Regulation of the gene: Glutathione S-transferase

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Introduction

Increased pollution adversely affects aquatic organisms and may exert effects on the entire ecosystem. Molecular assays are a procedure used to discover the presence, amount, and functionality of a particular gene. They are used to determine if an organism's genes are affected by exposure to pollutants. Aquatic amphipods that experience exposure to pollution and continue to reproduce will be the most sensitive reporters of environmental contamination and as a result may be used as a highly specific molecular bioassay (Beaty *et al.*, 1998). When these amphipods reproduce, substances present in the environment affect their genes. These changes will occur on a fast timescale due to the speed at which amphipods reproduce.

In aerobic organisms, glutathione serves as the main redox agent, which moves electrons from one substance to another, to detoxify various foreign substances (Deponte, 2013). Currently, there is substantial research focused on glutathione-S-transferase (GST), using multiple species to indicate its high potential as a biomarker due substantial evidence that GST is "induced or inhibited in response to environmental contaminants" (Lee *et al.*, 2007). When exposed to hydrogen peroxide, arsenic, cadmium, or copper, the mRNA expression for GSTs were all upregulated in *Tigriopus japonicas*, another crustacean species (Lee *et al.*, 2008). There are also various classes and subclasses of GST, with the subclasses Sigma, Mu, and Delta/Epsilon relevant to ecotoxicology. Each subclass has as different function, all of which have all been upregulated in different concentrations of various pollutants. Therefore there is evidence that GST can be an indicator of metal pollution.

Parhyale hawaiensis is a marine amphipod that has recently had its genome sequenced (deposited into Joint Genome Institute) and has well-established culture methods. It is hypothesized that exposure to environmentally relevant concentrations of contaminants will cause up-regulation of GST genes and therefore can function as a molecular bioassay for the detection of pollution exposure.

Materials and Methods

***Parhyale hawaiensis* maintenance:** The *P. hawaiensis* cultures were cleaned and fed every other day for the duration of the project. Pipettes were used to remove all of the food particles and debris that accumulated at the bottom of the tanks. The debris was first transferred to a 150ml beaker and examined under a light to ensure no neonates (amphipod less than 1-2 days of age) were accidentally removed with the debris. If neonates were discovered amongst the debris, a narrow stemmed pipette was used to gently remove the neonates and place them back into the culture tanks. Once the tank was clear of debris, half of the remaining water was removed and discarded. Fresh saltwater, with a refractive index of 1.025, was then added to replace the water content. Lastly, 20-50 pellets of food were scattered around the tank depending on the amount of *P. hawaiensis* present.

Locating Glutathione S-transferase (GST) genes: Since the genome of *P. hawaiensis* is not annotated, other crustaceans were used to find potential GST genes. The crustaceans used were: *Daphnia magna*, *Daphnia pulex*, *Tigriopus japonicas*, and *Calanus finmarchicus*. Each of the above crustaceans has already had their genome published on the NCBI database. Therefore, the NCBI database was used in order to locate GST genes. Once the genes were located, the coding sequence of those particular genes were converted to FASTA format, using the features of the NCBI database, and then copied into a Word document labeled with the name of the crustacean it was obtained from. Three sets of genes were collected: Sigma subclass, Mu subclass, and Delta/Epsilon subclass.

Aligning the GST sequences: All the sequences of a particular subclass were aligned in order to determine the similarity between the genes. To do this, the ClustalW program was used. All sequences in FASTA format were copied into ClustalW. The program was set to analyze DNA sequences using a slow alignment algorithm to ensure greater accuracy.

Primer Design and Creation: Primers were created for all *Daphnia* spp. GST. Primer3 was used to generate primers to *Daphnia* spp. GST. Each of the sequences for the *D. pulex* and *D. magna* were placed into Primer3 and programed to find a PCR product of ~300-500 bp length. The primers selected had an annealing temperature within 2°C of each other.

Results

Figure 1. Sample of GST Mu sequences

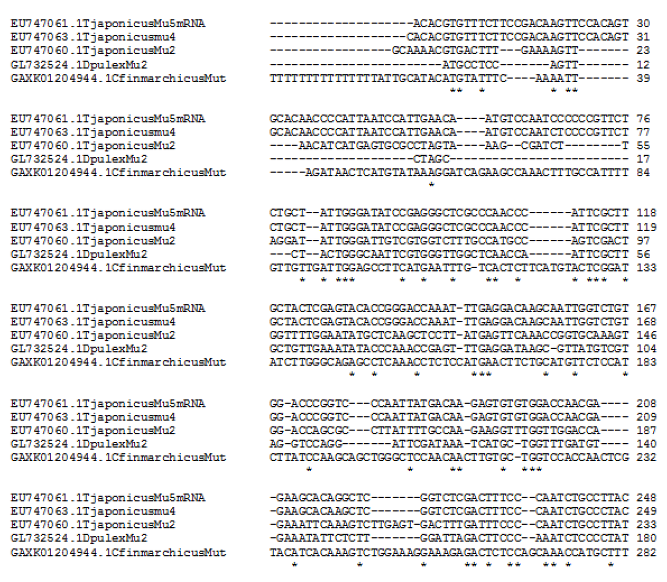

Figure 2. Sample of the alignment of GST Mu sequences

SeqA	Name	Length	SeqB	Name	Length	Score
1	EU747061.1TjaponicusMu5mRNA	849	2	EU747063.1Tjaponicusmu4	850	97.88
1	EU747061.1TjaponicusMu5mRNA	849	3	EU747060.1TjaponicusMu2	871	58.66
1	EU747061.1TjaponicusMu5mRNA	849	4	GL732524.1DpulexMu2	645	65.89
1	EU747061.1TjaponicusMu5mRNA	849	5	GAXK01204944.1CfinmarchicusMutranscribedrna	957	52.65
2	EU747063.1Tjaponicusmu4	850	3	EU747060.1TjaponicusMu2	871	58.47
2	EU747063.1Tjaponicusmu4	850	4	GL732524.1DpulexMu2	645	65.74
2	EU747063.1Tjaponicusmu4	850	5	GAXK01204944.1CfinmarchicusMutranscribedrna	957	52.0
3	EU747060.1TjaponicusMu2	871	4	GL732524.1DpulexMu2	645	61.4
3	EU747060.1TjaponicusMu2	871	5	GAXK01204944.1CfinmarchicusMutranscribedrna	957	50.86
4	GL732524.1DpulexMu2	645	5	GAXK01204944.1CfinmarchicusMutranscribedrna	957	58.14

Figure 3. Alignment results of GST Mu sequences

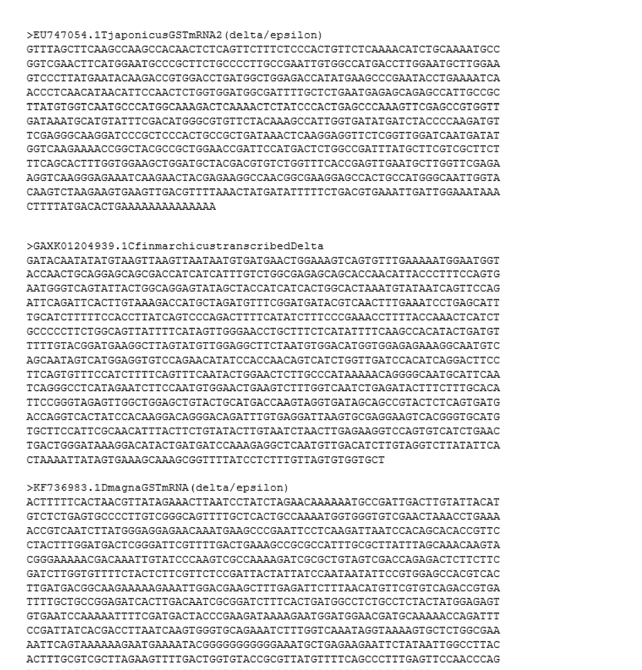

Figure 4. Sample of GST Delta/Espsilon sequences

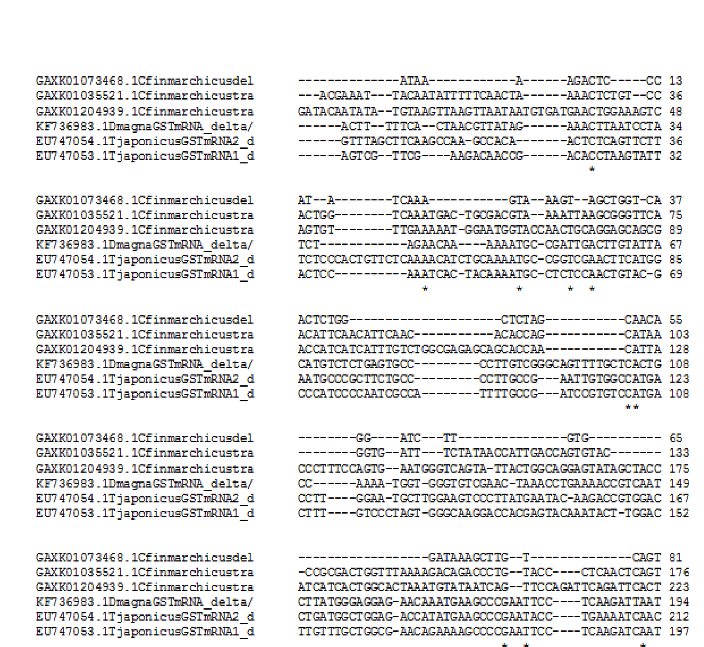

Figure 5. Sample of the alignment of GST Delta/Epsilon sequences

SeqA	Name	Length	SeqB	Name	Length	Score
1	KF736983.1DmagnaGSTmRNA_delta/epsilon_	900	2	EU747053.1TjaponicusGSTmRNA1_delta/epsilon_	811	58.45
1	KF736983.1DmagnaGSTmRNA_delta/epsilon_	900	3	EU747054.1TjaponicusGSTmRNA2_delta/epsilon_	798	61.28
1	KF736983.1DmagnaGSTmRNA_delta/epsilon_	900	4	GAXK01073468.1CfinmarchicusdeltatranscribedRNA	401	67.33
1	KF736983.1DmagnaGSTmRNA_delta/epsilon_	900	5	GAXK01035521.1Cfinmarchicustranscribeddelta	823	49.82
1	KF736983.1DmagnaGSTmRNA_delta/epsilon_	900	6	GAXK01204939.1CfinmarchicustranscribedDelta	1033	49.0
2	EU747053.1TjaponicusGSTmRNA1_delta/epsilon_	811	3	EU747054.1TjaponicusGSTmRNA2_delta/epsilon_	798	59.27
2	EU747053.1TjaponicusGSTmRNA1_delta/epsilon_	811	4	GAXK01073468.1CfinmarchicusdeltatranscribedRNA	401	67.58
2	EU747053.1TjaponicusGSTmRNA1_delta/epsilon_	811	5	GAXK01035521.1Cfinmarchicustranscribeddelta	823	49.82
2	EU747053.1TjaponicusGSTmRNA1_delta/epsilon_	811	6	GAXK01204939.1CfinmarchicustranscribedDelta	1033	53.51
3	EU747054.1TjaponicusGSTmRNA2_delta/epsilon_	798	4	GAXK01073468.1CfinmarchicusdeltatranscribedRNA	401	63.84
3	EU747054.1TjaponicusGSTmRNA2_delta/epsilon_	798	5	GAXK01035521.1Cfinmarchicustranscribeddelta	823	51.38
3	EU747054.1TjaponicusGSTmRNA2_delta/epsilon_	798	6	GAXK01204939.1CfinmarchicustranscribedDelta	1033	59.15
4	GAXK01073468.1CfinmarchicusdeltatranscribedRNA	401	5	GAXK01035521.1Cfinmarchicustranscribeddelta	823	71.57
4	GAXK01073468.1CfinmarchicusdeltatranscribedRNA	401	6	GAXK01204939.1CfinmarchicustranscribedDelta	1033	72.82
5	GAXK01035521.1Cfinmarchicustranscribeddelta	823	6	GAXK01204939.1CfinmarchicustranscribedDelta	1033	56.74

Figure 6. Alignment results of GST Delta/Epsilon sequences

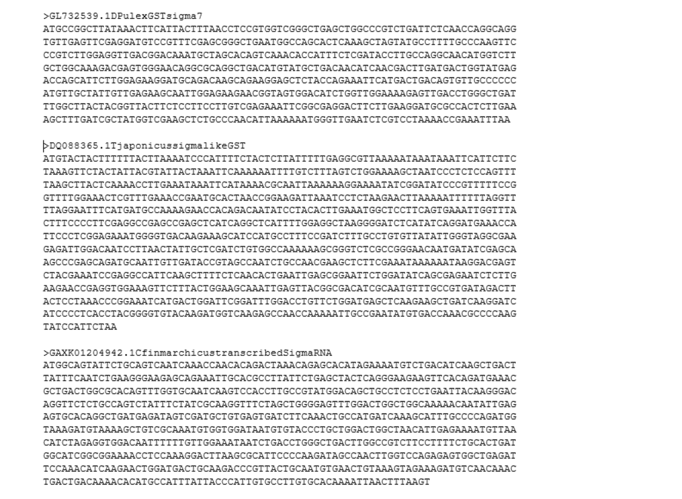

Figure 7. Sample of GST Sigma sequences

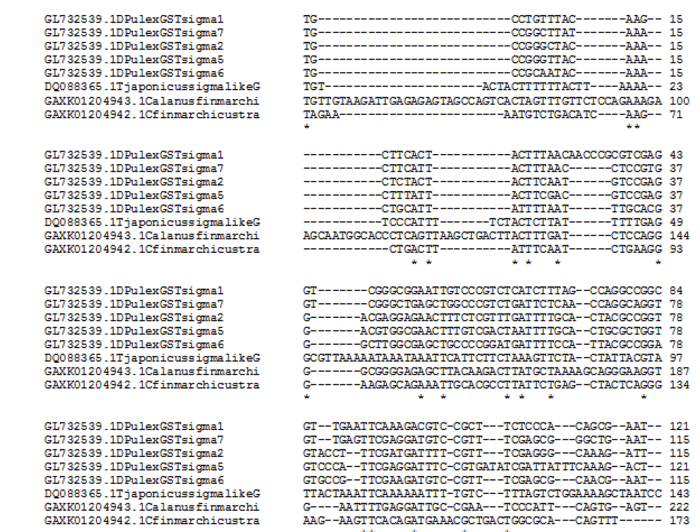

Figure 8. Sample of the alignment of GST Sigma sequences

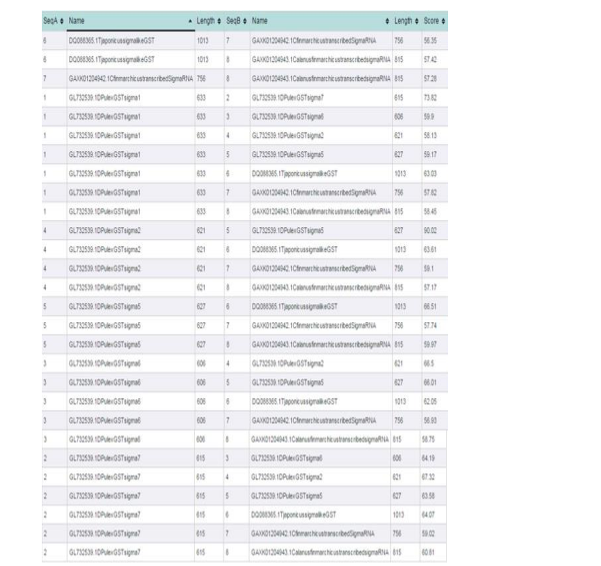

Figure 9. Alignment results of GST Sigma sequences

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OLIGO            start  len     tm     gc%   any    3' seq
LEFT PRIMER        136   20  60.12   55.00  4.00  1.00 CCCACTACGCCCTTTAGTCA
RIGHT PRIMER       452   20  59.76   45.00  4.00  0.00 TGCTTCCCAACCAAATATCC
SEQUENCE SIZE: 633
INCLUDED REGION SIZE: 633

PRODUCT SIZE: 317, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 0.00

OLIGO            start  len     tm     gc%   any    3' seq
LEFT PRIMER        256   20  60.05   40.00  2.00  0.00 GGCACGAATGAAAAGGAAAA
RIGHT PRIMER       625   20  59.97   45.00  4.00  0.00 GATTGTTTAATGGGCGTGCT
SEQUENCE SIZE: 645
INCLUDED REGION SIZE: 645

PRODUCT SIZE: 370, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 1.00

OLIGO            start  len     tm     gc%   any    3' seq
LEFT PRIMER        171   20  60.04   45.00  6.00  2.00 AAGCCCGAATTCCTCAAGAT
RIGHT PRIMER       549   20  59.97   55.00  4.00  2.00 TAGAGGCAGAGGCCATCAGT
SEQUENCE SIZE: 900
INCLUDED REGION SIZE: 900

PRODUCT SIZE: 379, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 1.00
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Figure 10. Sample of primers

Conclusion

In this experiment, the alignment percentage being 80% or higher would ensure that the gene was highly conserved; this means that the sequences would be easy to locate within the *P. hawaiensis* genome. However, the alignment percentages obtained suggest that the genes are not highly conserved between species since they were all under 70% identity. Since the GST genes were not highly conserved across multiple species, *Daphnia* sequences were examined to determine whether the genes are conserved within a genus. It was determined that GST genes are not conserved, even at the genus level. Instead, *Daphnia* primers were designed and will be tested for proof of principle, to show that GST is a potential ecotoxicology model to test for contaminant exposure. It is expected that once the primers are tested with *Daphnia* it will be easier to manipulate those same primers to find the exact gene within the *P. hawaiensis*. As a result, *P. hawaiensis* may still become a new model for detecting pollutants in the water.

References

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