Dynamic transcriptome and LC-MS/MS analysis revealed the important roles of taurine and glutamine metabolism in response to environmental salinity changes in gills of rainbow trout (*Oncorhynchus mykiss*)

Yuan Tian[1], Na Xue[1], Hancheng Bao[1], Qinfeng Gao[1], and Yangen Zhou[1]

[1] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China,

Correspondence: qfgao@ouc.edu.cn; tianyuan@ouc.edu.cn

1

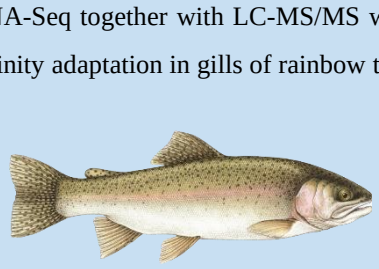
Highlights

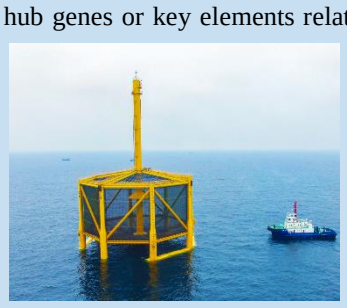
- **Salinity dramatically changed gene expression patterns in gills of rainbow trout.**
- **There were 18 independent modules with distinct expression patterns.**
- **7 hub genes are known as important regulators of taurine and glutamine metabolism.**
- **Concentrations of taurine and glutamine in gills were affected by salinity changes.**
- **Taurine and glutamine play important roles in salinity adaptation in rainbow trout.**

2

Introduction

Recently, Chinese researchers started to carry out salmon mariculture far offshore, within deep cages, in the central depression of the Yellow Sea area full of large cold-water mass. Hence, rainbow trout (*Oncorhynchus mykiss*) has been transferred from freshwater to seawater. However, the molecular mechanism of rainbow trout in salinity adaptation remained largely unexplored. In the present study, RNA-Seq together with LC-MS/MS were applied to determine the hub genes or key elements related to salinity adaptation in gills of rainbow trout.





3

Materials & Methods

A total of 180 rainbow trout ((body length: 24.90 ± 2.17 cm and body weight: 243.50 ± 35.95 g) adults were selected for salinity experiment. These individuals were cultured in fresh water (0‰) fro two weeks. Then, the salinity was gradually increased to 30‰ by adding sea salt at a steady rate of 6 ‰/day. Three individuals each tank were anesthetized with MS-222 and dissected for gills at 6 different salinities, including 0‰ (0 d), 6‰ (1 d), 12‰ (2 d), 18 ‰ (3 d), 24 ‰ (4 d) and 30 ‰ (5 d).

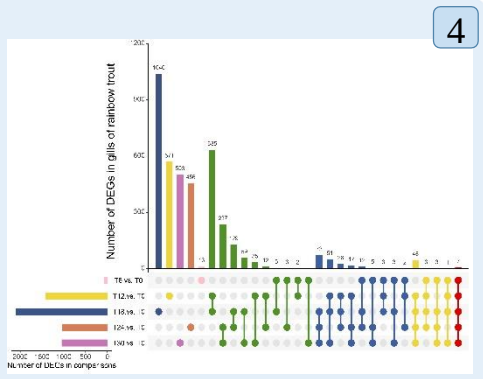
Total RNA of gill samples was extracted for library construction and RNA-Seq. The standard bioinformatics pipeline was employed to determine differentially expressed genes (DEGs). The genes were ranked by median absolute deviation from large to small, and the top 5,000 genes were selected for weighted gene co-expression network analysis (WGCNA).

Moreover, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed to determine the concentrations taurine, glutamine, glutamic acid and α-ketoglutaric acid.

4

DEGs

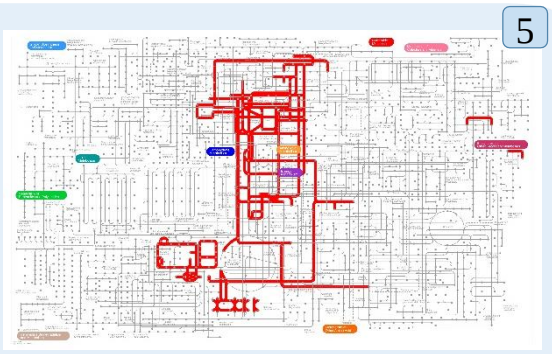
There were 63, 1,411, 2,096, 1,031 and 1,041 DEGs in the pairwise comparisons of T6 vs. T0, T12 vs. T0, T18 vs. T0, T24 vs. T0, and T30 vs. T0, respectively. It clearly showed that the number of DEGs was dramatically increased at 12‰, reached the top at 18‰, and then decreased at 24‰ and 30‰. UpSet plot was performed to visualize the intersections of DEGs among these comparisons .



5

Enrichment

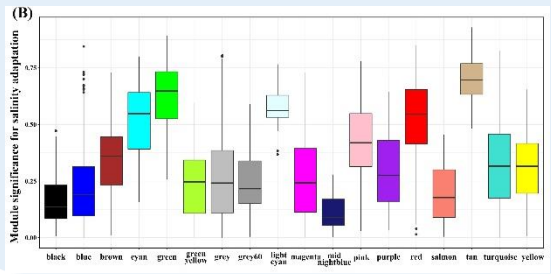
Enrichment analysis of KEGG pathways was performed to evaluate the biological and functional implications of DEGs. The interactive pathway analysis showed that some other enriched pathways in these comparisons were tightly related to amino acid metabolism.



6

WGCNA

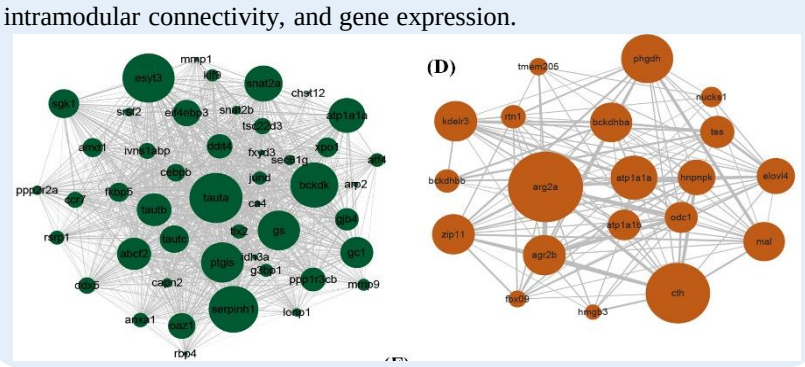
There were 18 independent with distinct expression patterns. Of them, green and tan modules exhibited the highest positive and negative correlations with salinity changes (R^2= 0.76 and R^2 = -0.86). Hence, they were considered as the salinity-related modules for the identification of hub genes.



7

Hub genes

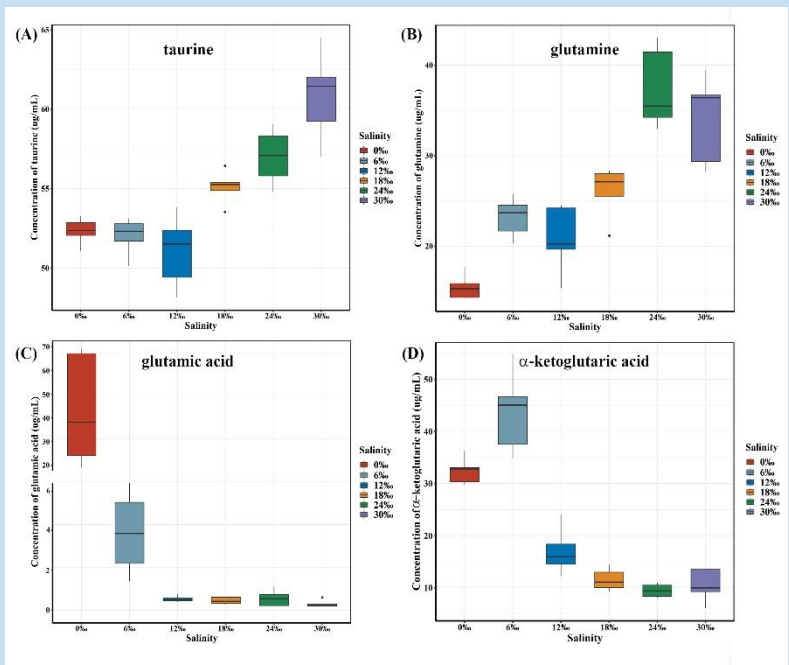
Several genes, tightly related to taurine and glutamine metabolism, were regarded as hubs based on their gene significance, module membership, intramodular connectivity, and gene expression.



8

LM-MS/MS analysis

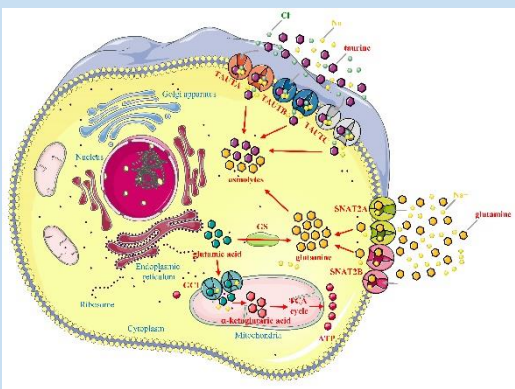
The concentrations of taurine, glutamine, and their metabolism-related glutamic acid and α-ketoglutaric acid in gills of rainbow trout were tightly related to the salinity changes. Glutamine and taurine were gradually increased since 6‰ or 18 ‰, while dramatic decreases were detected in the concentration of glutamic acid and α-ketoglutaric acid.



9

Conclusion

Confronted to salinity increases, taurine mediated by active transport of *tauta*, *tautb* and *tautc* may work as osmolytes to maintain the balance of osmotic pressure during salinity changes. Glutamine derived from active transport and biosynthesis may act as important osmolytes for osmoregulation. Simultaneously, some glutamic acid could be transported into mitochondria by *gc1* gene and converted to α-ketoglutaric acid for meeting the energy demand.



Acknowledgments: This study was funded by the National Key Research and Development Program of China, and the National Natural Science Foundation of China.

Reference: Tian Y, Gao Q, Yu H, Liu D, Dong S, Zhou Y, Yang W, Xue N, Bao H, Yu Y. Dynamic transcriptome and LC-MS/MS analysis revealed the important roles of taurine and glutamine metabolism in response to environmental salinity changes in gills of rainbow trout (*Oncorhynchus mykiss*). Int J Biol Macromol. 2022 Sep 16;221:1545-1557.