Differentially expressed genes of carpet shell clam *Ruditapes decussatus* against *Perkinsus olseni*

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CONSEJO SUPERIOR
DE INVESTIGACIONES
CIENTÍFICAS

Bivalve culture in Galicia (2007)

Ruditapes decussatus

One of the most important
aquaculture product

Carpet shell clam production in Galicia

Natural beds and ongrowing parks

(1/3 of total production)

Capture volume of *R. decussatus* depends on environmental changes

Increase of the rainfalls

Presence of pathogens

Introduction

Introduction

Perkinsus species are associated with mass mortalities in molluscs

Susceptible Bivalve species in Europe

Ruditapes decussatus

Ruditapes philippinarum

Ruditapes pullastra

Venerupis aureus

Cardium edule

Aquatic Animal Health Code 2009 (OIE)

Infection with P. olsenii is a molluscs disease listed

Ruditapes decussatus is a susceptible specie to the infection of *P. olsenii*

Perkinsus olsenii → *Ruditapes decussatus* in Spain

Studies of presence in tissue lesions

Molecular response against the parasite

Identification of genes involved in a *Perkinsus olsenii* infection

Naturally infected clams

150 organisms

Infection level: gills in fluid thioglycollate medium (FTM)

Gills and hemocytes were maintained in **liquid nitrogen** until the results of the infection level

2 groups of 20 – 25 clams

Highly infected

Non infected

Haemocytes and gills

Haemocytes and gills

Construction of cDNA libraries **Suppression subtractive hybridization**

Consecutive hybridizations of control and infected samples

Differentially enrichment of expressed transcripts by PCR in

infected samples: forward SSH → over-expressed ESTs

control sample: reverse SSH → down-expressed ESTs

ESTs sequencing and Functional classification of ESTs identification

Adiponectin

DAD-1 (*Defender against death*)

Prosaposin and saposin

Multidomain protein complement related

Thrombin

Astacin

Chaperonin

Serine-protease (gill)

IAP (inhibitor of apoptosis protein)

Ferritin

Study of expression of the interested genes **qPCR, index of expression**

Adiponectin-C1q; DAD-1; prosaposin; multidomain protein complement related; thrombin

**Chronic
infected
sample**

Experimental infection of haemocytes with *Perkinsus* zoospores

Experimental design

Level of infection in clams from a commercial shellfish farm by PCR

infected clams

gills

***Perkinsus* zoosporulation**

- Incubation in FTM, 5 days, dark, RT
- Centrifugation at 3000g & digestion with NaOH for 1 hour.
- Maintenance in filtered sea water after sporulation → zoospores

non infected clams

haemocytes

***in vitro* exposition**

- 2 pools of 10 individuals
- Exposition to 10^8 zoospores/ml.
- RNA extraction after 30min, 1, 3 & 24 h

Study of expression of the interested genes qPCR, index of expression

Experimentally
infected
samples

Chronic
infected
samples

Adiponectin

- collagen domain and C1q domain
- similar structure to C1q and TNF α

633 pares of bases
211 aa

C1q domain (130
residuos)

Rd-adiponectin-C1q

1	ccgtctctttttatcggaaggtggtgaagcaatttagcacagaaataatacagaaaaaaac	60
61	cacttcagaatgatgatgatattttgcttcgtgtagttttatcgcaaggaacgctaagt	120
	M M M I F C F V L V L S Q G T L S▼	
121	gcgtcacttccggagagtagcgatacaagcaagaaccttgagtcgtttgtagagaatttg	180
	A S L P E S S D T S K N L E S F V Q N L	
181	tttacgggaagcagtgatctatcaaagagttcagttttgaggaaaccgaggatgaaatt	240
	F T G S S D L S N E F S F E E T E D E I	
241	ccaccacatgaagaagaaacacaggacgtctccattcctgagaacggacttttattttaa	300
	P P H E E E T Q D V S I P E N G L L F K	
301	caaggattttcatgcctacctcagcgggtccacgggtgctaccacacagggaacccaattaaa	360
	Q G F H A Y L S G P R C Y H T G N P I K	
361	tttgacgtggcatcggtaaatattggtaacagatacagtggtccatactggtgtatatgtg	420
	F D V A S V N I G N R Y S V H T G V Y V	
421	gtggccacacctggactctacttttttacctggaccgttgctgcccgaagaaaagaactgg	480
	V A T P G L Y F F T W T V A A E E K N W	
481	tttgtgtctgatttaatggtgaacgggggttctgagaggaaacacccatgaccgactcatat	540
	F V S D L M V N G V L R G N T M T D S Y	
541	ggaaatggaaaagggaacggcatccatccagcgaccggatttgccttggtaaatgtcaaaa	600
	G N G K G N G I H P A T G F V L V N V K	
601	cgcggggatcatgtttacataagatttgcaccgggtcatggatgcattataaaaagcaac	660
	R G D H V Y I R F V T G H G C I I K S N	
661	aggatgactcgatctacgttttctggatggcggttgactaagcaacttgggcgacagtc	720
	R M T R S T F S G W R L H *	
721	tactgatgctggaaaaaaggagaagcttatcgacacaaccgttaaaccattaattcttgc	780
781	cgaatctcacacgttcttcagtttattcgtttctaacattttttctttatttttatttct	840
841	aaaatacgtacctcg	

Phylogenetic study. Adiponectina-C1q

DAD-1 (Defender against death)

- apoptosis inhibition
- tissular damage
- heat shock

339 pares of bases
113 aa

DAD domain

Rd-DAD-1

Phylogenetic study

1	cagcggagaaccagcgatcggtgtctacttccgggaaattgccacgtccaattttttctca	60
61	cttgtttgcaagaaatagcattattttaacaatgccagagaaactaacggggagtcatatcc	120
	M P E K L T G V I F	
121	aagttttatgacgagtagacataaacagtactcctaagagactcaaaattatagacgcatac	180
	K F Y D E Y I N S T P K R L K I I D A Y	
181	ctgctctatatattttcctgacaggtgtattttcagttttgctattgtgcatgtggttggaact	240
	L L Y I F L T G V F Q F C Y C A L V G T	
241	ttoccattcaattccttctgtcaggggttcattctcatgtgttaggatcttttggttattgca	300
	F P F N S F L S G F I S C V G S F V I A	
301	gtatgttttaagattacaagtaaatccacagaataagtcagacttttccggcatcagccca	360
	V C L R L Q V N P Q N K S D F S G I S P	
361	gaaagagcatttgctgattttatcttcgcaaatttggttctacacttggtggttatgaac	420
	E R A F A D F I F A N L V L H L V V M N	
421	ttcattgggttaaattggaccatcttgggttgagta	453
	F I G *

TUNEL

Brown precipitates on DNA breaks

Highly infected clam

Moderately infected clam

- Most of the identified sequences were found in haemocytes and less in gill
- Similar gene expression pattern in natural and experimentally infected samples
- 1 h of exposition provides the highest expression of related immune genes
- Chronic infected clams seem to be in an alert state
- Apoptosis: important process during *Perkinsus* infection
- Future studies: new identified genes could be used like candidate genes against disease

Thank you for your attention