

Supplementary Material

Transcriptome, intestinal microbiome and histomorphology profiling of differences in the response of Chinese sea bass (*Lateolabrax maculatus*) to *Aeromonas hydrophila* infection

Supplementary Table 1

Sequence information of primers for qRT-PCR.

Primer No.	Primer name	Sequence (5' - 3')
1	C6-F	ACCAAGTTCTGCCTGTTTCCA
2	C6-R	CCCTGAGCTGTAGTAGTGCCTC
3	LBP-F	AGCGTTTCCCAGGTCTGATGATGA
4	LBP-R	AGGGTGGCGTTGGGTGGATAG
5	NFATC1-F	CAACATGCGGGCCATGATCG
6	NFATC1-R	ACCATCCGCACTCGTGTGTT
7	RIPK2-F	GACCTGCGGCTGGACCTCTAACC
8	RIPK2-R	GACCCCTCATCACTCCTCTTCTCACA
9	SIPA1L2-F	GACAGCGGCATCGACACTAATCC
10	SIPA1L2-R	GGACATAATGGCAGAGGCGTAGC
11	C7-F	TGACAAAACGCCTCCCAACTC
12	C7-R	CTGCACTGCCCTCCGAAACTC
13	IRAK4-F	CGACTTGCTTGCTTGGAGGG
14	IRAK4-R	ACGCTCTCGTCAGCCCAAAG

15	β -actin-F	CGTGCTGTCTTCCCCTCCA
16	β -actin-R	TCACCAACGTAGCTGTCCTTCTG

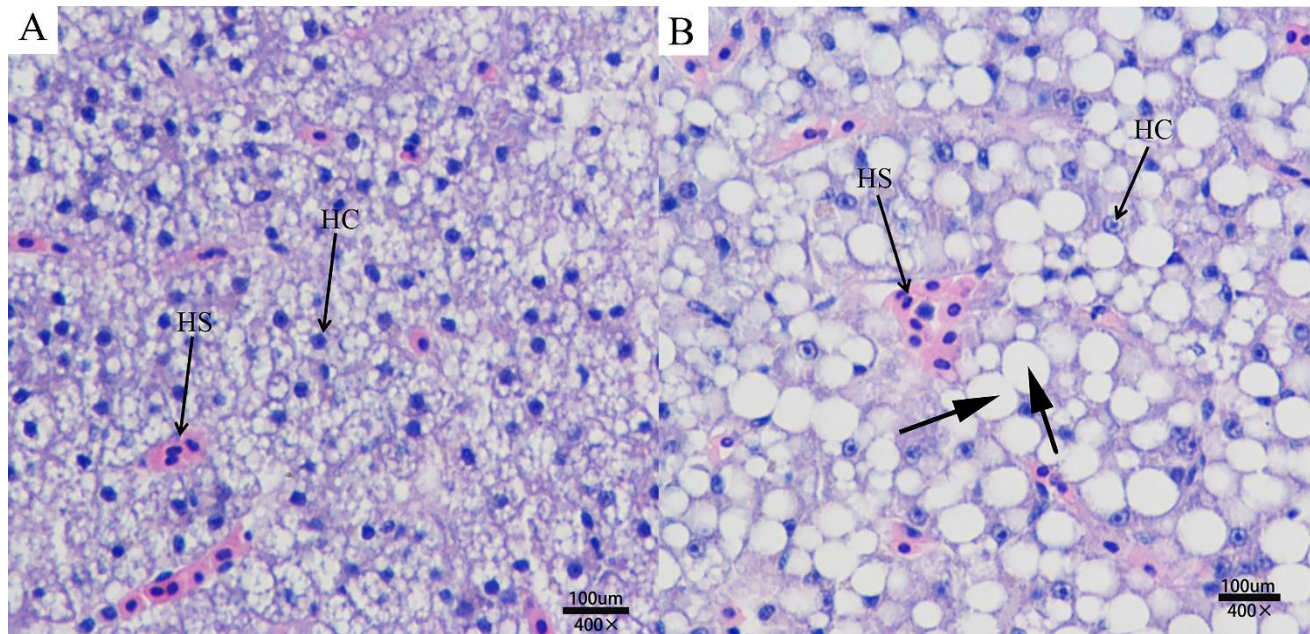
Supplementary Table 2 Partial of DEGs involved in signaling pathway related to immune response

Gene name	Gene ID in transcriptome	Log ₂ FC	Regulated	<i>p</i> -value
Toll-like receptor signaling pathway				
<i>TLR5</i>	evm.TU.scaffold_129.16	7.15	up	1.16×10 ⁻⁶
<i>CASP8</i>	evm.TU.scaffold_255.49	1.54	up	1×10 ⁻³
<i>CD40</i>	evm.TU.scaffold_11.322	1.34	up	2.81×10 ⁻³
NOD-like receptor signaling pathway				
<i>HSP90</i>	evm.TU.scaffold_1.108	-1.52	down	1.06×10 ⁻³
<i>ASC</i>	evm.TU.scaffold_14.2	2.00	up	1.43×10 ⁻⁴
<i>IL-1β</i>	evm.TU.scaffold_287.11	4.55	up	5.43×10 ⁻⁶
<i>IRF3/7</i>	evm.TU.scaffold_4.273	2.71	up	1.17×10 ⁻⁵
C-type lectin receptor signaling pathway				
<i>IκBα</i>	evm.TU.scaffold_137.4	3.24	up	1.79×10 ⁻¹¹
<i>MALT1</i>	evm.TU.scaffold_6.267	1.41	up	1.22×10 ⁻³
<i>RELB</i>	evm.TU.scaffold_56.112	1.54	up	1.46×10 ⁻³
<i>Cox-2</i>	evm.TU.scaffold_12.30	1.67	up	4.31×10 ⁻³

Supplementary Table 3 Relationship between differentially expressed genes and microbial community composition on genus level

Gene ID	NR	Chr	regulated	Correlation	P-value	Genus
				n		
evm.TU.scaffold_6.46	CD44 antigen	6	Down	0.828	0.042	<i>Ralstonia</i>
evm.TU.scaffold_76.153	Zinc finger protein 362-like isoform X1	4	Down	0.848	0.033	<i>Ralstonia</i>
evm.TU.scaffold_19.80	Ankyrin repeat and SOCS box protein 5-like isoform X1	14	Down	0.863	0.027	<i>Ralstonia</i>
evm.TU.scaffold_188.45	Growth/differentiation factor 10-like	13	Up	-0.872	0.024	<i>Ralstonia</i>
novel.1379	Unknown	23	Up	-0.831	0.040	<i>Ralstonia</i>
novel.2442	Uncharacterized protein LOC108881995(predicted)	scaffold_44 6	Down	0.814	0.049	<i>Ralstonia</i>
evm.TU.scaffold_25.115	Serum amyloid A	1	Up	0.983	0.000	<i>Pseudomonas</i>
evm.TU.scaffold_98.14	Metalloreductase STEAP4 isoform X2	7	Up	0.955	0.003	<i>Pseudomonas</i>
evm.TU.scaffold_24.251	Unknown	7	Up	0.944	0.005	<i>Pseudomonas</i>
evm.TU.scaffold_25.76	Haptoglobin-like isoform X1	1	Up	0.992	0.000	<i>Pseudomonas</i>

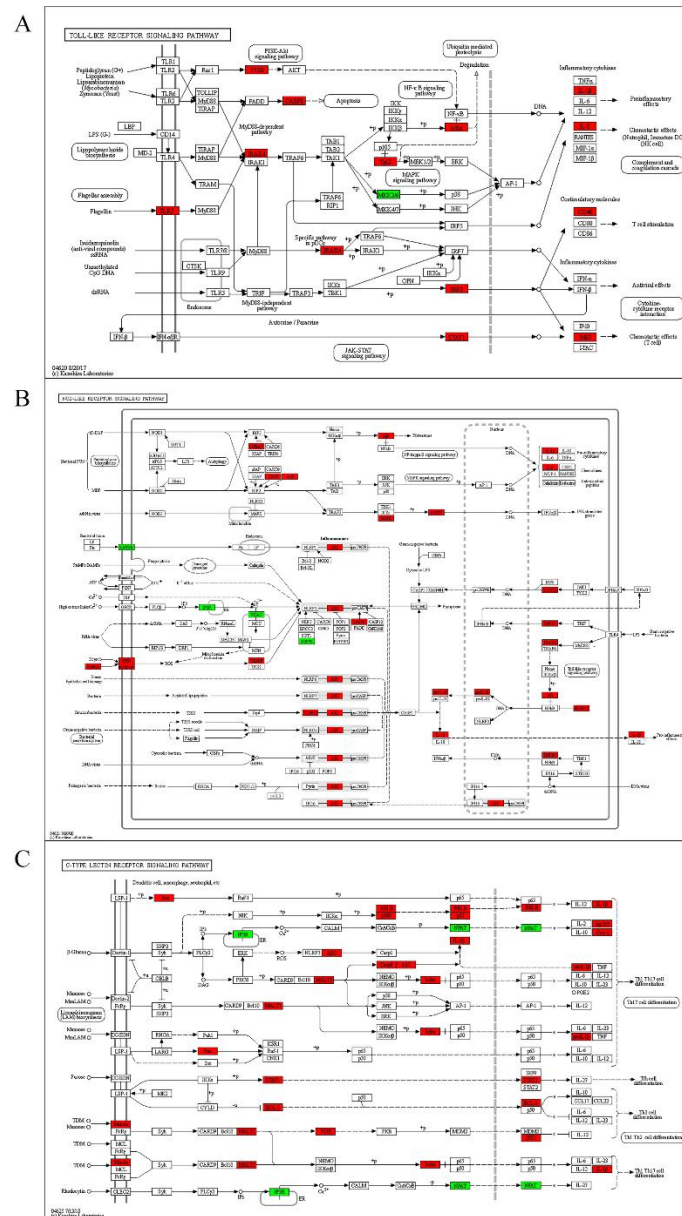
Supplementary Fig. 1



Supplementary Fig.1 Histopathological changes in the liver of the control and *A. hydrophila* groups.

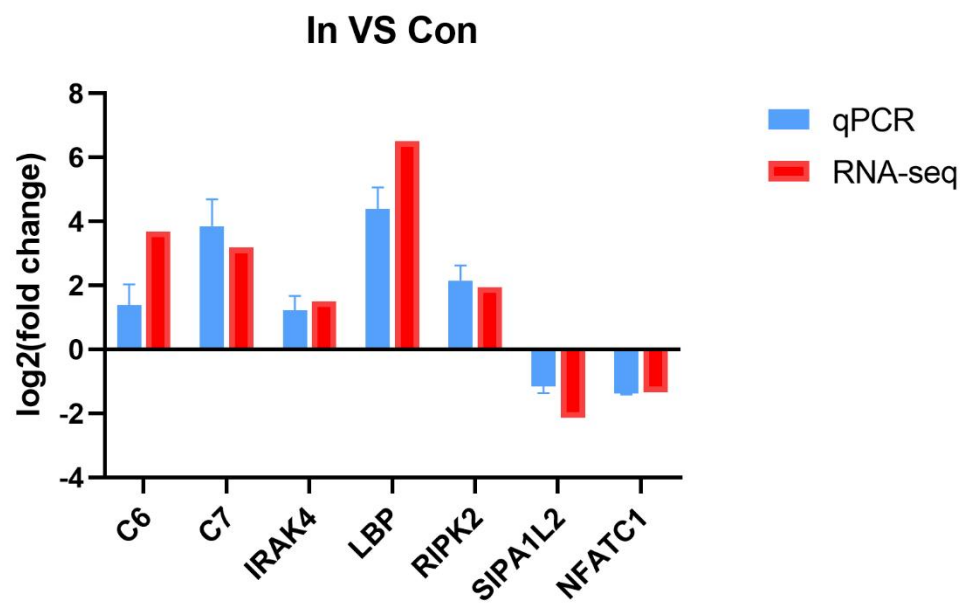
Note: A. The liver of the control group. B. The liver of the *A. hydrophila* group. Thick arrows= vacuolated hepatocytes, HS = hepatic sinusoid, HC = hepatocyte.

Supplementary Fig. 2



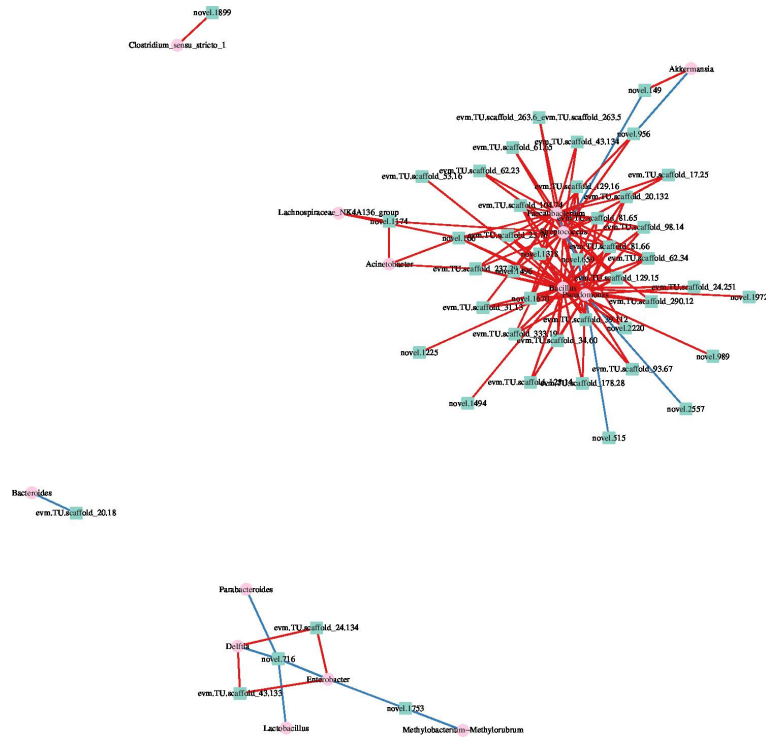
Supplementary Fig. 2 KEGG pathways annotation of differentially expressed genes. (A) DEGs are annotated to the Toll-like receptor signaling pathway. (B) DEGs are annotated to the NOD-like receptor signaling pathway. (C) C-type lectin receptor signaling pathway. For the infected group, the red box labeled protein is related to the up-regulated gene, and the green box labeled protein is related to the down-regulated gene. According to the differences of samples, the differential expression of genes related to metabolic pathway was mainly studied.

Supplementary Fig. 3



Supplementary Fig. 3 Validation of RNA-seq data by qRT-PCR analysis. X-axis, gene names; Y-axis, log₂(fold change) in gene expression. The relative expression of seven DEGs related to complement pathways and defense responses were determined by qRT-PCR with the *β-actin* as the internal reference.

Supplementary Fig. 4



Supplementary Fig. 4 Network interaction diagram of association analysis with the top 30 genera and significantly differentially expressed genes. The genes with significant differences ($|\log_2\text{foldchange}| \geq 5$) were screened for association analysis with the top 30 genera of relative abundance.