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Full Length Research Paper

Comparative proteomic analyses of *Anticarsia gemmatalis* and *Spodoptera frugiperda* guts

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***Anticarsia gemmatalis* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) are serious defoliators of a number of crops. In order to better understand the digestion process of these pests and considering that the midgut is one of the most important and attractive targets for biological pesticides, this report shows the evaluation of midgut proteins from *A. gemmatalis* and *S. frugiperda* by proteomic analyses. Larval midguts were removed and total proteins (approximately 650 µg) were extracted and further submitted to two-dimensional electrophoresis (2-DE). Proteins were identified by tandem mass spectrometry (MS/MS) including catalase, enolase, glyceraldehyde-3-phosphate dehydrogenase, arginine kinase, pyrophosphatase and farnesoic acid O-methyl transferase. All proteins identified seem to be involved in insect development in different ways, being directly involved in their growth or used in metabolic pathways that may influence pest development. The identification of those proteins could contribute to a better understanding of pest physiology, leading to the development of novel strategies for pest management. In short, the identification of proteins by 2D may lead to the use of new approaches for pest management.**

Key words: *Anticarsia gemmatalis*, *Spodoptera frugiperda*, proteomics, mass spectrometry (MS).

INTRODUCTION

Extensive monocrops such as soybean, maize, cotton and cabbage require enormous agrochemicals input to control insect-pests, especially Lepidoptera such as the velvet bean caterpillar *Anticarsia gemmatalis* Hübner and the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Chemical pesticides pose a major risk to the environment and human health, while biological pesticides such as bacteria, fungi, and viruses generally present natural effectiveness in controlling the targeted pest and are safe for the environment (Bravo et al., 2007, 2011; Roh et al., 2007; Moscardi et al., 2011).

The high costs involved in insect control sometimes make crop cultivation financially unviable, since a wide diversity of insect-pests is able to reduce crop production. Among insect-pests, *A. gemmatalis* and *S. frugiperda* are serious defoliators of soybean in Latin America and other regions (Moscardi, 1999, 2011; Cruz et al., 1999; Hoffmann-Campo et al., 2000; Silva et al., 2003, Farinelli and Fornasieri Filho, 2006; Alves and Lopes, 2008; Barrera et al., 2011), causing considerable losses in previously described crops (Martins et al., 2009).

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Although, it is clearly vital to know more about these two pests, no proteome database has yet been constructed for either of them.

In particular, knowledge of insect gut proteins is extremely important in a pest management program, in that it involves understanding food digestion, nutrient absorption and protection from pathogen invasion. The midgut has been observed as a common target of biopesticides, since it can be disrupted in several ways. This disruption process could lead to feeding cessation, growth reduction and increased susceptibility to toxins and pathogens involved in biological control (Nan Gong et al., 2005; Bravo et al., 2007; Guo et al., 2007).

In recent years, proteomic analysis has been used to boost biological control of insect-pests (Pigott and Ellar, 2007; Murad et al., 2008; Yao et al., 2009; Zhang et al., 2011). Proteomic technologies have shown several practical applications in pest-management, including the identification of novel compounds involved in biological control of insect-pests by entomopathogens (McNall and Adang, 2003; Baum et al., 2007; Krishnamoorthy et al., 2007; Murad et al., 2008; Barros et al., 2010; Gao et al., 2011, Yuan et al., 2011,). However, in addition to studies focusing on bioinsecticidal development, the target insect must also be investigated. Studies on protein expression profiles in the midgut can facilitate the identification of molecular targets for use in developing novel and environmentally benign control strategies (Rabilloud, 2002; Dechklar et al., 2011). In this work, the midgut proteins of *A. gemmatalis* and *S. frugiperda* were evaluated by proteomic analyses, aiming to contribute to pest management programs.

MATERIALS AND METHODS

Preparation of protein extracts from *A. gemmatalis* and *S. frugiperda* midguts

The noctuid larvae of about 2-day-old fifth instars of *A. gemmatalis* and *S. frugiperda* were provided by the Insect Rearing Laboratory (Caballero, 2005) of Embrapa Genetic Resources and Biotechnology, Brasília- DF, Brazil, after being reared on an artificial diet.

Protein extraction from the gut of *S. frugiperda* and *A. gemmatalis*

Larvae were anaesthetized by chilling on ice, and the midguts were dissected by using a binocular microscope. After dissecting and homogenizing guts by using saline solution, the extraction enzymes of the midgut were estimated for determining protein concentration (Bradford, 1976).

Gel electrophoresis analyses

Approximately 650 µg of total protein of the samples were precipitated in TCA 75% and incubated at 4°C for 30 min, then centrifuged at 12000 *g* for 15 min. The pellet was washed with 1 ml of cold acetone and centrifuged at 12000 *g* for 15 min. Aiming to remove carbohydrates and other possible interferences, samples were submitted to a novel precipitation step using 2-D Clean-Up Kit (GE Healthcare) according to the manufacturer's instructions. Isoelectric focusing and molecular mass separation were conducted by using 13 cm immobilized pH gradient (IPG) strips with a non linear pH ranging from 3 to11 (GE HealthCare, Biosciences AB, Sweden). Strips were re-hydrated with samples re-suspended in rehydration solution (250 ml solution of 2% CHAPS, 8 M urea, 2 M thiourea, 1% DTT, traces of bromophenol blue) for 16 h. Isoelectric focusing was carried out at Ettan IPGphor3 (GE Healthcare Life Sciences) following a voltage step-gradient (50 V for 3 h, 500 V for 1 h, 1000 V for 1 h, 2000 V for 1 h, 4000 V for 1 h and 6000 V for 12 h) for six strips. After the first dimension, strips were equilibrated in solutions of 1% DTT and 1% iodoacetamide for 15 min (1.5 M Tris–HCl pH 8.8, 6 M urea, 30% glycerol, traces of bromophenol blue and 2% SDS). A second dimension was performed using 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) as described by Laemmli (1970), which contained acrylamide 30%, 1.5 M tris–HCl pH 8.8, 10% sodium dodecyl sulfate (SDS), 10% ammonium persulfate (APS), 10% tetramethyl-ethylenediamine (TEMED) and Milli-Q water. For pI standard, the Broad pI Kit (3 to 10) (GE Healthcare) was used as marker and for molecular weight marker, Unstained Protein Molecular Weight Marker (Fermentas) was used. Electrophoresis was performed at 15°C using Ettan DAL Tsix vertical electrophoresis system (GE Healthcare Life Sciences). The first phase was set at 600 V, 90 mA, 100 W for 30 min and the second phase was set at 700 V, 240 mA, 100 W for 6 h, which was set for 6 gels. The gels were fixed in 30% methanol, 10% acetic acid and stained by Coomassie Brilliant Blue G-250 (0.1%). The number of biological and technical replicates was 6 and 12, respectively.

In silico gel analyses

Gels were digitalized using the HP Scanjet Model 8290 scanner and further analyzed with BioNumerics software v. 5.1 (Applied-Maths). Each protein sample was analyzed in triplicate. First, calibration with a gray scale was necessary to transform gray levels into values for each pixel of the gel picture. The calibration method used a calibration curve from BioNumerics software. All gel pictures were analyzed as tiff files. The six gel images were placed in one folder and the wizard detection method proposed by the software was used for spot detection. Automatically detected spots were manually checked, and some of them manually added or removed according to the size (>0.2 cm was used as the cutoff limit for inclusion), shape (circular) and density (>2 pixel cm^{-1}). Following the detection procedure, the normalization step was carried out to attribute a common protein identity to identical spots from different gel images. For this procedure, a reference gel was constructed and automatically matching options of BioNumerics software were used. For each sample, when a protein was detected in all gel images, this protein was automatically added to the reference gel.

Protein digestion

The most significant spots were excised from the gels using a scalpel. In-gel digestion with Gold sequencing-grade trypsin (Promega) was conducted following the protocol described by Shevchenko et al. (1996). Briefly, after removing the Coomassie from spots by de-staining solution, 400 µl of 50% ACN (acetonitrile) and 25 mM NH_4HCO_3 were added to the tubes and incubated for 20 min on the vortex. Supernatants were removed and 200 µl of 100% ACN was added to the tubes and incubated for 10 min, then spots were dried in a speed vacuum for 20 min. Next, 600 ηg/2 µl (30% acetic-acid 50 mM and 195 µl of 50 mM NH_4HCO_3) were added to the tubes and incubated on ice for 30 min. After this process, 40 µl

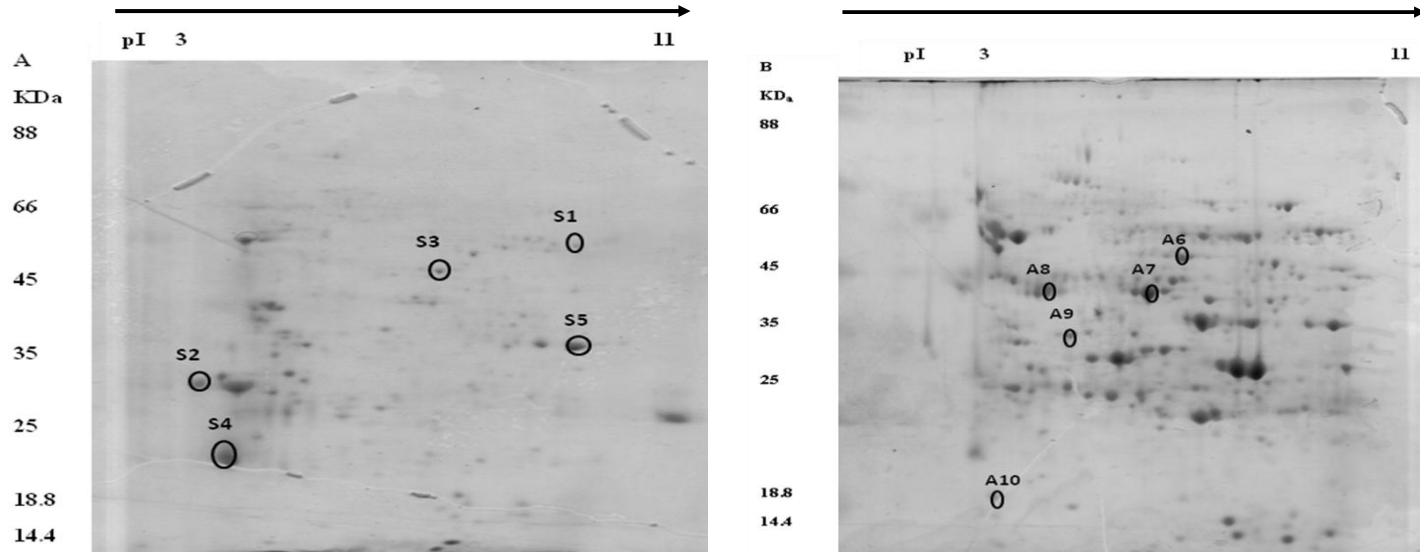

Figure 1. Two-dimensional gels of proteins guts extracted from the fifth-instar larvae of (A) *Spodoptera frugiperda* and B). *Anticarsia gemmatalis.* Numbers indicate the differential spots further identified by mass spectrometry technology (Table 1). Numbers on the left side of the gel correspond to the molecular mass marker. Numbers on the top correspond to pI marker.

of 50 mM NH_4HCO_3 was added to each tube and they were incubated in a water bath at 37°C for 22 h. The supernatant was used for mass spectrometry analysis.

Protein identification by mass spectrometry

Monoisotopic masses of the molecular components from digested peptides ranging from 600 to 6000 m/z were determined by mass spectrometry using a 4700 MALDI-TOF/TOF (Applied Biosystems, Framingham, MA) controlled by the manufacturer's software. A sample of 2 ml was mixed with 6 ml of 0.1% a-cyano-4-hydroxycinnamic acid, 0.1% trifluoroacetic acid and acetonitrile (1:1). A volume of 0.5 ml was applied to a MALDI plate and air-dried at room temperature. The spectrometer was operated in linear mode for MS acquisition and reflected mode for MS/MS acquisitions using modulated power with 200 random shots. Data were saved in standard Bruker's software format.

Protein identification

Spots were identified using peptide mass fingerprinting (PMF) and "*de novo*" sequencing. The mass list for each sample was analyzed using the program MASCOT v2.1.0, Matrix Science, London (http://www.matrixscience.com) assuming one missed cleavage, carboxymethylation and methionine oxidation was used as modifications. The lists of masses were compared against the non-redundant NCBI database. Results were evaluated by comparing the molecular mass and isoelectric point of the hit of the highest score data reported and observed in 2D gels. *De novo* sequencing was conducted manually, subtracting the masses from y series and comparing to amino acid masses. Sequences were then compared to the Swiss-Prot Data base (www.expasy.org) using the MPSrch tool from the European Bioinformatics Institute (EBI). Only protein hits with molecular mass and isoelectric point values similar to those found on gels, together with coverage, similarity and tryptic digestion pattern were considered as a match.

RESULTS

Comparative analyses of protein maps

Proteins were extracted from midguts of 2-day-old, fifth instar larvae of *A. gemmatalis* and *S. frugiperda*. After extraction, two-dimensional gels from midguts of *S. frugiperda* (Figure 1a) and *A. gemmatalis* (Figure 1b) larvae were obtained using a pH range varying from 3.0 to 11.0 and molecular mass varying from 14.4 to 116 kDa. Protein maps obtained from two independent samples were scanned and further compared to identify differences in protein expression using the BioNumerics software. Each protein sample was analyzed in triplicate and corresponding gels showed correlation values higher than 0.9 (data not shown). Two-dimensional gels from the *S. frugiperda* analyzed showed 206 spots, distributed throughout the gel. pIs and molecular masses of identified proteins varied from 3.0 to 11.0 and from 11 to 78 kDa, respectively (Figure 1a). Most of the spots were situated between pIs 5.0 and 8.0, where the most abundant proteins are also located. A similar profile was observed for *A. gemmatalis* (Figure 1b), where a high quantity of spots were resolved (385 spots), showing proteins with pH varying from 3.0 to 11 and molecular masses ranging from 6.67 to 88 kDa (Figure 1b). Most of the spots were situated between pIs 6.0 and 8.0, where the most abundant proteins are also located.

Spots were sliced for mass spectrometry protein identification, trypsinized, submitted to mass spectrometry analyses (MALDI-ToF-ToF) and further analyzed. Thirty spots were analyzed, and 10 of these were identified by mass spectrometry. The spot proteins

Table 1. Proteins identified by MALDI ToF MS from Spodoptera frugiperda (proteins spots 1-5) and Anticarsia gemmatalis (Protein spots 6-10).

Spot	Protein class (species)	Species	Accession number	Score	Gel molecular mass (KD_a)	Theoretical molecular mass (D_a)	Gel pI	Partial sequences
S1	Catalase 1	*A. gambiae*	gi|40792585	50	54.48	54958	7.98	R.FSTVGGESGSADTVRDPR.G
S2	Hypothetical protein	*L. loa*	gi|312071189	37	34.00	24550	3.00	R.SAIPAAIQAPCTVVHIR.D
S3	Putative enolase protein	*S. frugiperda*	gi|158451691	149	49.63	40899	6.78	PMF
S4	Phaseolin	*V. legumin*	gi|640273	147	24.57	44965	3.51	PMF
S5	Glyceraldehyde-3-phosphate dehydrogenase	*R. norvegicus*	gi|56188	105	39.84	36098	8.98	K.LISWYDNEYGYSNR.V
A6	Putative enolase	*S. incertulas*	gi|254934399	106	83.49	40802	11.61	PMF
A7	Arginine kinase	*K. rumia*	gi|269116647	99	36.42	19248	9.51	PMF
A8	Hypothetical protein	*S. moellendorffii*	gi|300156048	86	49.02	95526	4.59	PMF
A9	Inorganic pyrophosphatase	*E. cuniculi*	gi|19074643	74	59.04	32079	8.16	R.GDNDPLDVIEIGRK.R
A10	Farnesoic acid O-methyl transferase	*B. mori*	gi|148298754	51	27.58	25359	8.07	R.VGTDADGDEIYAGR.A

Peptide mass fingerprint (PMF) and MS/MS ion search was performed and further submitted to Mascot software. Mpsrch score, sequence, pI and molecular mass were used for protein identification. Proteins that showed no sequence, identified by PMF, were only identified by Mascot scores.

identified from *S. frugiperda* were named S1, S2, S3, S4 and S5. Spots were named A6, A7, A8, A9 and A10 for the proteins identified from *A. gemmatalis* (Table 1). Peptide mass fingerprint (PMF) and MS/MS ion search were performed with trypsinized samples, and Mascot search and EBI databanks were used for further data mining (Table 1).

Mass spectrometry protein identification of *S. frugiperda*

Protein S1 from *S. frugiperda* showed a clear similarity to catalase I protein from *Anopheles gambiae* (Table 1) (David and Ranson, 2004). The second spot (S2) was also identified by MS/MS analyses, as a hypothetical protein, showing similarities to proteins from *Loa loa* (Nematoda: Onchocercidae) (Table 1, spot S2) being impossible to relate a clear function (Nutman et al., 2010). Protein spot S3 obtained from *S. frugiperda* showed similarity to putative enolase protein from *S. frugiperda* (Regier et al., 2009). Lastly, protein spot S5 from *S. frugiperda* was identified as glyceraldehyde-3-phosphate dehydrogenase protein (Piechaczyk et al., 1984).

Mass spectrometry protein identification of *A. gemmatalis*

The midgut of *A. gemmatalis* also generates differential proteins. Protein spot A6, obtained from *A. gemmatalis*, showed similarity to a putative enolase protein from *Scirpophaga incertulas* (Regier et al., 2009). Arginine kinase (AK) protein (spot A7) is one of the proteins which are identified by fingerprint analyses (Wahlberg et al., 2009). The next spot, A8, was also identified by fingerprint analyses as hypothetical proteins, showing similarities to proteins from the nematode *Selaginella moellendorfii* (Table 1, spot A8) (Banks et al., 2011). Spot A9 from *A. gemmatalis* was identified as an inorganic pyrophosphatase GB-M1 protein (Katinka et al., 2001), which is responsible for catalyzing the conversion of one molecule of pyrophosphate to two phosphate ions (Harold, 1966). Finally, the last protein identified in *A. gemmatalis*, named A10, showed homology to putative farnesoic acid O-methyl transferase from *Bombyx mori* (Xu et al., 2008). Farnesoic acid O-methyltransferase (FaMeT) is the enzyme responsible for the conversion of farnesoic acid (FA) to methyl farnesoate (MF) in the final step of MF synthesis (Kuballa et al., 2007). The FAMeT gene was well identified in crustaceans in which MF

production is specific to the mandibular organ, and homologous to the insect brain gland *Corpora allata* (Chang, 1993; Kuballa et al., 2007). Vannini et al. (2010) reported that this gene has been found in the penultimate step of insect juvenile insect development and reproduction. Additionally, JH hormone is effective in insect metamorphosis, regulating modification processes in larval instars.

DISCUSSION

Protein S1 from *S. frugiperda* showed a clear similarity to catalase I protein, which are commonly accumulated inside peroxisomes, which are present in a wide variety of eukaryotic organisms, including insects. They commonly contain oxidases that generate hydrogen peroxide and catalase that degrades hydrogen peroxide to oxygen and water (Switala and Loewen, 2002; Chelikani et al., 2004; Kurisu et al., 2003), being important for detoxification. Moreover, peroxysomes play an indispensable role in the oxidation of very long-chain fatty acids (Reddy and Mannaerts, 1994), as well as playing an anabolic role in the biosynthesis of cholesterol, bile acids and plasmalogen (van den Bosch et al., 1992) suggesting that this enzyme is also involved in lipid metabolism. Finally, since catalases are involved in cholesterol biosynthesis, this enzyme is also important for biosynthesis of ecdysion, one of the most important hormones which control the ecdysis process in insects (van den Bosch et al., 1992; Nation, 2002).

Protein spot S3 which was identified from larvae of *S. frugiperda* showed similarity to putative enolase protein from *S. frugiperda.* Enolase is a well-known enzyme in cell metabolism, mediating the activation of multiple enzymes involved in tissue response to invasion by pathogens and tumor cells, being controlled by host immune response (Falabella et al., 2009). Some protein spots showed clear similarity to proteins not directly related to midgut proteins. For example, spot 4 showed similarity to a common *Vicili legumin* structure of seed storage proteins (Lawrence et al., 1990), indicating that proteins previously digested from broth may also be observed in this kind of approach.

Glyceraldehyde-3-phosphate dehydrogenase protein was another protein obtained from this species (S5). This ~37 kDa enzyme catalyzes the sixth step of glycolysis and thus serves to break down glucose for energy and carbon molecule production. In addition to this long established metabolic function, GAPDH has recently been implicated in several non-metabolic processes, including transcription activation and initiation of apoptosis (Tarze et al., 2007).

Arginine kinase (AK) protein (spot A7) is one of the proteins identified by fingerprint analyses. AK belongs to a class of kinases that play a role in the maintenance of ATP levels by phosphorylation of "phosphagens", which then serve as a high energy source from which ATP can be rapidly replenished. In a wide variety of invertebrates, phosphoarginine is the most important phosphagen, in contrast to phosphocreatine normally found in vertebrates (Pereira et al., 2000). Furthermore, Pereira et al. (2000) showed that AK is important for chitin biosynthesis. Chitin is one of the polysaccharides that are used in cuticle structures of insects, and it provides stability and firmness of the cuticle, inhibiting the impact of external factors such as pathogens, predators, parasites and pesticides (Nation, 2002).

Spot A9 from *A. gemmatalis* was identified as an inorganic pyrophosphatase GB-M1 protein (Katinlka et al., 2001), which is responsible for catalyzing the conversion of one molecule of pyrophosphate to two phosphate ions (Harold, 1966). This is a highly exergonic reaction, and can therefore be coupled to unfavorable biochemical transformations in order to drive these transformations to completion (Terkeltaub, 2001). The functionality of this enzyme plays a critical role in lipid metabolism (including lipid synthesis and degradation), calcium absorption and bone formation (Orimo et al., 1971; Poole and Reeve, 2005) and DNA synthesis (Nelson and Cox, 2000), as well as other biochemical transformations (Ko et al., 2007; Usui et al., 2010).

Finally, the last protein identified in *A. gemmatalis*, named A10, showed homology to putative farnesoic acid O-methyl transferase (FaMeT) from *Bombyx mori*, which is responsible for the conversion of farnesoic acid (FA) to methyl farnesoate (MF) in the final step of MF synthesis (Kuballa et al., 2007). The FAMeT gene was well identified in crustaceans in which MF production is specific to the mandibular organ, homologous to the insect brain gland *Corpora allata* (Chang, 1993; Kuballa et al., 2007). Vannini et al. (2010) reported that this gene has been found in the penultimate step of insect juvenile hormone (JH) biosynthesis, thus being a key regulator in insect development and reproduction. Additionally, JH hormone is effective in insect metamorphosis, regulating modification processes in larval instars.

This proteomic work indicates novel targets that could be used for insect-pest control. For example, the blockage of farnesoic acid O-methyltransferase by different inhibitors could lead to the creation of novel insect growth regulators (IGRs). These normally cause delay in larval transformation to the next instar, be it pupa or adult, making them effective as a natural control. If larvae manage to overcome this product and enter the next instar, this novel IGR could affect size and reproduction system, decreasing the oviposition period (Nation, 2002). Furthermore, other identified proteins, such as catalases and arginine kinases, were effective in physiological process including lipid metabolism metamorphosis, ecdysis and reproduction. So, manipulation in the biosynthesis of these pathways by inhibitors could also be an effective step in controlling these pests.

Conclusion

Our report indicates different targets found in the midgets of two important insect-pests that could be used for bioinsecticidal development. These data encourage us to take a new route toward creating different strategies for insect-pest control. In short, the identification of proteins by 2D can lead to new approaches involving synthetic or chemical plant proteinase inhibitors in pest management.

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REFERENCES

Alves SB, Lopes RB (2008). Controle microbiano de pragas na América Latina: Avanços e desafios. Piracicaba: FEALQ (Biblioteca de Ciências Agrárias Luiz de Queiroz), p. 14.

Banks JA, Nishiyama T, Hasebe M (2011). The *Selaginella* genome identifies genetic changes associated with the evolution of vascular plants. Science, 332(6032): 960-963.

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.

Barrera G, Simón O, Villamizar L, Williams T, Caballero P (2011). *Spodoptera frugiperda* multiple nucleopolyhedrovirus as a potential biological insecticide: genetic and phenotypic comparison of field isolates from Colombia. Biolog. Control., 58: 113–120.

Barros BHR, da Silva SH, Marques E dos R, Rosa JC, Yatsuda AP, Roberts D W, Braga GU (2010). A proteomic approach to identifying proteins differentially expressed in conidia and mycelium of the entomopathogenic fungus *Metarhizium acridum*. Fungal Biol., 114: 572–579.

Baum JA, Bogaert T, Clinton W (2007). Control of coleopteran insect pests through RNA interference. Nat. Biotechnol., 25: 1322–1326.

Bravo A, Gill SS, Soberón M (2007). Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon., 49:423-435.

Bravo A, Likitvivatanavong S, Gill SS, Soberón M (2011). *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochem. Mol. Biol., 41:423- 431.

Chelikani P, Fita I, Loewen PC (2004). Diversity of structures and properties among catalases. Cell. Mol. Life Sci., 61 (2): 192 -208.

Cruz I, Figueiredo MLC, Matoso MJ (1999). Controle biolo´gico de *Spodoptera frugiperda* utilizando o parasitoide de ovos Trichogramma. Embrapa Milho e Sorgo, Sete Lagoas, 40 p.

David JP, Ranson H (2004). Molecular coloning of oxidative stress response genes from the mosquito *Anopheles gambiae*. Involvement in resistance to insecticide, un published, http://www.ncbi.nlm.nih.gov/protein/40792585.

Dechklar M, Tiewsiri K, Angsuthanasombat C, Pootanakit K (2011). Functional expression in insect cells of glycosylphosphatidylinositol-linked alkaline phosphatase from *Aedes aegypti* larval midgut: a *Bacillus thuringiensis* Cry4Ba toxin receptor. Insect Biochem. Mol. Biol., 41: 159-166.

Falabella P, Riviello L, Luisa De Stradis M, Stigliano C, Varricchio P, Grimaldi A, Eguileor M, Graziani F, Gigliotti S, Pennacchio F (2009). *Aphidius ervi* teratocytes release an extracellular enolase. Insect Biochem. Mol. Biol., 39(11): 801-813.

Farinelli R, Fornasieri Filho D (2006). Avaliação de dano de *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) em cultivares de milho. Científica, 34: 197-202.

Gao Q, Jin K, Ying S-H, Zhang Y, Xiao G (2011). Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum.* PLoS Genetics 7(1): e1001264. doi:10.1371/journal.pgen.1001264.

Guo, HF, Fang JC, Wang JP, Zhong WF, Liu BS (2007). Interaction of *Xestia c-nigrum* granulovirus with peritrophic matrix and *Spodoptera litura* nucleopolyhedrovirus in *Spodoptera litura*. J. Econ. Entomol., 100: 20–25.

Harold FM (1966). Inorganic polyphosphates in biology: structure, metabolism, and function. Bacteriol. Rev., 30: 772-794.

Hoffmann-Campo CB, Moscardi F, Correa-Ferreira BS, Sosa-Gómez DR, Panizzi AR, Corso IC, Gazzoni DL, Oliveira EB (2000). Pragas da soja no Brasil e seu manejo integrado. Londrina: Embrapa Soja, 70p. (Circular Técnica n.30/Embrapa Soja, ISSN pp 1516-7860).

Katinka MD, Duprat S, Cornillot E (2001). Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. Nature, 414(6862): 450-453.

Ko, KM, Lee WYu JR, Ahnn J (2007). PYP-1, inorganic pyrophosphatase, is required for larval development and intestinal function in *C. elegans*. FEBS Lett., 581(28): 5445-5453.

Krishnamoorthy M, Jurat-Fuentes JL, McNall RJ, AndachtT, Adang MJ (2007). Identification of novel Cry1Ac binding proteins in midgut membranes from *Heliothis virescens* using proteomic analyses. Insect Biochem. Mol. Biol., 37: 189-201.

Kuballa AV, Guyatt K, Dixon B, Thaggard H, Ashton AR, Paterson B, Merritt DJ, Elizur A (2007). Isolation and expression analysis of multiple isoforms of putative farnesoic acid O-methyltransferase in several crustacean species. Gen. Comp. Endocrinol., 150(1): 48-58.

Kurisu M, Morita M, Kashiwayama Y, Yokota S, Hayashi H, Sakai Y, Ohkuma S, Nishimura M, Imanaka T (2003). Existence of catalase-less peroxisomes in Sf21 insect cells. Biochem. Biophys. Res. Commun., 306: 169–176.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature, 227: 680–685.

Lawrence MC, Suzuki E, Varghese JN, Davis PC, Van Donkelaar A, Tulloch PA, Colman PM (1990). The three-dimensional structure of the seed storage protein phaseolin at 3 A resolution. EMBO J., 9(1): 9-15.

McNall RJ, Adang MJ (2003). Identification of novel *Bacillus thuringiensis* Cry1Ac binding proteins in *Manduca sexta* midgut through proteomic analysis. Insect Biochem. Mol. Biol., 33: 999-1010.

Martins GLM, Toscano LC, Tomquelski GV, Maruyama WI (2009). Inseticidas no controle de *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) e impacto sobre aranhas predadoras em soja, Rev. Bras. Ciên. Agrá., 4(2):128-132.

Moscardi F (1999). Assessment of the application of baculoviruses for control of lepidoptera Ann. Rev. Entomol., 44: 257-289.

Moscardi F, Souza ML, Castro MEB, Moscardi ML, Szewczyk B (2011). Baculovirus pesticides: present state and future perspectives. In: Ahmad et. al (Org.). Microbes and Microbial Technology .Springer, 415-445.

Murad AM, Noronha EF, Miller RNG, Costa FT, Pereira CD, Mehta A, Caldas RA, Franco OL (2008). Proteomic analysis of *Metarhizium anisopliae* secretion in the presence of the insect pest *Callosobruchus maculates*. Microbiology, 154: 3766–3774.

Nan Gong ZY, Wang QY, Song P, Yang J, Mao WJ (2005). Histopathological effects of the protein toxin from *Xenorhabdus nematophila* on the midgut of *Helicoverpa armigera*. Sci. Agric. Sin., 38: 2240–2245.

Nation JL (2002). Insect Physiology and Biochemistry. CRC, Boca Raton, FL.

Nelson DL, Cox MM (2000). Lehninger Principles of Biochemistry, third editions. New York, Worth Publishers, pp. 937.

Orimo H, Ohata M, Fujita T (1971). Role of inorganic pyrophosphatase in the mechanism of action of parathyroid hormone and calcitonin. Endocrinology, 89(3): 852-858.

Pereira CA, Alonso GD, Paveto MC, Iribarren A, Cabanas ML, Torres HN, Flawia MM (2000). *Trypanosoma cruzi* arginine kinase characterization and cloning. Biol. Chem., 275: 1495-1501.

Piechaczyk M, Blanchard JM, Marty L, Dani C, Panabieres F, El Sabouty S, Fort P, Jeanteur P (1984). Post-transcriptional regulation of glyceraldehyde-3-phosphate-dehydrogenase gene expression in rat tissues. Nucleic Acids Res., 12 (18): 6951-6963.

Pigott CR, Ellar DJ (2007). Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol. Mol. Biol. Rev., 71: 255-281.

Poole KE, Reeve J (2005). Parathyroid hormone - a bone anabolic and catabolic agent. Cur. Opin. Pharm., 5 (6): 612-617.

Rabilloud T (2002). Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. Proteomics, 2: 3–10.

Reddy JK, Mannaerts GP (1994). Peroxisomal lipid metabolism. Ann. Rev. Nutr., 14: 343–370.

Regier JC, Zwick A, Cummings MP (2009). Toward reconstructing the evolution of advancd moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. BMC Evol. Biol., 9: 280.

Roh, JY, Choi JY, Li MS, Jin BR, Je YH (2007). *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J. Microbiol. Biotechn., 17: 547-59.

Silva MTB da, Costa EC, Boss A (2003). Controle de *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) com reguladores de crescimento de insetos. Ciên. Rur. Santa Maria, 33(4): 601-605.

Shevchenko A, Wilm M, Vorm O, Mann M (1996). Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels. Anal. Chem., 68 (5): 850–858.

Switala J, Loewen PC (2002). Diversity of properties among catalases. Arch. Biochem. Biophys., 401:145-154.

Terkeltaub RA (2001). Inorganic pyrophosphate generation and disposition in pathophysiology. Am. J. Physiol. - Cell Ph. 281 (1): C1 - C11.

Tarze A, Deniaud A, Le Bras M, Maillier, E, Molle D, Larochette N, Zamzami N, Jan G, Kroemer G, Brenner C (2007). GAPDH, a novel regulator of the pro-apoptotic mitochondrial membrane permeabilization. Oncogene, 26 (18): 2606–2620.

Usui Y, Uematsu T, Uchihashi T, Takahashi M, Takahashi M Ishizuka M, Doto R, Tanaka H, Komazaki Y, Osawa M, Yamada K, Yamaoka M, Furusawa K (2010). Inorganic polyphosphate induces osteoblastic differentiation. J. Dent. Res., 89(5): 504 -509.

Van den Bosch H, Schtgens RB H, Wanders RJA, Tager JM (1992). Biochemistry of peroxisomes. Annu. Rev. Biochem., 61: 157-197.

Vannini L, Ciolfi S, Spinsanti G, Panti C, Frati F, Dallai R **(**2010). The putative-farnesoic acid O-methyl transferase (FAMeT) gene of *Ceratitis capitata*: characterization and pre-imaginal life expression. Arch. Insect Biochem., 73 (2):106-117.

Wahlberg N, Leneveu J, Kodandaramaiah U, Pena C, Nylin S, Freitas AV, Brower AV (2009). Nymphalid butterflies diversify following near demise at the Cretaceous/Tertiary boundary. Proc. Biol. Sci., 276 (1677): 4295-4302.

Yao, HP, Xiang XW, Chen L, Guo AQ, He FQ, Lan LP, Lu XM, Wu XF (2009). Identification of the proteome of the midgut of silkworm, *Bombyx mori* L. by multidimensional liquid chromatography (MDLC) LTQOrbitrap MS, Bios. Rep., 29: 363–373.

Yuan C**,** Ding X**,** Xia L**,** Yin J**,** Huang S**,** Huang F (2011). Proteomic analysis of BBMV in *Helicoverpa armigera* midgut with and without Cry1Ac toxin treatment. Bio. Sci. Techn., 21: 139-151

Xu J, Nie Z, Chen J, He P, Wang D, Lv Z, Jiang C, Liu L, Wu X, Zhang Y (2008). The construction of putative protein database from cDNA library of silkworm pupa and verification by LC/MS/MS, direct submission.

Zhang S, Xu Y, Fu Q, Jia L, Xiang Z, He N (2011). Proteomic analysis of larval midgut from the silkworm (*Bombyx mori*). Comp. Funct. Genomics. Article ID 876064, 1-13.