



Expression patterns of S1 family serine peptidases across *Tribolium castaneum* life cycle

Kosimov M.N.

Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University;

Email: mihamsik00@gmail.com



Introduction

Serine peptidases (SPs) of the chymotrypsin S1 family is a group of peptidases that catalyze the peptide bond cleavage reaction which allows them to participate in digestive and protective processes. Catalytic activity of SPs is performed by His/Asp/Ser catalytic triad and substrate-specificity depends on the structure of the S1 substrate-binding subsite.

One of the model organisms for the study of SPs is *Tribolium castaneum*, a major storage products pest responsible for considerable loss of stored grains worldwide. For *T. castaneum*, S1 serine peptidases are important components of the digestive system. The cases of *T. castaneum* resistance to most control products have been identified so it is essential to develop alternative control strategies based on inhibition of the most important SPs. In order to determine most highly expressed enzymes and classify them, the differential expression analysis and further alignment of the detected SPs was performed.

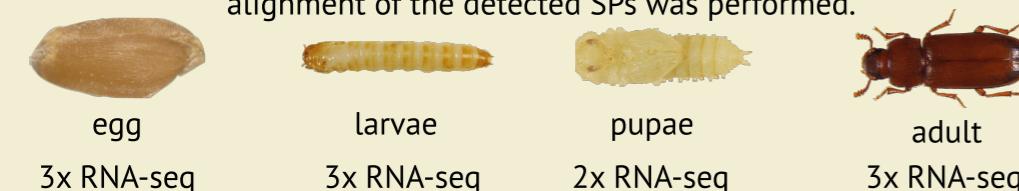


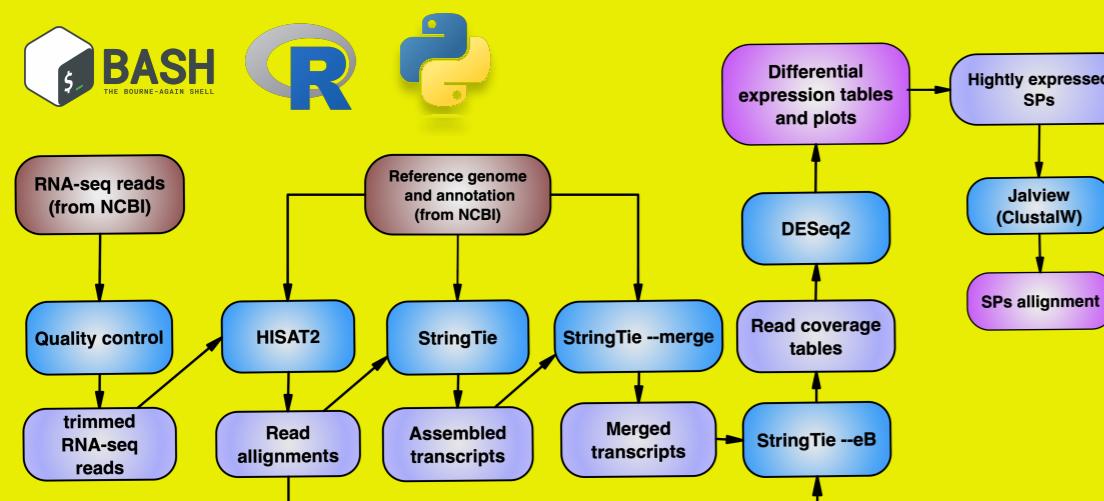
Figure 1. Life stages of *T.castaneum*

Picture credits:<https://www.grainscanada.gc.ca/en/grain-quality/manage/identify-an-insect-primary-insect-pests/red-flour-beetle.html>

Aims

Assembly of the transcriptome and differential expression analysis of *T.castaneum* transcripts corresponding to SPs in order to find the most highly expressed ones, predict the functions of enzymes and classify them based on catalytic triad and S1 substrate-binding subsite.

Methods



Raw reads were trimmed (nucleotide quality > 10, read length > 20bp) and genome guided transcriptome assembly for 11 samples was performed. Samples were subsequently merged. The loss of reads over these steps is depicted in Fig 2.

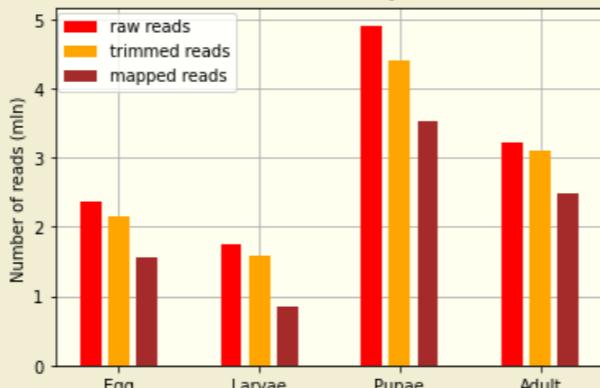


Figure 2. Average reads loss after trimming and mapping steps for each stage

Given the merged transcriptome and alignments, expression of transcripts was evaluated and a table of normalized counts was obtained for a PCA based comparison of samples (Fig. 3) and for SPs expression comparison at different stages.

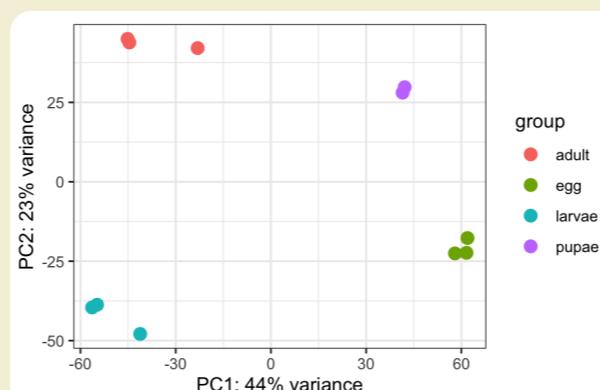


Figure 3. PCA based comparison of transcriptome data

Results

SPs with high levels of gene expression at the feeding stages and low at others are evident (Fig. 4A), and this group is the most prevalent. These SPs may function as digestive enzymes. There are also SPs genes which are mostly expressed during the pupa (Fig.4B) and egg (Fig.4C) stages. These SPs might participate in *T.castaneum* metamorphosis.

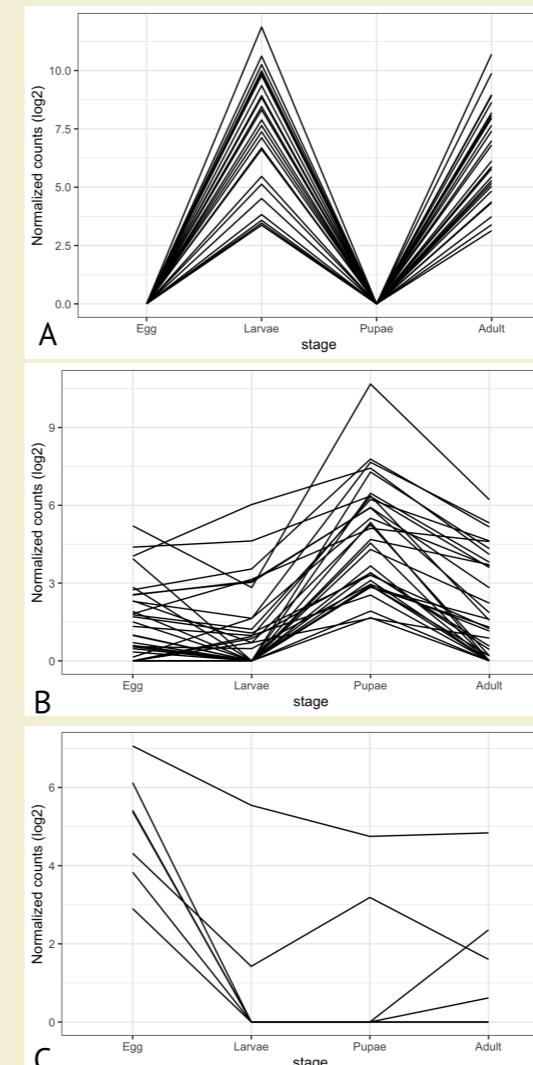


Figure 4. Highly expressed SPs at A) larvae and adult B) pupae and C) egg stages

These potentially crucial for *T.castaneum*'s life cycle SPs were multiply aligned (Fig. 5) and further classified based on their substrate-binding subsite and whether they have canonical His/Asp/Ser catalytic triad or there are replacements indicating its SPs homologs.

In a given alignment, 27 homologs and 4 polypeptidases are evident and it is unknown whether homologs have catalytic activity or not. Others were classified based on S1 subsite. (Table 1)



Figure 5. Highly expressed SPs multiple alignment with catalytic triad and S1 subsite being colored

Type of peptidase	S1 subsite	SPs
Trypsin	DGG	13
Trypsin-like	DGA	2
Chymotrypsin	SGS, SGA, SGG	1
Elastase	GIS, SVS, GVS	1
Collagenase	GGD	3
Homologs	-	27
Polypeptidase	-	4
not annotated	-	10
Overall	-	61

Table1. SPs classification based on S1 subsite and catalytic triad

Conclusions

Highly expressed SPs at the feeding larval and imago stages that are presumably digestive, as well as peptidases specific to the egg and pupal stages, were identified and classified based on S1 substrate-binding subsite and catalytic triad.