alone served as the control in each experiment.

Enzymes preparation

Enzymes were prepared by homogenization of 10 larvae in 40 mL ice-cold phosphate buffer (0.05 M, pH 7.0) at 30 W, 10 s by using an ultrasonic homogenizer. After centrifuging for 30 min (4°C, 10000×g), the final supernatant was used as enzyme solution. Protein content of enzyme solution was measured according to the method of Bradford (1976). All chemicals used were analytical grade and were obtained from Sigma.

Biochemical analysis for the activities of enzymes

All experiments were replicated three times each with ten larvae. Assays also were performed as three technical replicates per preparation for determination of TYR, AChE CarE and GSTs activities. Assays of enzymes activities of TYR, AChE, CarE and GSTs were assessed following standard methods described by Sugumaran and Nellaiappan (2000), Ellman et al. (1961), van Asperen (1962) and Habig et al. (1974), respectively. Activities were expressed as units, mmol or µmol per milligram of protein.

Statistical analysis

SPSS version 17.0 software (SPSS for Windows, Inc., Chicago, IL, USA) was used for all statistical analyses. All results are expressed as mean ± SEM. Statistical analysis of the data was performed using standard 1-way ANOVA, followed by LSD post-hoc test. Bonferroni's correction was used to adjust for multiple comparisons. A P value < 0.05 was considered to be statistically significant.

RESULTS

TYR activity of *G. mellonella* larvae after infecting with *H. beicherriana*

Ten larval *G. mellonella* infected with 0, 20, 40, or 80 surface-sterilized IJs of *H. beicherriana* were randomly selected from each group, at the timepoint of 0, 8, 16, 24, 32 and 40 h postinfection, respectively. Enzymes were readily isolated from the infected insect larvae and the activity of TYR was measured immediately. As shown in Figure 1, TYR activity was significantly increased compared with the controls (P < 0.05), and increasing IJs doses and treatment time resulted in a progressive enhancement of TYR enzyme activity. At the initial

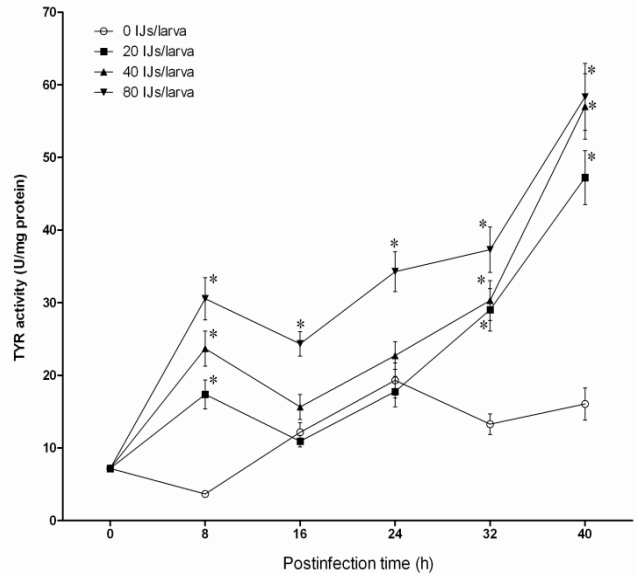

Figure 1. Effects of *H. beicherriana* infection on TYR activity of *G. mellonella* larvae. TYR activity of *G. mellonella* larvae infected with *H. beicherriana*. Data represent the means ± SEM of three replicates with 10 larvae per replicate. **P* < 0.05 vs. control (*LSD* test).

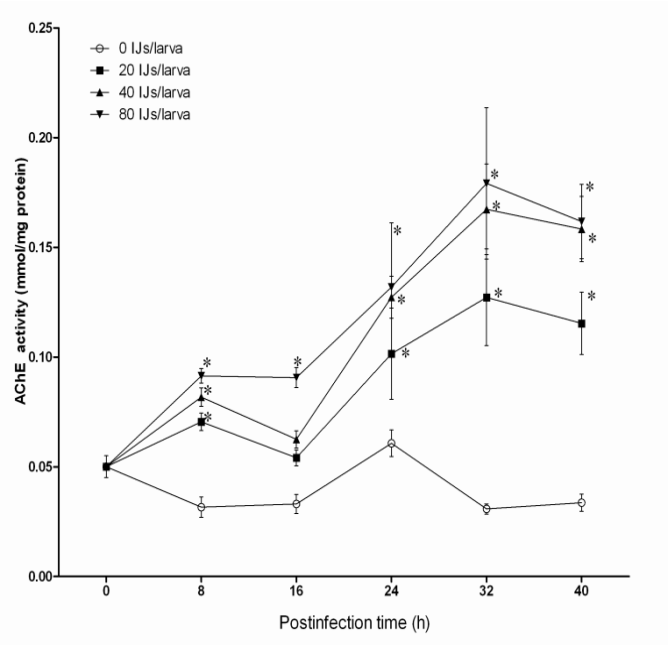

Figure 2. Effects of *H. beicherriana* infection on AChE activity of *G. mellonella* larvae. AChE activity of *G. mellonella* larvae infected with *H. beicherriana*. Data represent the means ± SEM of three replicates with 10 larvae per replicate. **P* < 0.05 vs. control (*LSD* test).

timepoint 0h, no differences were observed between different treatment groups. However, infection of entomopathogenic nematodes (EPNs) for more than 8 h resulted in a significant dose- and time-dependent promotion in enzyme activity of TYR. The first significant promotion was observed at the dose of 20, 40 and 80 IJs/larva after treatment with EPNs for 8 h, with an enhancement of 4.73, 6.44 and 8.31 folds versus the control group 0 IJs/larva, respectively ($P < 0.05$). At 40h (the endpoint of the experiment), TYR activities of the four treatments (0, 20, 40, 80 IJs/larva) reached 2.24, 6.57, 7.94, 8.12 folds of that at 0 h, respectively ($P < 0.05$). Furthermore, at 16 and 24h, only the dose of 80 IJs/larva showed significant increasing TYR activity compared with the 0 IJs/larva, whereas 20 IJs/larva treatment even demonstrated a slightly inhibition of TYR activity.

AChE activity of *G. mellonella* larvae infected with *H. beicherriana*

Similar trend was observed in AChE activity of *G. mellonella* larvae after infecting with *H. beicherriana*. As shown in Figure 2, AChE activity was also significantly increased compared with the controls ($P < 0.05$). At 0h, no differences were observed between different treatment groups. Infection of EPNs for more than 8 h resulted in a significant dose- and time-dependent promotion in enzyme activity of AChE. The first significant enhancement was observed at the dose of 20, 40 and 80 IJs/larva after treatment with EPNs for 8 h, with an increasing of 2.22, 2.56 and 2.84 folds versus the control group 0 IJs/larva, respectively ($P < 0.05$). At 40h (the endpoint of the experiment), AChE activities of the four groups (0, 20, 40, 80 IJs/larva) reached 0.68, 2.30, 3.16, 3.24 folds of that value at 0 h, respectively ($P < 0.05$). However, at 16 h, only the dose of 80 IJs/larva significantly increased AChE activity compared with the 0 IJs/larva.

CarE activity of *G. mellonella* larvae after infecting with *H. beicherriana*

As shown in Figure 3, CarE activity was significantly increased compared with the controls ($P < 0.05$). At the initial timepoint 0h, no differences were observed between different treatment groups. Infection of entomopathogenic nematodes (EPNs) for more than 8 h resulted in a significant dose- dependent promotion in activity of CarE. The first significant promotion was observed at the dose of 20, 40 and 80 IJs/larva after treatment with EPNs for 8 h, with an enhancement of 1.64, 1.85 and 2.22 folds versus the control group 0 IJs/larva, respectively ($P < 0.05$). Unlike the results of TYR and AChE, the activity of CarE decreased at 24 h and then increased at 32h, 40h, however, the values were still smaller than that of 16h, and the peak value appeared at 16 h instead of at the endpoint 40 h.

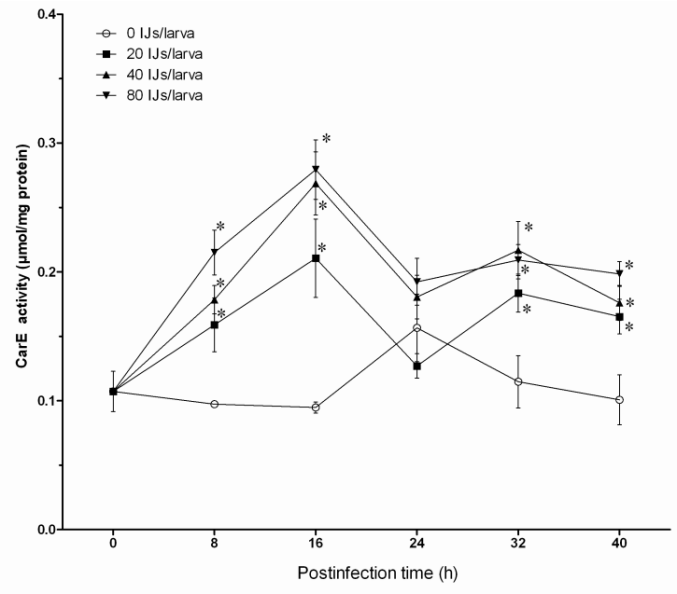

Figure 3. Effects of *H. beicherriana* infection on CarE activity of *G. mellonella* larvae. CarE activity of *G. mellonella* larvae infected with *H. beicherriana*. Data represent the means ± SEM of three replicates with 10 larvae per replicate. **P* < 0.05 vs. control (*LSD* test).

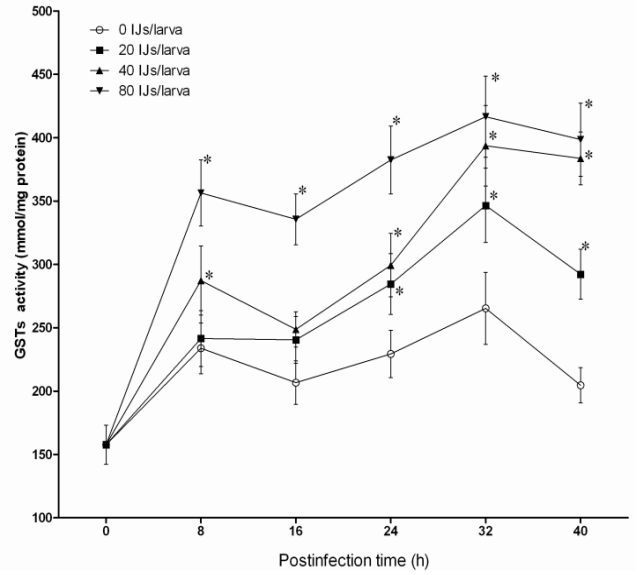

Figure 4. Effects of *H. beicherriana* infection on GST activity of *G. mellonella* larvae. GST activity of *G. mellonella* larvae infected with *H. beicherriana*. Data represent the means ± SEM of three replicates with 10 larvae per replicate. **P* < 0.05 vs. control (*LSD* test).

GST activity of *G. mellonella* larvae after infecting with *H. beicherriana*

As shown in Figure 4, GST activity was significantly increased compared with the controls (*P* < 0.05), and increasing IJs doses and treatment time resulted in a progressive enhancement of GST activity. At the initial timepoint 0h, no differences were observed between different treatment groups. However, infection of entomopathogenic nematodes (EPNs) for more than 8 h resulted in a significant dose- and time-dependent promotion in enzyme activity of GST. The first significant promotion was observed at the dose of 40 and 80 IJs/larva after treatment with EPNs for 8 h, with an enhancement of 1.23 and 1.52 folds versus the control group 0 IJs/larva, respectively (*P* < 0.05). No significant differences were shown between 20 IJs/larva and 0 IJs/larva group. The peak value of GST activity appeared at 32h instead of at the endpoint 40h, reaching 1.68, 2.20, 2.50 and 2.64 folds of that at 0 h, respectively (*P* < 0.05). Furthermore, at 16 h, only the dose of 80 IJs/larva showed significant increasing GST activity compared with that of 0 IJs/larva (*P* < 0.05).

DISCUSSION

Tyrosinase (TYR) is an enzyme ubiquitously distributed in insects, and is responsible for some physiologically important processes (Asano and Ashida, 2001). TYRs in most insects are stored in the haemolymph, epidermis and midgut with the form of zymogen under normal physiological conditions, which could be activated by various factors to detoxify the invasive substances (Brunet, 1980; Hall et al., 1995). In this study, TYR activity was significantly increased compared with the control, and increasing IJs doses and treatment time resulted in a progressive dose- and time-dependent enhancement of TYR enzyme activity. At 40 h (the endpoint of the experiment), TYR activities of the four treatments (0, 20, 40 and 80 IJs/larva) reached the maximum point. At 16 and 24 h, only the dose of 80 IJs/larva showed significant increasing TYR activity compared with the 0 IJs/larva, whereas 20 IJs/larva treatment even demonstrated a slightly inhibition of TYR activity. The increased TYR activity in *Galleria* larvae infection with *H. beicherriana* may be an overreactive and adaptive response to EPNs infection. However, the decreased TYR activity 16 h post-infection indicates that EPNs infection may consume superfluous TYR. Balasubramanian et al. (2010) found that a trypsin-like serine protease extracted and purified from secretions of *S. carpocapsae* could inhibit activity of TYR of *G. mellonella* larvae, which indicates that the changes of TYR activity in insects infected with EPNs are context dependent.

AChE is a very important enzyme in nerve conduction of insects, which rapidly hydrolyzes excitatory neurotransmitter acetylcholine and maintained the normal function of synaptic transmission. Some toxic substances such as organophosphate insecticides will inhibit the activity of AChE, resulting in excessive accumulation of ACh, leading to hyperactivities and consequently

paralysis and death (Fournier and Mutero, 1994). The study of toxic substance on activity of AChE of insect mainly focused on pesticide toxicology, and there were few reports of EPNs on the AChE activity of host insects. In this study, AChE activity of *G. mellonella* larvae after infecting with *H. beicherriana* was significantly increased compared with the control. Infection of EPNs for more than 8 h resulted in a significant dose- and time-dependent promotion in activity of AchE. At 40 h post-infection (the endpoint of the experiment), AchE activities of the four groups (0, 20, 40 and 80 IJs/larva) reached the peak. The slightly decreased AChE activity 16 h post-infection compared with 0 h indicates that EPNs infection may consume too much AChE. The increase in AChE activity in *G. mellonella* larvae infection with *Heterorhabditis beicherriana* n.sp. may be an overreactive response to EPNs infection, which would eliminate much more ACh, consequently resulting in paralysis and death as well.

CarE is a crucial detoxification enzyme for insects to degrade toxic substances. Studies have indicated that increased CarE activity is the major mechanism of insecticide resistance by increasing the hydrolysis and/or sequestration of insecticides in insects (Herath et al., 1987). In this study, changes of CarE activity in *G. mellonella* larvae infected with *H. beicherriana* fluctuated with the increase of infection time. CarE activity was significantly increased compared with the control, and increasing IJs doses resulted in a progressive enhancement of CarE activity. Infection of EPNs for more than 8 h resulted in a significant dose- dependent promotion in enzyme activity of CarE. Unlike the results of TYR and AChE, the activity of CarE decreased at 24 h and then increased at 32 and 40 h, however, the values were still smaller than that of 16 h, and the peak value appeared at 16 h instead of at the endpoint 40 h. The increase in CarE activity in *G. mellonella* larvae infection with *H. beicherriana* may be an adaptive response to neutralize EPNs-induced oxidative stress. However, the decreased CarE activity 24 h post-infection indicates that EPNs infection may consume CarE through detoxification reactions. It seems that the host insects overreacted to the EPNs infection by enhancing the CarE activity first, indicating that many lipids would be disintegrated, which would damage organs and tissues, and lead to the death of host insects. Then the EPNs-infection response would consume the CarE, resulting in the decreased CarE activities.

GST, known as a detoxication enzyme, widely existed in a variety of organisms. It plays an important role in the degradation of endogenous and exogenous toxic substances, and also in intracellular material transportation, hormone synthesis and protection from oxidative stress (Motoyama and Dauterman, 1980; Comoy et al., 1997; Hemingway, 2000; Vontas et al., 2001; Enayati et al., 2005). It has been found that GST is vital for maintaining the homeostasis of oxidation and antioxidant in insects by removing redundant free radicals (Singh et al., 2001; Vontas et al., 2001; Ortelli et al., 2003). Previous studies reported that elevated GST activity was associated with resistance to insecticides (Comoy et al., 1997; Hemingway, 2000). In this study, activity of GST was significantly increased compared with the controls, and increasing IJs doses and treatment time resulted in a progressive dose- and time-dependent promotion of GST activity. The increase in GST activity in *G. mellonella* larvae infected with *Heterorhabditis beicherriana* n.sp. may be an adaptive response to neutralize EPNs-induced oxidative stress. However, the decreased GST activity 16 h post-infection indicates that EPNs infection may consume GST through detoxification reactions of glutathione-dependent enzymes. The increase in GST activity of control after 0 h may be related with some certain physiological changes.

In conclusion, our data suggest that the activities of TYR, AChE, CarE and GST would be significantly enhanced in a dose-dependent manner in *G. mellonella* larvae infected with *H. beicherriana,* which may be the overeactive stress response to the EPNs infection. This overreaction often leads to death of the host insects. There may be synergistic and (or) antagonistic effects of these four enzymes, however, further study needs to be carried out to elucidate the exact mechanism. Since the entomopathogenic nematodes can be mass-produced using *in-vivo* or *in-vitro* methods, commercial *H. beicherriana* can be applied as infective juveniles (IJs) in aqueous suspensions using various approaches to protect the crops by infecting and extinguishing targeted harmful insects.

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REFERENCES

Asano T, Ashida M (2001). Cuticular pro-phenoloxidase of the silkworm, *Bombyx mori*. Purification and demonstration of its transport from hemolymph. The J. Biol. Chem. 276:11100-11112.

Balasubramanian N, Toubarro D, SimÕes N (2010). Biochemical study and *in vitro* insect immune suppression by a trypsin-like secreted protease from the nematode *Steinernema carpocapsae.*Parasite. Immunol. 32:165-175.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. biochem.72:248-254.

Brown SE, Cao AT, Dobson P, Hines ER, Akhurst RJ, East PD (2006). Txp40, a ubiquitous insecticidal toxin protein from *Xenorhabdus* and *Photorhabdus* bacteria. Appl. Environ. Microbiol. 72:1653-1662.

Brown SE, Cao AT, Hines E R, Akhurst R J, East PD. (2004). A novel secreted protein toxin from the insect pathogenic bacterium *Xenorhabdus nematophila*. TheJ. Biol. Chem. 279:14595-14601.

Brunet PCJ (1980). The metabolism of the aromatic amino acids concerned in the cross-linking of insect cuticle. Insect. Biochem. 10:467-500.

Comoy EE, Vendeville C, Capron A, Thyphronitis G (1997). Infection with *Salmonella typhimurium* modulates the immune response to *Schistosoma mansoni* glutathione S-transferase. Infect. Immun. 65:3261-3266.
Ellman GL, Courtney KD, Andres VJr, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity.Biochem. Pharmacol. 7:88-95.
Enayati AA, Ranson H, Hemingway J (2005). Insect glutathione transferases and insecticide resistance. Insect. Mole. Biol. 14: 3-8.
Ffrench-Constant RH, Dowling A, Waterfield NR (2007). Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. Toxicon. 49: 436-451.
Fournier D, Mutero A (1994). Modification of acetylcholinesterase as a mechanism of resistance to insecticides.Compar. Biochem. Physiol. 108:19-31.
Grewal P, Ehlers R-U, Shapiro-Ilan DI (Eds.), (2005). Nematodes as Biological Control Agents. CABI Publishing, Wallingford, UK.
Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J.Biol.Chem. 249:7130-7139.
Hall M, Scott T, Sugumaran M, Söderhäll K, Law JH (1995). Proenzyme of *Manduca sexta* phenol oxidase: purification, activation, substrate specificity of the active enzyme, and molecular cloning. *Proceedings of the* National Academy of Sciences of the U.S. A.92:7764-7768.
Hemingway J (2000). The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. Insect Biochem. Mole. Biol. 30: 1009-1015.
Herath PRJ, Hemingway J, Weerasinghe IS, Jayawardena KGI (1987).The detection and characterization of malathion resistance in field populations of *Anopheles culicifacies* B in Sri Lanka. Pesticid.e Biochem. Physiol. 29:157-162.
Maxwell PW, Chen G. Webster JM, Dunphy GB (1994). Stability and activities of antibiotics produced during infection of the insect Galleria mellonella by two isolates of Xenorhabdus nematophilus. Appl. Environ. Microbiol. 60:715-721.
Mracek Z, Webster JM. (1993), Survey of Heterorhabditidae and Steinernematidae (Rhabditida: Nematoda) in Western Canada. J. Nematol. 25:710-717.
Motoyama N, Dauterman WC (1980). Glutathione S-transferases: Their role in the metabolism of organophosphorus insecticides. Rev. Biochem. Toxicol., 2:49-69.
Ortelli F, Rossiter LC, Vontas J, Ranson H, Hemingway J (2003). Heterologous expression of four glutathione transferase genes genetically linked to a major insecticide-resistance locus from the malaria vector *Anopheles gambiae*. The Biochem. J. 373:957-963.
Singh SP, Coronella JA, Benes H, Cochrane BJ, Zimniak P (2001). Catalytic function of *Drosophila melanogaster* glutathione S-transferase DmGSTS1-1 (GST-2) in conjugation of lipid peroxidation end products. Europ. J. Biochem. 268:2912-2923.
Soreq H, Seidman S (2001). Acetylcholinesterase-new roles for an old actor. Nature Rev. Neurosci. 2:294-302.
Sugumaran M, Nellaiappan K, Valivittan K. (2000). A new mechanism for the control of phenoloxidase activity: inhibition and complex formation with quinone isomerase. Arch. Biochem. Biophys. 379: 252-260.
Sugumaran M, Nellaiappan K (2000). Characterization of a new phenoloxidase inhibitor from the cuticle of *Manduca sexta*. Biochem. Biophys. Res. Commun. 268:379-383.
Toubarro D, Lucena-Robles M, Nascimento G, Costa G, Montiel R, Coelho AV, Simões N (2009). An apoptosis-inducing serine protease secreted by the entomopathogenic nematode Steinernema carpocapsae.Int. J.Parasitol. 39:1319-1330.
Van Asperen K (1962). A study of housefly esterases by means of a sensitive colorimetric method. J. Insect. Physiol. 8:401-414.
Vontas JG, Small GJ, Hemingway J (2001). Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. The Biochem. J. 357: 65-72.