Pectinases From Sphenophorus levis Vaurie, 1978 (Coleoptera: Curculionidae): Putative Accessory Digestive Enzymes

Danilo Elton Evangelista,1 Fernando Fonseca Pereira de Paula,1 André Rodrigues,2 and Flávio Henrique-Silva1,3

1Laboratory of Molecular Biology, Department of Genetics and Evolution, Federal University of São Carlos, Road Washington Luís Km 235, São Carlos, 13565-905 São Paulo, Brazil
2Department of Biochemistry and Microbiology, UNESP-São Paulo State University, Av. 24A, n. 1515-Bela Vista, Rio Claro, São Paulo 13506-900, Brazil
3Corresponding author, e-mail: dfhs@ufscar.br

ABSTRACT. The cell wall in plants offers protection against invading organisms and is mainly composed of the polysaccharides pectin, cellulose, and hemicellulose, which can be degraded by plant cell wall degrading enzymes (PCWDEs). Such enzymes are often synthesized by free living microorganisms or endosymbionts that live in the gut of some animals, including certain phytophagous insects. Thus, the ability of an insect to degrade the cell wall was once thought to be related to endosymbiont enzyme activity. However, recent studies have revealed that some phytophagous insects are able to synthesize their own PCWDEs by endogenous genes, although questions regarding the origin of these genes remain unclear. This study describes two pectinases from the sugarcane weevil, Sphenophorus levis Vaurie, 1978 (Sl-pectinases), which is considered one of the most serious agricultural pests in Brazil. Two cDNA sequences identified in a cDNA library of the insect larvae coding for a pectin methyl esterase (PME) and an endo-polygalacturonase (endo-PG)—denominated Sl-PME and Sl-endoPG, respectively—were isolated and characterized. The quantitative real-time reverse transcriptase polymerase chain reaction expression profile for both Sl-pectinases showed mRNA production mainly in the insect feeding stages and exclusively in mid gut tissue of the larvae. This analysis, together Western blotting data, suggests that Sl-pectinases have a digestive role. Phylogenetic analyses indicate that Sl-PME and Sl-endoPG sequences are closely related to bacteria and fungi, respectively. Moreover, the partial genomic sequences of the pectinases were amplified from insect fat body DNA, which was certified to be free of endosymbiotic DNA. The analysis of genomic sequences revealed the existence of two small introns with 53 and 166 bp in Sl-endoPG, which is similar to the common pattern in fungal introns. In contrast, no intron was identified in the Sl-PME genomic sequence, as generally observed in bacteria. These data support the theory of horizontal gene transfer proposed for the origin of insect pectinases, reinforcing the acquisition of PME genes from bacteria and endo-PG genes from fungi.

Key Words: plant cell wall degrading enzyme, insect pectinase, pectin methylesterase, endo-polygalacturonase, horizontal gene transfer

The cell wall in plants offers structural support to the cell and protection against pathogens and phytophagous organisms. Pectic substances are the major component of the middle lamella and are composed of partially methyl-esterified galacturonic acid residues linked by α-1,4 glycosidic bonds (Kashyap et al. 2001). These substances are naturally degraded by pectinases. Pectin methyl esterase (PME) is responsible for the removal of methyl-ester groups, and endo-polygalacturonase (endo-PG) is responsible for the random hydrolysis reaction of α-1,4 bonds (Reignault et al. 1994, Crelier et al. 2001).

Pectinases and other plant cell wall degrading enzymes (PCWDEs) have been extensively studied in plants, bacteria, and fungi. These enzymes constitute an arsenal that can determine the virulence of pathogens (Rogers et al. 2000). A wide range of microorganisms that produce PCWDEs live in symbiotic relationships in the gut of certain insect species, supplementing the nutritional capacity of the host (Calderon-Cortes et al. 2012). Thus, until a few years ago, all PCWDEs found in insect sources were believed to have an endosymbiotic origin. However, studies have shown that some invertebrates, including insects, can synthesize these enzymes by endogenous genes (Watanabe et al. 1998; Girard and Jouanin 1999; Watanabe and Tokuda 2001, 2010; Allen and Mertens 2008; Celorio-Mancera Mde et al. 2009; Willis et al. 2011).

The first insect pectinases described were a PME and an endo-PG, initially purified from extracts of entire adult specimens of the rice weevil (Sitophilus oryzae) (Shen et al. 1996, 1999). Since this discovery, pectinolytic enzymes from phytophagous beetles have been identified in several representative members of the superfamilies Chrysomeloidae and Curculionoidae (Pauchet et al. 2010, 2014). Pectinases and other PCWDE genes have been identified in several herbivorous insect species with distinct feeding habits, which suggests the involvement of PCWDEs in the evolution of plant–insect interactions (Calderon-Cortes et al. 2012).

In Brazil, the sugarcane weevil (Sphenophorus levis) is one of the most economically important agricultural pests from the family Curculionidae. The larvae of this beetle feed on the sugarcane rhizome and stem base, building galleries that eventually cause the death of the plant, which leads to a reduction in productivity of one of the most important agricultural crops in the country (Cerda et al. 1999). Unfortunately, there are no effective methods for controlling this coleopteran. Thus, our group has built and sequenced a cDNA library from S. levis larvae (F.H.S, unpublished data) to identify new molecular strategies for the biotechnological control of this insect. Sequence analyses have revealed a single full-length PME (GenBank: KF697077) and an endo-PG (GenBank: KF697078) denominated Sl-PME and Sl-endoPG, respectively. The genomic coding sequences of these enzymes were characterized, and gene expression analysis by real-time reverse transcriptase polymerase chain reaction (qRT-PCR) was performed in different developmental stages as well as different larval tissues. Phylogenetic analyses were also performed to investigate the evolutionary relationships of both gene families.

Materials and Methods

Clone Isolation and Characterization. Clones were obtained from an S. levis cDNA library constructed from an RNA of a pool of larvae...
reaching the pupal stage (~30-d-old larvae) using the CloneMiner kit (Invitrogen, CA) and 5'-sequenced using DYEnamic ET Dye Terminator Kit in a MegaBACE 1000 Automatic Sequencer (GE Healthcare, USA). After data processing and the assembly of clusters in the dCAS platform (Guo et al. 2009), the pectinase clones were identified using BLASTX and tBLASTX (http://www.ncbi.nlm.nih.gov/blat). The clones were sequenced entirely and the amino acid-deduced sequences were analyzed in the SIGNALP 4.0 (Petersen et al. 2011), NetOGlyc 3.1 (R. Gupta, E. Jung, and S. Brunak, unpublished data), and NetOGlyc 1.0 programs (Julenius et al. 2005).

**Multiple Sequence Alignment and Phylogenetic Analyses.**

Multiple alignment was carried out using homologous sequences selected from the NCBI-GenBank database with the aid of the Multalin program (Corpet 1988) with default settings. The sequences were selected to investigate the evolutionary trends of PMEs and endo-PGs using organisms from distinct taxa. Analyses were performed using 36 PME sequences and 34 endoPG sequences.

To infer evolutionary relationships, multiple alignments were carried out in the MUSCLE program, version 3.8.31 (Edgar 2004a,b), using default parameters and the same dataset. Phylogenetic analyses were performed in MEGA 5.0 (Tamura et al. 2011) using the neighbor joining method (Saitou and Nei 1987) and the Poisson correction model. Regions with gaps and missing data were excluded from the analysis. The robustness of the tree was assessed by 1,000 bootstrap pseudoreplicates. The final graphic representation of the phylogenetic tree (Figs. 2 and 3) was created in Adobe Illustrator v. 6.0.

**Analysis of Relative Gene Expression of SI-Pectinases.**

The analysis of the transcript levels of the SI-pectinases genes was performed as described by Fonseca et al. (2012). Seven development stages of *S. levis* and six different tissues from 30-d-old larvae were analyzed. RNA was extracted from eggs, larvae 10 d after eggs hatching, 20-d-old larvae, 30-d-old larvae, prepupae, pupae, and female adult insects using the Trizol reagent (Invitrogen, CA). RNA integrity was verified in a 1.5% agarose gel, and quantification was performed in a Nanodrop water for a final volume of 25 μl. 5 μg of insect protein extracts and 0.25 μl of the purified recombinant enzyme (positive control) were used in Western blotting. The samples were separated for 2 h at 150 mA in 12% SDS-PAGE and then transferred to a PVDF membrane by electroblotting in transfer buffer (200 mM of Tris-HCl, 50 mM of glycine and 20% methanol). The membrane was incubated overnight in a blocking solution containing 5% defatted milk in TBS (50 mM of Tris-HCl, pH 8.0, 150 mM of NaCl) and washed with 1X TBS. Next, the membrane was incubated for 2 h with the primary specific antibody raised in mice (1:2,500), washed in TBS, and then incubated for 90 min with the secondary antibody Anti-Mouse IgG conjugated with the alkaline phosphatase (Sigma-Aldrich Inc., MO) (1:1,000), washed with 1X TBS, and revealed with the NBT-BCIP (Thermo Scientific Pierce, IL) substrate for alkaline phosphatase.

**Genomic DNA Analysis.**

Genomic DNA was extracted from the fat body of *S. levis* larvae to investigate the presence of introns. To prevent contamination from an exogenous source (especially endosymbionts in the gut), the portion of this tissue that was not in direct contact with the integument and midgut was used. Approximately 2–3 mg of tissue was solubilized in 700 μl of saline buffer (150 mM of NaCl, 25 mM of ethylenediaminetetraacetic acid, 0.2% SDS) and treated with 15 μl of proteinase K (10 mg/ml) followed by 5 h of incubation at 60°C and 300 rpm. In total, 400 μl of phenol:chloroform:isoamyl alcohol (24:24:1) was added to the resulting solution, and this mixture was incubated at 37°C and 300 rpm for 30 min. After 5 min of centrifugation at 15,000 × g, the supernatant was transferred to a new tube and precipitated with ethanol, dried, resuspended in 0.1 mg/ml of RNAse, and kept at 37°C for 30 min. DNA was quantified using a NanoDrop spectrophotometer, and integrity was verified by 1% agarose gel electrophoresis. To certify that the DNA samples were indeed free of contamination by endosymbiotic DNA, a simple diagnosis test was performed by PCR using 16S and internal transcribed spacer (ITS) ribosomal primers. ITS1 and ITS4 (Piterina et al. 2010) primers were used to amplify DNA extracted from the fat body, genomic DNA samples extracted from the gut (as the positive control), and a reaction without DNA (as the negative control). For ITS analysis, genomic DNA extracted from the yeast *P. pastoris* was used as an extra positive control. PCR were carried out using 50 ng of the DNA, 0.2 mM of dNTPs (Invitrogen, CA), 1 μl PCR buffer (20 mM of Tris HCl, pH 8.4, 1.5 mM of MgCl2 and 30 mM of KCl), 0.4 μM of each primer, 1 U of Taq DNA polymerase (Invitrogen, CA), and milli-Q water for a final volume of 25 μl. The PCR cycling conditions were...
94°C for 8 min, followed by 35 cycles of 1 min at 94°C, 45 s at 51°C, 5 min at 72°C, and a final step of 10 min at 72°C. The fragments were analyzed in a 1% agarose gel for the presence or absence of amplicons for the 16S region and the size of amplicons in the ITS region.

The presence of introns in the pectinase genes was verified by the amplification of the genes using different combinations of the primers employed for cloning in a pPICZα vector and qRT-PCR. cDNA synthesized from RNA from 20-d-old larvae was used as nonintron control. PCR was conducted as described above. The amplicons were analyzed in a 1.5% agarose gel stained with ethidium bromide. Genomic fragments were excised from the gel, purified, sequenced, and compared with the respective ORFs.

Results and Discussion

Characterization of Sl-Pectinase Sequences. The full-length ORF of Sl-PME is composed of 1,158 bp coding a 386-amino acid polypeptide, including a putative 16-residue signal peptide. Sl-endoPG exhibited 1,092 bp coding a 364-amino acid polypeptide, also including a putative signal peptide of 19 residues (Fig. 1). The analysis of mature amino acid sequences, excluding the peptide signal sequence, revealed a predicted 36-kDa protein with an isoelectric point of 8.77, four N-glycosylation sites (182NSSG185, 212NLTC215, 239NVTF241, and 340NWSG343), and no O-glycosylation site (Fig. 1). The presence of a signal peptide at the N-terminus of both S. levis pectinases and glycosylation sites indicates that these are secreted proteins. The features were verified in PCWDEs from other insects (Pauchet et al. 2010), as expected for digestive enzymes.

Inference of the Occurrence of Insect Pectinases. The alignment showed in Supp Fig. S1 (online only) demonstrates the high similarity among insect endo-PGs and, particularly, that the insect group is closely related to the fungal group. Indeed, several amino acid residues are common in all insect and fungal sequences but are not found in bacteria or plant sequences. An extended N-terminus has been found only in bacterial and plant endo-PGs. Studying 43 homologous sequences, Cho et al. (2001) found this extension in all 17 plants and five bacteria analyzed, whereas all 21 fungal endo-PGs lacked this feature. In addition, although less frequent, a long C-terminal extension was also only found in bacteria and plants but not in fungi, except Claviceps purpurea (Van Santen et al. 1999, Cho et al. 2001).

Another feature shared only by insects and fungi is the presence of eight conserved cysteine residues distributed along the primary structure of the enzyme responsible for the formation of four disulfide bonds (Cys27-Cys42, Cys197-Cys215, Cys331-Cys336, and Cys355-Cys364) (Van Santen et al. 1999, Cho et al. 2001). Bacterial and plant endo-PGs may also have disulfide bonds, but the cysteine residues positions are not conserved as in insects and fungi (Pickersgill et al. 1998, Cho et al. 2001). Except in Ph. cochleariae, all insect endo-PGs have two extra cysteine conserved residues (Cys142 and Cys146) that enable other disulfide bonds (Pauchet et al. 2010). These exclusive two extra cysteine conserved residues in the insect group strongly indicate that endo-PG sequences are not derived from endosymbiotic contamination and, therefore, are indeed derived from the insect genomes.

<table>
<thead>
<tr>
<th>Sl-PME</th>
<th>Sl-endoPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 MKIVLELVLVACACA</td>
<td>1 MLLVASYLLVLVARAYA</td>
</tr>
<tr>
<td>101 NTVPILYIAGSRPDTNTVILMPAQTPASAYNLVNPNDAFFKPGPA</td>
<td>101 TVPKAGAKMLKSSGGT7VSKGATIKFAVSEKGPLVEISGKIFSGAGG</td>
</tr>
<tr>
<td>151 YSIYNGCASSSTIGTSTCSTVFNLAPVQIVNLQNSAKKEDQQA</td>
<td>151 YFDDQGASWYGDIQGGKTIIKFKKFTIKTTGSSHNNKLLNCLHCQVCIN</td>
</tr>
<tr>
<td>201 LQN5RDIQVINNVLHGQTHDGTCAGGSSGTDTQIAHTYNTYIEGDIDYVFG</td>
<td>201 PASDTILTWDVSAAGSDKGKNDTFGDLSNSGTTIKHAYVVKQDCOV</td>
</tr>
<tr>
<td>251 G7TAFESCFTYKSKADKNDSEVFAPFDTDHQMYGLVTIKSTTDASAN</td>
<td>251 AVNQGSHYFONLTCSSGHLSLVSQVQSSGNPNTVKNLTSCTVNTS</td>
</tr>
<tr>
<td>301 SSSKVGLRSDWAGVKSANAYVFGTSPENQLVIRETCTINGLVFNSAFWT</td>
<td>301 RNIHVTINHDAHTGAISDITVYKNIKLSGITNYHGNNQEDYENGGGSGNP</td>
</tr>
<tr>
<td>351 TALSRTGGNANATRDLNQNFREVEYANTGS</td>
<td>351 KNPFIOSHNLQVTVGSMSGGSSMPYLYLCNSGCNKNHNSGSVSGSNK</td>
</tr>
</tbody>
</table>

Fig. 1. Predicted primary structure of Sl-pectinases. Deduced amino acid sequences of Sl-PME and Sl-endoPG. Residues in bold indicate putative peptide signals for extracellular secretion and underlined residues refer to probable glycosylation sites.
Figure 2 displays the SI-PME tree inferred from the amino acid sequences of PMEs from several different organisms. This analysis suggests that bacterial PMEs, unlike what occurs with most of the taxa, did not originate from a unique episode of genetic introgression, demonstrating a polyphyletic origin with at least three different emergence events. Moreover, the results show that insect PMEs constitute a single clade among two splitting bacterial groups, suggesting a close phylogenetic relationship between the PMEs of insects and bacteria.

Figure 3 displays the SI-endoPG tree inferred from amino acid sequences of endo-PGs from several different organisms. This phylogenetic analysis reveals that insect endo-PGs form a cohesive group associated with two fungal sister groups (ascomycetes and basidiomycetes). However, the endo-PGs from insects are closer to those of ascomycetes than basidiomycetes. Thus, insect endo-PGs may have originated from ascomycete endo-PGs through a horizontal transfer event.

The first phylogenetic studies on insect pectinases were performed in *S. oryzae* by Shen et al. (2003, 2005). The authors identified a PME and an endo-PG by Southern blotting using DNA extracted from the sterile legs of the beetle and demonstrated that these genes are indeed integrated into the insect genome. The authors also verified the close proximity between the insect and bacterial PME as well as the close proximity between insect and fungal endo-PG. Neither PME nor endo-PG activity have been identified in primitive animal taxa, suggesting a horizontal gene transfer origin from bacteria for PME and from fungi for endo-PG. The same phylogenetic relationship has been found for three endo-PGs from *Lygus lineolaris* (Allen and Mertens 2008). Therefore, the present results, which are supported by a larger, more diverse amount of analyzed sequences, furnish evidence corroborating the theory regarding the origin of PME and endo-PG in insects through horizontal transfer events.

Investigation of Introns in SI-Pectinase Genes. To investigate possible introns in SI-pectinases and clarify their prokaryotic or eukaryotic origin, the genes were amplified from the genomic DNA extracted from the fat body (Supp Item 1 and Fig. S3 [online only]) and compared with the respective amplicons obtained from cDNA. Differences between genomic amplicons and cDNA amplicons were observed for SI-endoPG (Fig. 4 B). Primer combination 3 generated an expected fragment with 100 bp from the cDNA sample and a 240 bp fragment for the genomic sample, indicating the presence of an intron of 150 bp. The amplicons generated using primer combination 4 also exhibited a difference in size, since the fragment obtained from genomic DNA (KF697076) was about 800 bp and the cDNA fragment was about 600 bp. Therefore, two introns were expected for the region flanked by primer combination 4: one of about 150 bp evidenced by combination 3 and a smaller one close to 50 bp.

The presence of the two deduced introns was confirmed by the sequencing of these genomic fragments (Fig. 4 C). The two introns in the coding sequence of SI-endoPG had 166 bp (509–675 bp) and 53 bp (1,003–1,056 bp), respectively. These introns exhibited the typical 5'-GT at the beginning and a 3'-AG at the end (Wu and Krainer 1999) (Fig. 4 C). As is well known, introns are very rare in prokaryotic organisms in contrast to fungi, in which introns are quite common. Therefore, the presence of introns in the SI-endoPG gene, but not in the SI-PME gene is additional evidence supporting the theory regarding the origin of insect pectinases.
Fig. 3. Phylogenetic analysis of endo-PGs. Phylogenetic reconstruction of 34 polygalacturonases from plants, fungi, bacteria, and insects performed using neighbor-joining method. Numbers in branches indicate bootstrap percentage values after 1,000 replicates. GenBank accession numbers are shown adjacent to each enzyme and *S. levis* is in bold.

Fig. 4. Pectinase fragments amplified from *S. levis* cDNA and genomic DNA. PCR conducted with primers used for cloning ORFs in pPICZα vectors and primers for qRT-PCR analysis. (A) Representative scheme of primer position on coding sequence of mature Sl-pectinases. (B) Gel separation of fragments generated by PCR; M, GeneRuler ladder 1 kb (Thermo Scientific Fermentas, CA); 1–4, primer combinations; I, reaction with genomic DNA template; II, reaction with cDNA template; III, negative control. (C) Alignment between Sl-endoPG sequences from both genomic and cDNA samples, showing two small introns. Sequences underlined in white indicate forward and reverse primers used for sequencing of genomic Sl-endoPG. Asterisks indicate 5’ and 3’ of two introns identified.
mRNA Expression Analysis. To improve the understanding of the physiological role of *S. levis* pectinases, seven developmental stages and tissues from 30-d-old larvae were analyzed by qRT-PCR. Both gene transcript levels were estimated by comparison to transcript levels of the GAPDH gene. Figure 5A and C show the expression pattern of the Sl-PME and Sl-endoPG gene transcripts, respectively, throughout the lifecycle of the insect. The highest Sl-PME expression was found in adults (0.481), followed by the larval stages, in which high transcript levels were detected. In contrast, very low transcripts levels were found in eggs as well as in the prepupal and pupal stages, suggesting a probable basal transcription level. The highest Sl-endoPG transcript levels were detected in the larval stage, with a peak (0.708) in 20-d-old larvae, followed by the 30-d-old larvae (0.350) and the 10-d-old larvae (0.320). Once again, low expression was found in eggs and prepupae. Moreover, similar values were found in pupae and adults, which were much lower than in the larval stages.

Both Sl-PME and Sl-endoPG transcripts are detected in feeding stages, which is expected for digestive enzymes. A similar pattern has been observed for the main digestive enzyme in *S. levis* larvae—a cathepsin L cysteine peptidase (Sl-CathL) (Fonseca et al. 2012). Different levels of Sl-pectinase gene expression were observed between larvae and adults. The predominance of pectinase mRNAs in the larval stage may be explained by different feeding habits and plant attack mechanisms, as adult insects use the rostrum to suck sugarcane sap instead of opening galleries as larvae do (Fonseca et al. 2012). The reasons for Sl-PME transcript detection in adults are not clear, but, as all adult insects analyzed were females, Sl-PME may be related to the invasion process and digging a cavity for egg deposition, since prior action of PME increases the endo-PG activity in methylated substrates.

Immunodetection of *S. levis* Pectinases by Western Blotting. Western blotting was performed using the same tissues analyzed by qRT-PCR to determine whether mRNA produced in the midgut indeed promotes Sl-pectinase synthesis and to detect the tissues in which these proteins are present. Native Sl-PME was detected in the midgut and hindgut tissues, with molecular mass of about 45 kDa (Fig. 6). The slight difference in molecular mass between the native Sl-PME and recombinant Sl-PME (which has ~50 kDa) is explained by extra residues at the C-terminus (15 residues from the c-myc epitope and 6 kDa).

Sugarcane is one of the major natural supplies of soluble sugars, such as sucrose, glucose, and fructose. These carbohydrates are easily assimilated by cells as an energy source because they are simple molecules the metabolism of which requires a lower level of energy, unlike the pectin complex, which is an insoluble long-chain molecule. In this context, it would be energetically infeasible for the insect to use pectinases to obtain energy from pectin in a diet rich in easily assimilated sugars. Moreover, no exo-polygalacturonases (Exo-PG) sequences were found in the cDNA library for *S. levis*. In fact, to the best of our knowledge, no Exo-PG has been identified in insects. Exo-PG and endo-PG catalyze the hydrolysis of α-1,4 glycosidic bonds between galacturonic acid monomers and also have enhanced activity after the prior action of PME (Crelier et al. 2001). However, Exo-PG acts sequentially on terminal monomers of the chain, releasing them as products (Kashyap et al. 2001). It is plausible to infer that endo-PG is more efficient at disrupting the main pectin chain than creating free sugar monomers as an energy source. Therefore, we suggest that Sl-pectinases are accessory digestive enzymes, the role of which is directly related to the disorganization of the cell wall to gain access to intracellular nutrients in the plant. The synergic PME and endo-PG activities could contribute to the breakdown of the cell, allowing access to the intracellular content by other digestive enzymes.
Fig. 6. Immunodetection of SI-PME in tissues of S. levis larvae. Immunodetection of SI-PME performed by Western blotting using polyclonal antibodies. M, Benchmark molecular weight marker (Invitrogen, CA); P, purified recombinant SI-PME, head—hindgut, total protein extracted from indicated tissues.

References Cited

residues of His-tag) added from the expression vector and the glycosilation of recombinant SI-PME by P. pastoris. The synthesis of SI-PME mRNA in the midgut was expected, but no gene expression was detected in the hindgut, which suggests that this protein is carried to the hindgut during the digestion process. The same analysis was conducted for SI-endoPG (data not shown), but the native enzyme was not detected, probably due to low specificity of the polyclonal antibody generated against it. However, considering the SI-endoPG expression in the midgut and the fact these two pectinases act synergistically, it is probable that SI-endoPG also acts in the midgut.

The present results provide evidence that SI-pectinases are indeed integrated into the S. levis genome and corroborate the hypothesis put forth by Shen et al. on horizontal gene transfer for insect pectinases. SI-pectinases are expressed in all feeding stages, possibly as important accessory digestive enzymes acting in the midgut and contributing to the efficient herbivory of S. levis. Therefore, we suggest these enzymes as future targets for the control of this insect in sugarcane plantations.

Acknowledgments

This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grant # 98/14138-2. Flávio Henrique-Silva and André Rodrigues are research fellows from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).


Received 13 November 2013; accepted 24 November 2014.