Cysteine Cathepsins in Beetles: Vulnerable Targets for Insect Control?

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ABSTRACT

Nearly half of all species on earth are beetles, including many of the most problematic pests in agriculture. To digest protein, many beetles use cysteine cathepsin enzymes. In the tenebrionid beetles *Tenebrio molitor* and *Tribolium castaneum* (right), protein digestion begins with cysteine cathepsins found in the acidic anterior part of the larval midgut. Therefore, cysteine cathepsins are critical components of the digestive process in tenebrionid larvae. To explore the potential for new beetle control products based on cysteine cathepsin inhibitors or disruptors, our laboratory has used genomics, proteomics, transcriptomics, and bioinformatics to study tenebrionid digestive cysteine cathepsins. In the annotation of the *T. castaneum* genome, cysteine cathepsins were found in four major clusters expanded in five gene families. A microarray and proteomic analysis revealed that genes encoding three cathepsin L-like and two cathepsin B-like enzymes were highly expressed in the *T. castaneum* larval gut. Lacking a genome for *T. molitor*, we used targeted and high-throughput sequencing of the *T. molitor* larval gut to reveal that cathepsin L genes are expressed at higher levels than cathepsin B genes. Gene expression analyses in tenebrionid larvae demonstrated that cysteine cathepsins are differentially expressed in larvae exposed to cysteine protease inhibitors or toxins from *Bacillus thuringiensis*. In response to either dietary stressor, larvae generally decreased production of genes encoding cathepsin L-like enzymes and increased production of genes encoding cathepsin B-like enzymes. The data support the importance of cysteine cathepsins for protein digestion in tenebrionid larvae and suggest that these enzymes are good targets to inhibit feeding and subsequent damage to cereals.

GENOME ANNOTATION

Table 1. Genes encoding predicted cysteine proteases (CP) in the *T. castaneum* genome are members of the C1 cysteine peptidase family, clan CA, and features of active site and/or critical residues are listed, with a putative identification of functional activity. Those on the same linkage group were found in tandem, suggesting an expansion of at least 5 separate gene families. Yellow highlighted sequences were determined to be highly expressed in the gut by gene expression microarray analysis and whole gut proteomics (Data from references 1 and 2).

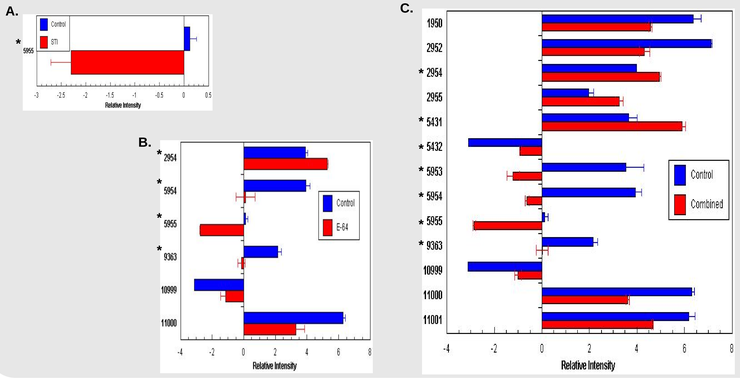
Annotated Gene #	Linkage Group	Drosophila Ortholog	Active Site Residues	Critical Residues	EST Support	Putative Functional Activity
01950	10	CG6692-PC	QCHN	n/a	partial	cathepsin L
02843	3	CG6692-PC	QCHN	n/a	partial	cathepsin L
02952	3	CG10992-PA	QCHN	HH	full	cathepsin B
02953	3	CG10992-PA	QCHN	HH	partial	cathepsin B
02954	3	CG10992-PA	QCHN	KG	full	cathepsin B homolog
02955	3	CG10992-PA	QCHN	HH	full	cathepsin B
05431	8	CG10992-PA	QCHN	NS	partial	cathepsin B homolog
05432	8	CG10992-PA	QCHN	DS	none	cathepsin B homolog
05953	8	CG10992-PA	QCHN	DG	none	cathepsin B homolog
05954	8	CG10992-PA	QSTN	R-	none	cathepsin B homolog
05955	8	CG10992-PA	QCSN	YA	none	cathepsin B homolog
05956	8	CG6692-PC	QCHN	n/a	none	cathepsin L
07214	4	CG12163-PB	QCHN	n/a	none	cathepsin O
09217	7	CG3074-PB	QSHN	CR	full	cathepsin B homolog
09362	7	CG6692-PC	QCHN	n/a	full	cathepsin L
09363	7	CG4847-PA	-CHN	n/a	none	cathepsin L homolog
09364	7	CG6692-PC	QCHN	n/a	none	cathepsin L
09365	7	CG6692-PC	QCHN	n/a	full	cathepsin L
09448	7	CG6692-PC	QCHN	n/a	full	cathepsin L
10999	10	CG6692-PC	QCHN	n/a	one	cathepsin L
11000	10	CG4847-PD	QCHN	n/a	full	cathepsin L
11001	10	CG6692-PC	QCHN	n/a	full	cathepsin L
11002	10	CG6692-PC	ESHN	n/a	full	cathepsin L homolog
11003	10	CG6692-PC	QCHN	n/a	full	cathepsin L
13582	5	CG5367-PA	QCHN	n/a	none	cathepsin K

GENE EXPRESSION STUDIES

Table 2. Comparison of uniESTs encoding CPs from the gut of *T. molitor* larvae fed a control diet or one with 0.1% *Bacillus thuringiensis* toxin Cry3Aa.

Gene	Control			Bt-treated		
	Contig #	# Reads	Total	Contig #	# Reads	Total
Cathepsin B	240	44		249	18	
	890	15		871	27	
	8975	40		1453	19	
	9310	26	125	8237	85	149
Cathepsin L	9	53		111	31	
	1354	51		1497	58	
	8897	627	731	7528	87	
				7583	324	
				7886	25	525

Fig. 1. When we feed *T. castaneum* larvae protease inhibitors (PI), they have different effects on the larvae (data not shown) and CP gene expression. Larvae fed 5% soybean trypsin inhibitor (STI, a serine protease inhibitor) demonstrate normal growth and development, and only one CP gene is downregulated (A). Larvae fed 0.1% E-64 (a cysteine protease inhibitor) grow slower, and more CP genes are affected (B). However, larvae fed a combination of 5% STI and 0.1% E-64 are stunted and eventually die; many CP genes are differentially expressed in these larvae (C), suggesting that larvae are attempting to compensate for PI in the diet. Protease "homologs", those that are predicted to be nonfunctional proteases based on the lack of homology in critical residues in the predicted protein (noted with stars), are numerous among the differentially expressed genes. In some cases, gene expression increases in PI-treated larvae, usually in cathepsin-B like genes or their homologs.



CONCLUSIONS

In summary, we have studied CP in tenebrionid insects to develop control methods based on disrupting digestion through inhibitors that target digestive enzymes. These data (and other presented elsewhere) demonstrate the following:

- In tenebrionid insects, the initial stages of digestion occur in the acidic anterior part of the midgut, where mostly CP are active.
- Digestive CP in tenebrionid insects are cysteine cathepsins B and L.
- Under normal dietary conditions, genes encoding cathepsin L are expressed at higher levels than those encoding cathpesin B (also true at the protein level).
- When tenebrionid larvae are fed a diet containing a microbial toxin (i.e., *B. thuringiensis* Cry3Aa) or PIs (i.e., STI and E-64), they respond by decreasing production of cathepsin L and increasing production of cathepsin B, a compensation response whereby insects shift protease production in response to inhibitors. Of interest is the number of genes encoding protease homologs that are involved in the compensation response, yet to be fully understood.
- Understanding how insects respond to toxins and inhibitors help us to understand the compensation response.

The data suggest that inhibitors of cathepsin B may be useful to combine with microbial toxins or other PIs as part of a integrated pest management strategy to control tenebrionid pests in agriculture.

References

1. *Tribolium* Genome Sequencing Consortium. 2008. The genome of the model beetle and pest *Tribolium castaneum*. Nature 452: 949-955.

2. Morris, K. M., Hiromasa, Y., Tomich, J. M., Oppert, C., Elpidina, E. N., Vinokurov, K., Lorenzen, M., Jurat-Fuentes, J. L., Fabrick, J. and Oppert, B. 2009. *Tribolium castaneum* larval gut transcriptome and proteome: A resource for the study of the coleopteran gut. J. Proteome Res. 8: 3889-3898.