

Transcriptome response to salinity stress in the Pacific oyster (*Crassostrea gigas*)

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Background

- As sessile marine animals of estuarine and intertidal regions, oysters can tolerate harsh and dynamically changing environments, such as temperature, salinity, and air exposure variations.
- **Salt stress** is an important component of intertidal environments. In such areas, the sea water varies from nearly fresh water to highly saline and dry salt conditions.
- **Low salinity** is important for the survival and distribution of marine animals.



Background

The Pacific oyster *Crassostrea gigas*

- A dominant species in many intertidal locations;
- A major aquaculture bivalve species worldwide;
- An excellent model system to study the osmoregulation mechanism.



Molecular
mechanisms?



Outline

Part 1. Gene co-expression network analysis reveals the correlation patterns among genes in euryhaline adaptation of the Pacific oyster

Part 2. Identification and expression of cysteine sulfinic acid decarboxylase, possible regulation of taurine biosynthesis in the Pacific oyster in response to low salinity

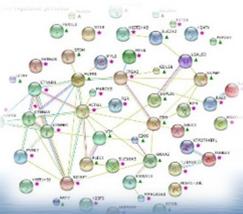


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Gene co-expression network analysis reveals the correlation patterns among genes in euryhaline adaptation

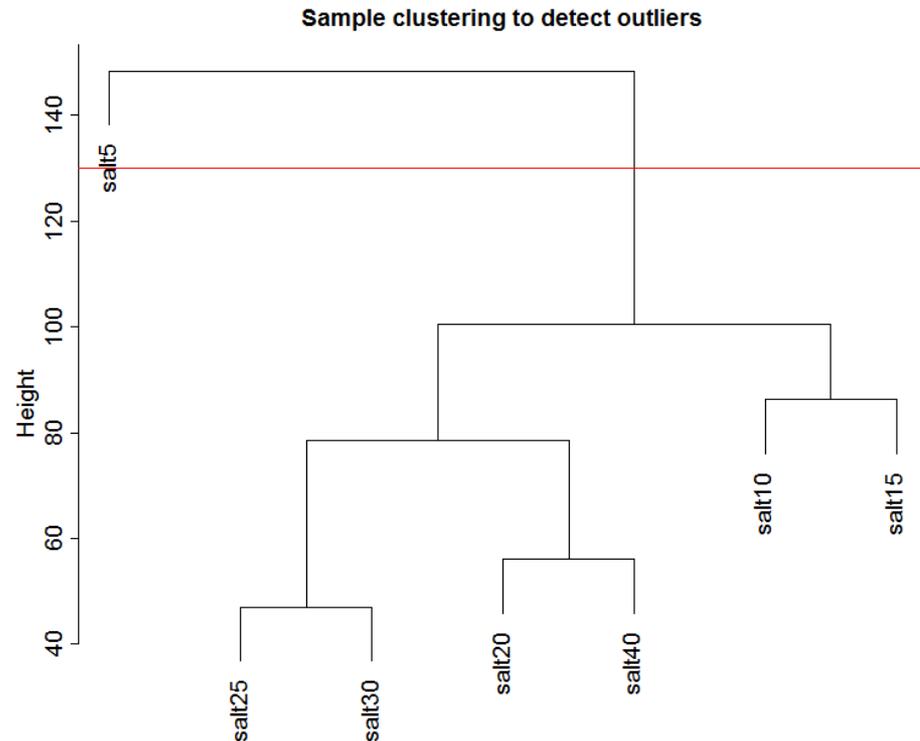
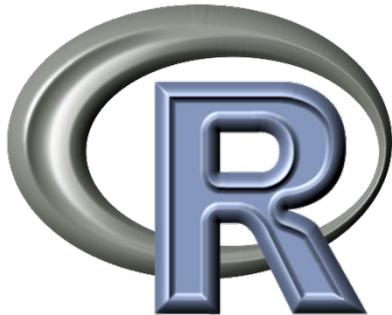
✚ **The co-expressed gene network of the Pacific oyster transcriptome was constructed to identify salinity stress-related modules and candidate key genes**

- Transcriptome data from seven different salinities (5, 10, 15, 20, 25, 30, and 40‰ for 7 days) were downloaded from GenBank.
- All the seven libraries were mapped to the genome of *C. gigas* (Zhang et al. 2012).
- The transcript expression levels (FPKM) were estimated, and differential expression analysis was performed.



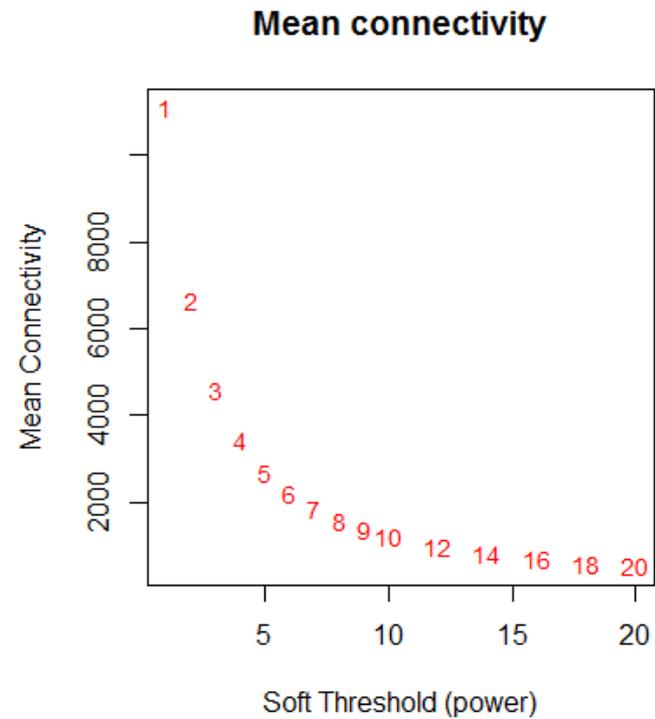
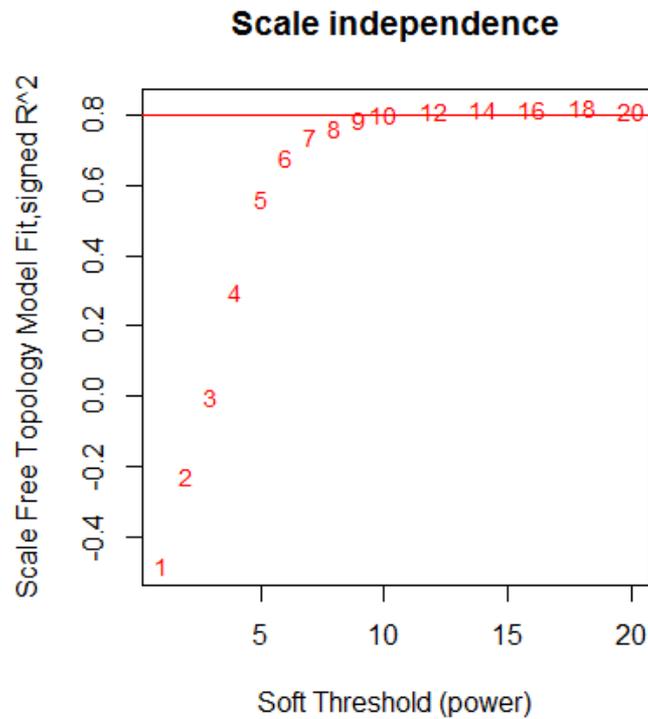
Gene Co-Expression Network Construction

- Gene network was constructed using R package WGCNA following the procedure described in Langfelder and Horvath (2008).
- We clustered the samples to see if there were any obvious outliers. As a result, the group of salt 5 was removed as an outlier.



Gene Co-Expression Network Construction

➔ A power of nine was chosen so that the resulting networks exhibited approximate scale-free topology (model fitting index $R^2 = 0.8$).



Gene Co-Expression Network Construction

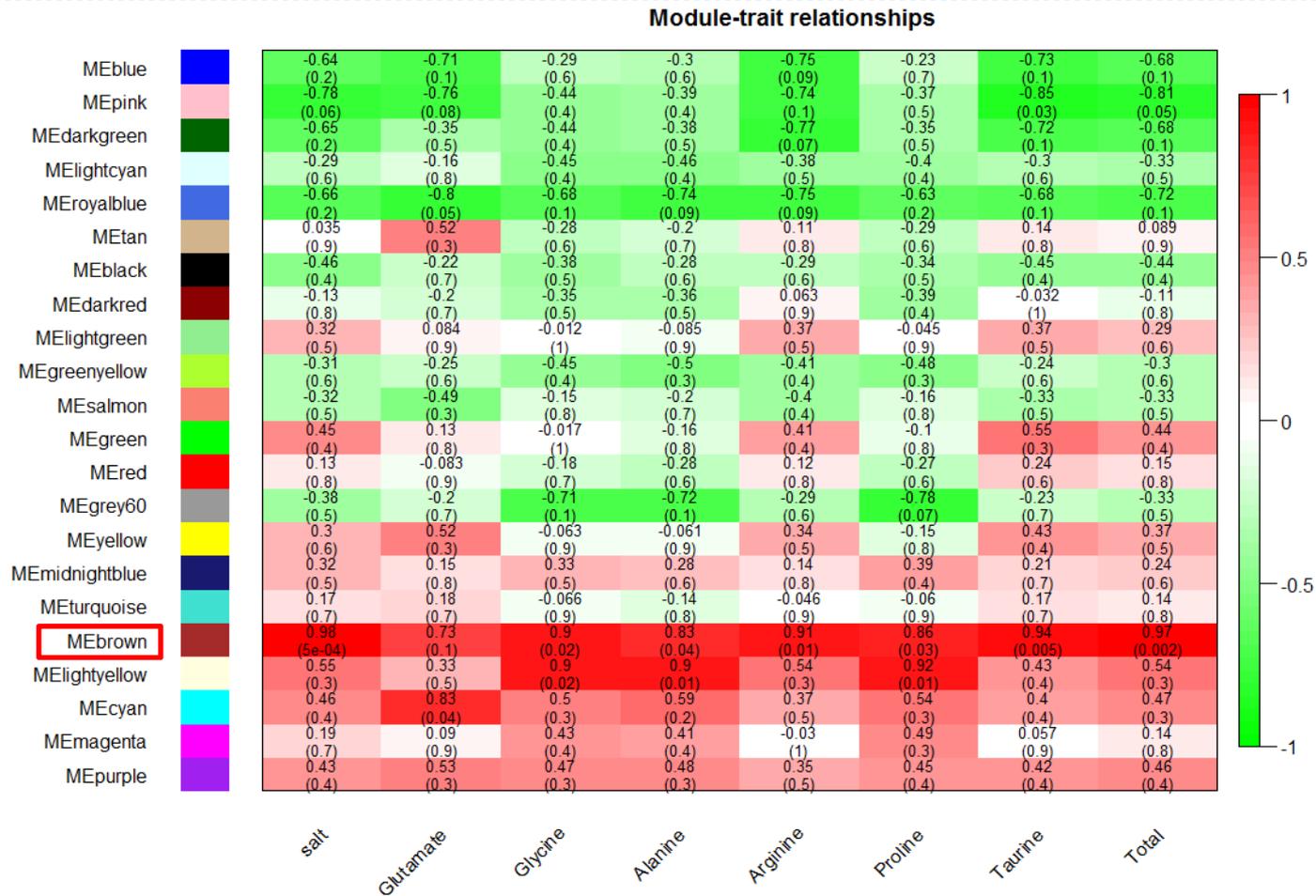
- All 25,463 coding sequences were parsed into 22 gene modules, with module size ranging from 128 to 12,942.

Module	Gene No.	Module	Gene No.	Module	Gene No.	Module	Gene No.
turquoise	12942	blue	2306	brown	1718	yellow	1115
green	936	red	808	black	765	pink	643
magenta	582	purple	534	greenyellow	474	tan	445
salmon	363	cyan	318	midnightblue	302	lightcyan	240
grey60	238	lightgreen	161	lightyellow	160	royalblue	151
darkred	134	darkgreen	128				



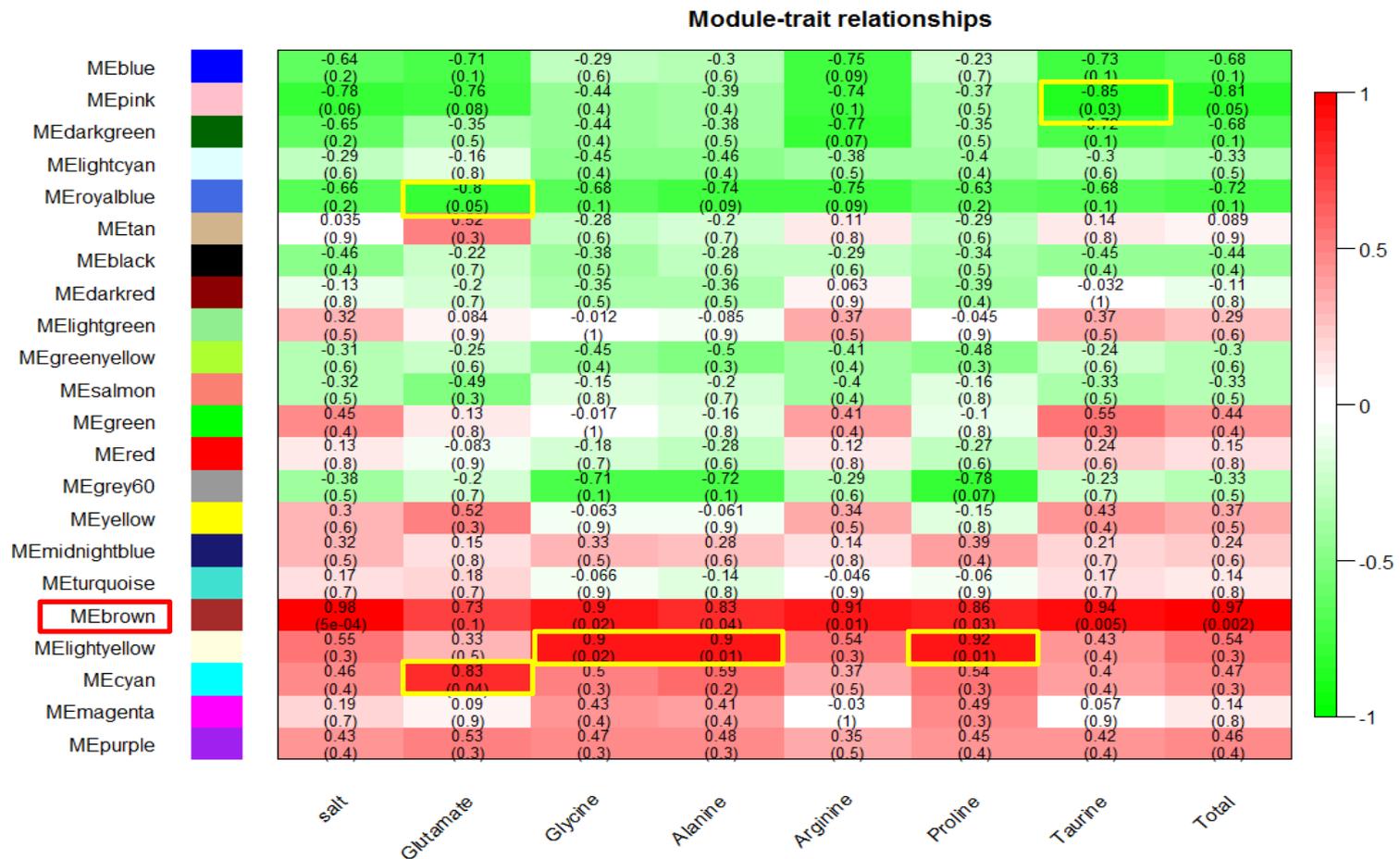
Identification of Salinity Stress-Related Modules

- ➔ The module-trait correlation analysis showed that **brown module** was significantly positively correlated with salinity stress and almost all the FAAs contents (except glutamate).



Identification of Salinity Stress-Related Modules

- ➔ In addition, **pink** module was correlated with taurine; **lightyellow** correlated with glycine, alanine, and proline; and **cyan** and **royalblue** correlated with glutamate.



- The four modules showed differential expression at different salinity: pink module responded at hypo-salinity (salt 10 and salt 15); cyan module responded at salt 20 and salt 40; lightyellow module responded at salt 40; and royalblue module responded at salt 25 and salt 40.

Module	Gene No.	Hypergeometric P value				
		Salt 10	Salt 15	Salt 20	Salt 25	Salt 40
Pink	643	0**	0**	0.994	0.961	0.957

It may imply that different FAAs play a dominant role at different salinity.

Cyan	318	0.998	1	5.13E-06**	0.447	0.0149
Royalblue	151	1	1	0.502	0.00018**	3.68E-07**



Functional Annotation of Salinity Stress-Related Modules

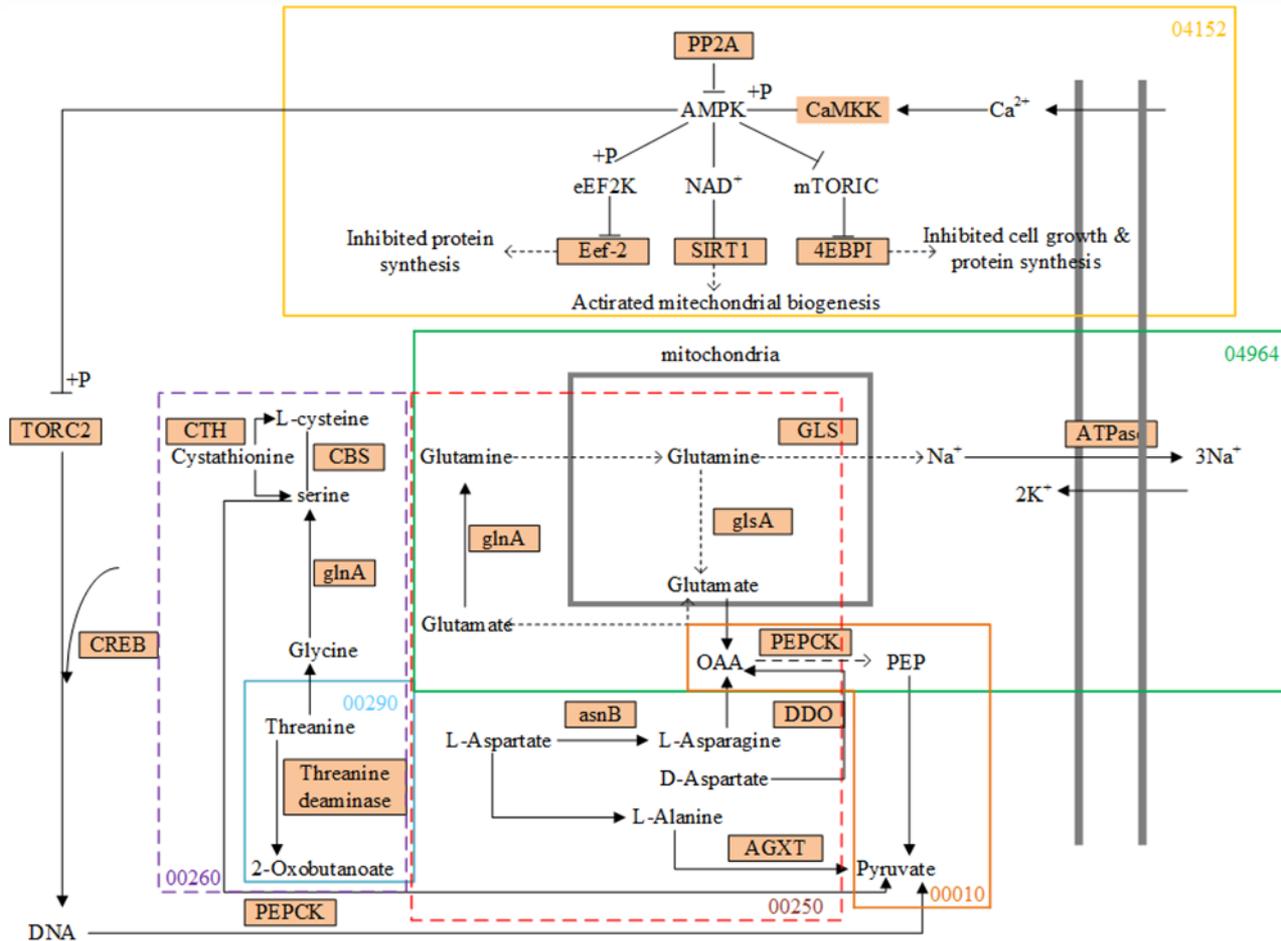
Module	Pathway name	Total entities	Overlap	P value
brown	Selenocompound metabolism	12	6	0.000365
	Methane metabolism	21	8	0.000371
	Biosynthesis of amino acids	62	15	0.000421
	Cysteine and methionine metabolism	37	9	0.005789
	Glycine, serine and threonine metabolism	40	9	0.009904
pink	Pathways in cancer	163	16	0.000707
	Cysteine and methionine metabolism	37	6	0.003198

It verified amino acids playing important roles in osmoregulation in the Pacific oyster.

lightyellow	Oxidative phosphorylation	57	4	0.002582
	Protein export	22	2	0.014986
royalblue	Starch and sucrose metabolism	28	2	0.015524
	Flavone and flavonol biosynthesis	3	1	0.020606
cyan	Ribosome	120	6	0.000653



Functional Annotation of Salinity Stress-Related Modules



- ➔ *C. gigas* response to salinity was initiated upon the sensing of ion via cell membrane receptors. The signals subsequently transmitted down through the AMPK signaling pathways and triggered the production of a series of FAAs as the end results. In parallel, energy metabolism was activated to provide energy.



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Identification and expression of cysteine sulfinic acid decarboxylase in the Pacific oyster in response to low salinity

- ◆ As an important osmolyte, taurine was found to participate in osmoregulation in many invertebrates, including oyster, mussels, and so on.
- ◆ Marine bivalves mainly use cysteine as raw material to maintain taurine pool. Cysteine sulfinic acid decarboxylase (CSAD) has been reported as the key enzyme that determines taurine biosynthesis capability in animals.
- ◆ There are two copies of CSAD genes in *C. gigas* genome.

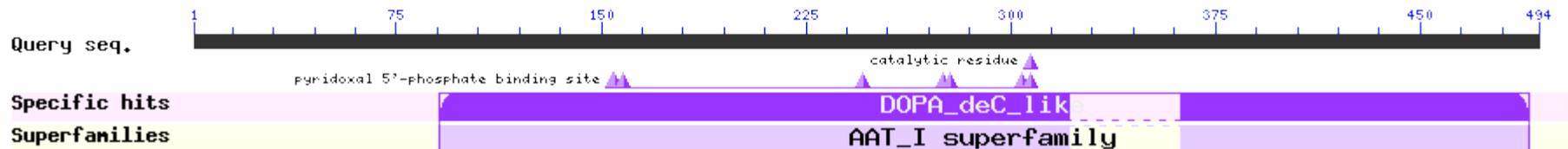
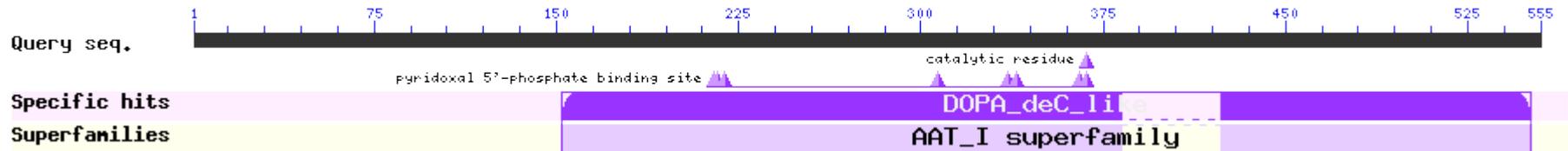


- 👉 To identify the roles of CSAD genes in taurine biosynthesis and oyster osmoregulation.

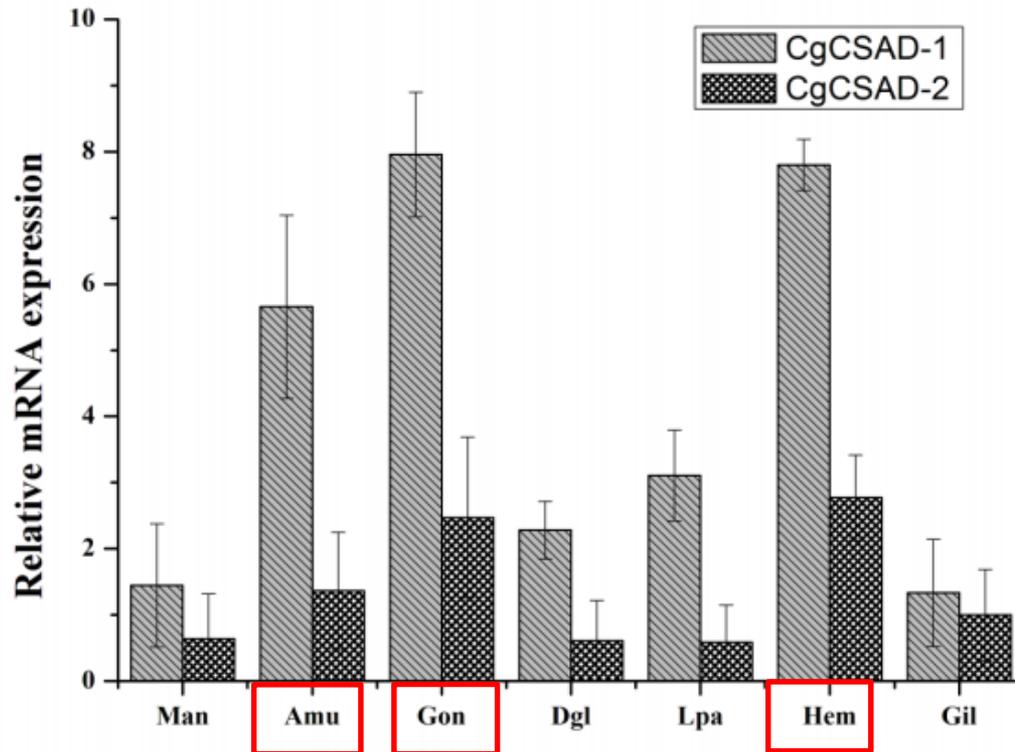


cDNA cloning and phylogenetic analyses

- ◆ Two copies of CSAD were cloned in *C. gigas* and were named as CgCSAD1 and CgCSAD2. The length of the ORF of the two cDNAs was 1668 bp, and 1485 bp, respectively.
- ◆ Both the two CSAD proteins contain pyridoxal phosphate (PLP)-dependent amino acid carboxylases domain.
- ◆ The identity of the two CSAD was 65%.



Tissue specificity of mRNA expression

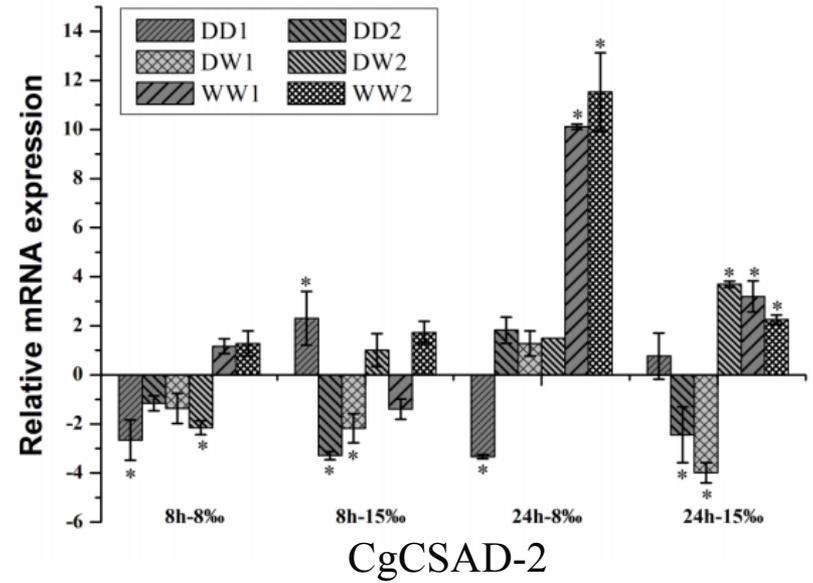
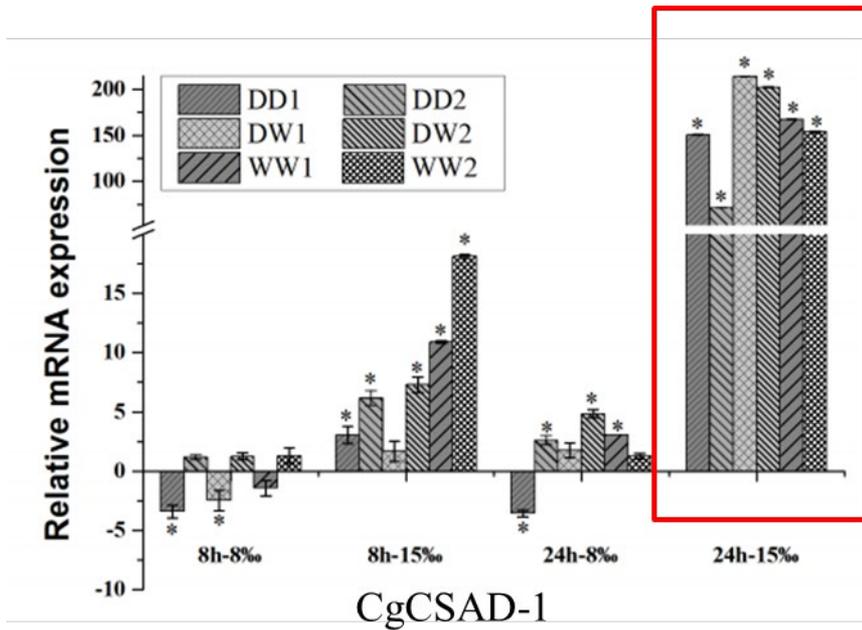


- ◆ The transcript level of CgCSAD1 was higher than that of CgCSAD2 in all tissues.
- ◆ As for CgCSAD1 gene, the transcript level was higher in the gonad, followed by the hemolymph and adductor muscle.

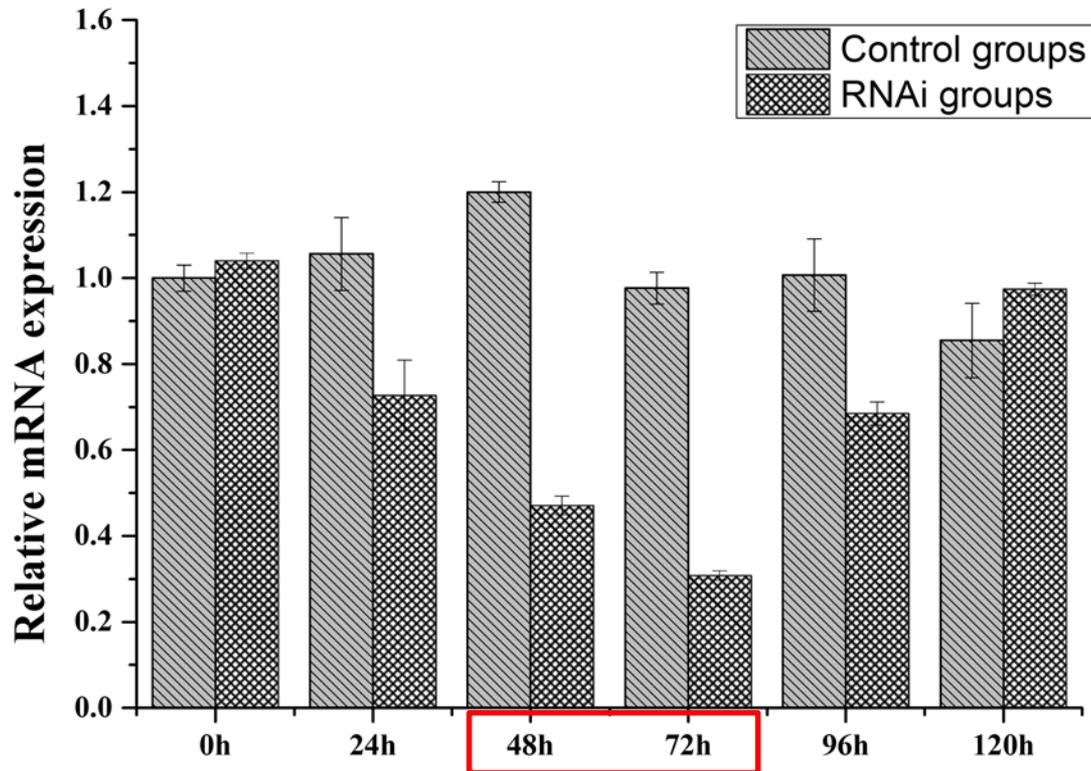


Effects of low salinity exposure

up-regulated

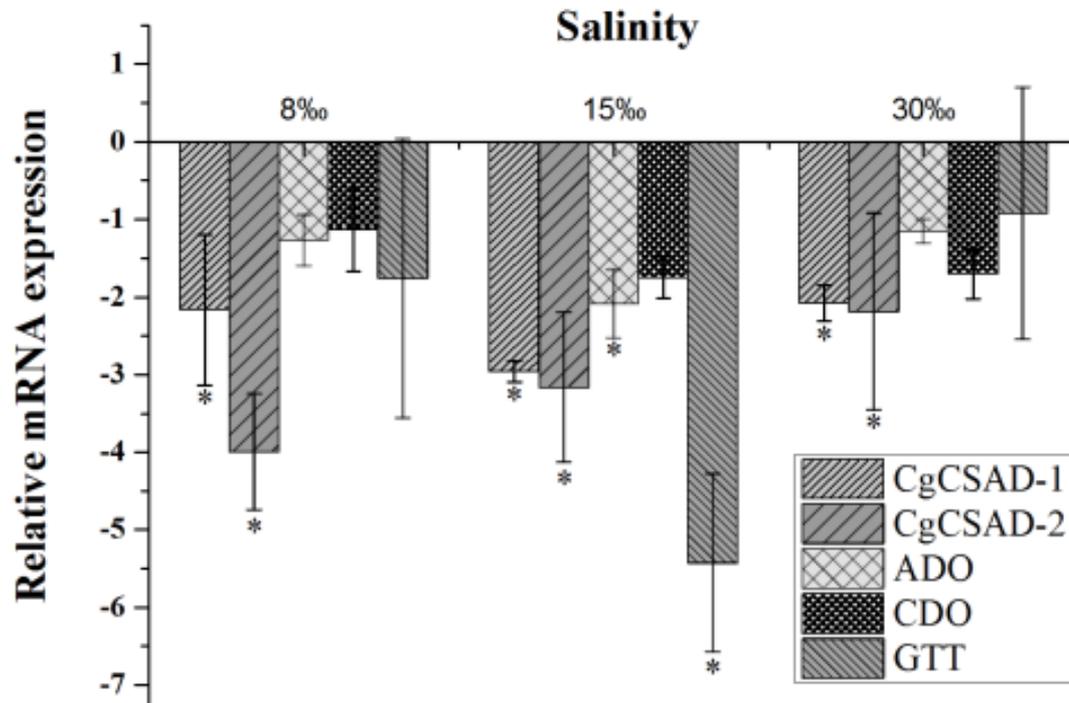


Effect on the expression of genes in taurine metabolism after CgCSAD1 interference



- ◆ Based on the result of pre-experiment, the expression of CgCSAD1 began to decrease at 24 h after dsRNA injection and it was maintained at a relatively low level from 48 to 72 h.

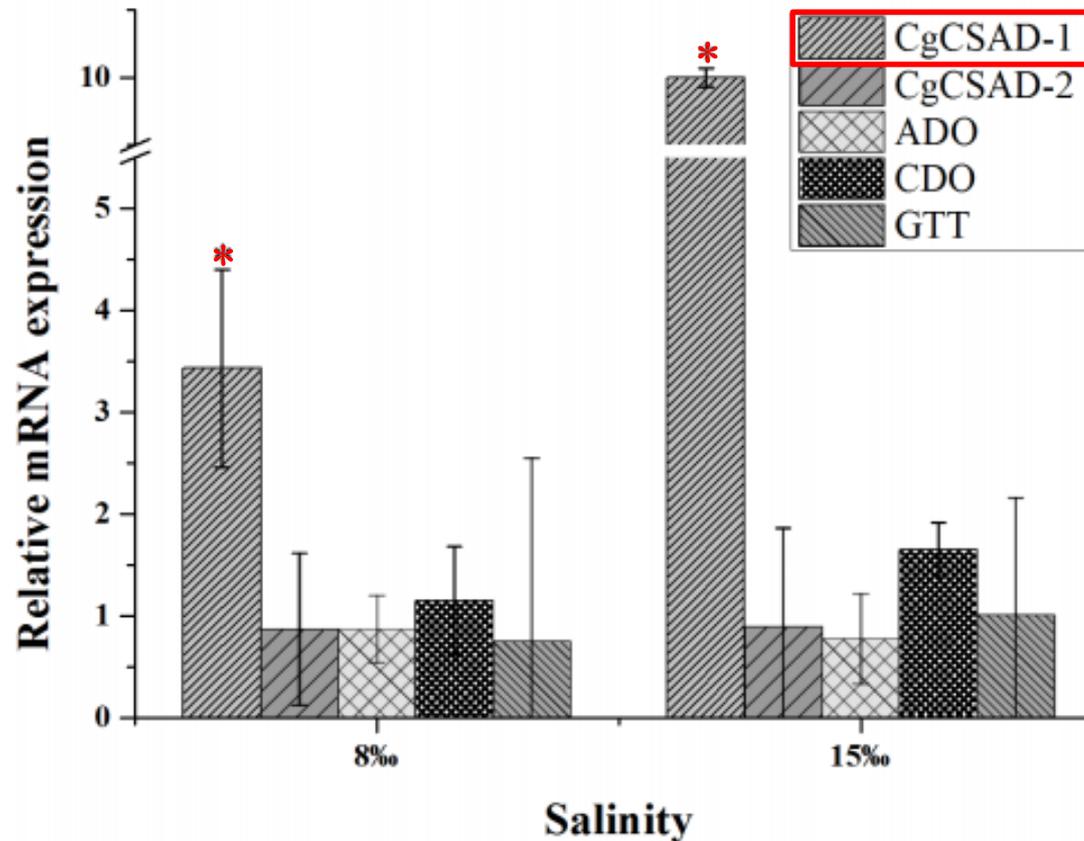
Effect on the expression of genes in taurine metabolism after CgCSAD1 interference



- ◆ The expression of CgCSAD1 decreased significantly after CgCSAD1 interference in different salinities. Meanwhile, the transcript level of CgCSAD2 decreased significantly as well.
- ◆ Under 15‰ condition, the expression of ADO and GTT decreased significantly and others had no significant change.



RNAi group in low salinity



- ◆ Compared with the RNAi group cultured in salt 30, the expression of CgCSAD1 increased significantly, especially 10 fold in salt 15, and other genes had no significant change.



Effect of CgCSAD1 suppression on taurine contents

Group Name	Taurine contents (mg/g dry weight) Average \pm SD (n = 6)
8‰C	62.02 \pm 3.28
8‰T	55.37 \pm 2.42
30‰T	56.06 \pm 4.13



CgCSAD1 may be the key enzyme in taurine biosynthesis to regulate taurine pool in response to low salinity of *C. gigas*.

- ◆ Compared with control groups, taurine contents decreased in groups of CgCSAD1 interference.
- ◆ There is a positive correlation between CgCSAD1 expression and taurine contents.



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Thank You !

