A transcriptome analysis focusing on inflammation-related genes of grass carp intestines following infection with

Aeromonas hydrophila

Xuehong Song^{1,#}, Xiaolong Hu^{1,2,#}, Bingyao Sun¹, Yunxuan Bo¹, Kang Wu¹, Lanying Xiao¹, Chengliang Gong^{1,2,*}

¹School of Biology and Basic Medical Sciences, Soochow University, Suzhou 215123, China

²National Engineering Laboratory for Modern Silk, Soochow University, Suzhou 215123, China

L

^{*} Corresponding author: gongcl@suda.edu.cn

List of Supplementary Information (Supplementary Tables and Figures)

- Table S1. Summary of sequencing data generated for grass carp transcriptome.
- Table S2. Summary of *de novo* grass carp transcriptome assembly results.
- Table S3. KEGG pathway analysis of the unigenes identified in the grass carp transcriptome.
- Table S4. Summary of the RNA-seq alignment statistics in the two libraries.
- Table S5. Gene ontology (GO) classification of the significantly differentially expressed genes.
- Table S6. The significantly enriched KEGG pathways affected by A. hydrophila infection.
- Table S7. The sequences of oligonucleotide primer pairs used in quantitative real-time PCR (qPCR).
- Figure S1. The size distribution of assembled contigs (A) and unigenes (B) from the grass carp transcriptome.
- Figure S2. Distribution of total clean tags from DGE libraries.
- Figure S3. The significantly DEG-enriched KEGG phagosome pathway.
- Figure S4. The significantly DEG-enriched KEGG lysosome pathway.
- Figure S5. The significantly DEG-enriched KEGG rheumatoid arthritis pathway.
- Figure S6. The significantly DEG-enriched KEGG antigen processing and presentation pathway.
- Figure S7. The significantly DEG-enriched KEGG oxidative phosphorylation pathway.
- Figure S8. The significantly DEG-enriched KEGG type I diabetes mellitus pathway.
- Figure S9. The significantly DEG-enriched KEGG ribosome pathway.

Total clean reads	Total clean nucleotides (nt)	Q20 percentage	N percentage	GC percentage
45,867,282	4,128,055,380	95.32%	0.00%	46.61%

Table S1. Summary of sequencing data generated for grass carp transcriptome.

Table S2. Summary of *de novo* grass carp transcriptome assembly results.

Sequence type	Total number	Total length (nt)	Mean length (nt)	N50	Total consensus sequences	Distinct clusters	Distinct singletons
Contig	120,964	44,621,250	369	614	-	-	-
Unigene	67,413	44,810,194	665	983	67,413	11,623	55,790

#	Pathway	number of unigenes with pathway annotation (28386 in total)	Pathway ID
1	Metabolic pathways	2946 (10.38%)	ko01100
2	Pathways in cancer	1388 (4.89%)	ko05200
3	Regulation of actin cytoskeleton	1140 (4.02%)	ko04810
4	Focal adhesion	1126 (3.97%)	ko04510
5	HTLV-I infection	983 (3.46%)	ko05166
6	Endocytosis	921 (3.24%)	ko04144
7	MAPK signaling pathway	894 (3.15%)	ko04010
8	Influenza A	879 (3.1%)	ko05164
9	Epstein-Barr virus infection	872 (3.07%)	ko05169
10	Herpes simplex infection	844 (2.97%)	ko05168
11	Tight junction	810 (2.85%)	ko04530
12	Transcriptional misregulation in cancer	782 (2.75%)	ko05202
13	Tuberculosis	744 (2.62%)	ko05152
14	Vascular smooth muscle contraction	679 (2.39%)	ko04270
15	Chemokine signaling pathway	661 (2.33%)	ko04062
16	RNA transport	649 (2.29%)	ko03013
17	Amoebiasis	642 (2.26%)	ko05146
18	Phagosome	641 (2.26%)	ko04145
19	Axon guidance	611 (2.15%)	ko04360
20	Purine metabolism	606 (2.13%)	ko00230
21	Adherens junction	599 (2.11%)	ko04520
22	Leukocyte transendothelial migration	595 (2.1%)	ko04670
23	Wnt signaling pathway	593 (2.09%)	ko04310
24	Insulin signaling pathway	591 (2.08%)	ko04910
25	<u>Spliceosome</u>	542 (1.91%)	ko03040
26	Huntington's disease	542 (1.91%)	ko05016

Table S3. KEGG pathway analysis of the unigenes identified in the grass carp transcriptome.

27 <u>Calcium signaling pathway</u>	530 (1.87%)	ko04020
28 Fc gamma R-mediated phagocytosis	525 (1.85%)	ko04666
29 <u>Toxoplasmosis</u>	516 (1.82%)	ko05145
30 Protein processing in endoplasmic reticulum	516 (1.82%)	ko04141
31 Ubiquitin mediated proteolysis	513 (1.81%)	ko04120
32 <u>Dilated cardiomyopathy</u>	512 (1.8%)	ko05414
33 <u>Cell adhesion molecules (CAMs)</u>	504 (1.78%)	ko04514
34 <u>Viral myocarditis</u>	493 (1.74%)	ko05416
35 <u>Salmonella infection</u>	485 (1.71%)	ko05132
36 ECM-receptor interaction	480 (1.69%)	ko04512
37 <u>Neurotrophin signaling pathway</u>	477 (1.68%)	ko04722
38 Hypertrophic cardiomyopathy (HCM)	468 (1.65%)	ko05410
39 <u>Alzheimer's disease</u>	460 (1.62%)	ko05010
40 <u>Measles</u>	456 (1.61%)	ko05162
41 Natural killer cell mediated cytotoxicity	440 (1.55%)	ko04650
42 <u>Cell cycle</u>	438 (1.54%)	ko04110
43 Cytokine-cytokine receptor interaction	433 (1.53%)	ko04060
44 <u>T cell receptor signaling pathway</u>	418 (1.47%)	ko04660
45 <u>NF-kappa B signaling pathway</u>	416 (1.47%)	ko04064
46 Osteoclast differentiation	413 (1.45%)	ko04380
47 Pathogenic Escherichia coli infection	412 (1.45%)	ko05130
48 Dopaminergic synapse	412 (1.45%)	ko04728
49 Phosphatidylinositol signaling system	408 (1.44%)	ko04070
50 <u>Hepatitis C</u>	401 (1.41%)	ko05160
51 Lysine degradation	398 (1.4%)	ko00310
52 <u>Small cell lung cancer</u>	398 (1.4%)	ko05222
53 <u>Prostate cancer</u>	395 (1.39%)	ko05215
54 Jak-STAT signaling pathway	394 (1.39%)	ko04630
55 <u>Pertussis</u>	393 (1.38%)	ko05133
56 Bacterial invasion of epithelial cells	392 (1.38%)	ko05100

57	Lysosome	387 (1.36%)	ko04142
58	Pancreatic secretion	383 (1.35%)	ko04972
59	<u>Melanogenesis</u>	378 (1.33%)	ko04916
60	Pyrimidine metabolism	377 (1.33%)	ko00240
61	mRNA surveillance pathway	371 (1.31%)	ko03015
62	B cell receptor signaling pathway	368 (1.3%)	ko04662
63	GnRH signaling pathway	362 (1.28%)	ko04912
64	Oocyte meiosis	359 (1.26%)	ko04114
65	Salivary secretion	354 (1.25%)	ko04970
66	TGF-beta signaling pathway	354 (1.25%)	ko04350
67	Renal cell carcinoma	352 (1.24%)	ko05211
68	Chagas disease (American trypanosomiasis)	347 (1.22%)	ko05142
69	ErbB signaling pathway	346 (1.22%)	ko04012
70	Cardiac muscle contraction	344 (1.21%)	ko04260
71	Protein digestion and absorption	343 (1.21%)	ko04974
72	Cholinergic synapse	338 (1.19%)	ko04725
73	Gastric acid secretion	336 (1.18%)	ko04971
74	Vibrio cholerae infection	335 (1.18%)	ko05110
75	Gap junction	333 (1.17%)	ko04540
76	Neuroactive ligand-receptor interaction	322 (1.13%)	ko04080
77	NOD-like receptor signaling pathway	321 (1.13%)	ko04621
78	Long-term potentiation	320 (1.13%)	ko04720
79	Fc epsilon RI signaling pathway	319 (1.12%)	ko04664
80	Chronic myeloid leukemia	319 (1.12%)	ko05220
81	Shigellosis	318 (1.12%)	ko05131
82	Progesterone-mediated oocyte maturation	316 (1.11%)	ko04914
83	Alcoholism	315 (1.11%)	ko05034
84	Complement and coagulation cascades	313 (1.1%)	ko04610
85	Serotonergic synapse	311 (1.1%)	ko04726
86	Leishmaniasis	308 (1.09%)	ko05140

87 <u>Amyotrophic lateral sclerosis (ALS)</u>	300 (1.06%)	ko05014
88 Notch signaling pathway	299 (1.05%)	ko04330
89 <u>VEGF signaling pathway</u>	296 (1.04%)	ko04370
90 <u>Glutamatergic synapse</u>	296 (1.04%)	ko04724
91 <u>Hematopoietic cell lineage</u>	293 (1.03%)	ko04640
92 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	293 (1.03%)	ko05412
93 <u>Apoptosis</u>	292 (1.03%)	ko04210
94 Adipocytokine signaling pathway	288 (1.01%)	ko04920
95 <u>Bile secretion</u>	286 (1.01%)	ko04976
96 Inositol phosphate metabolism	281 (0.99%)	ko00562
97 <u>Ribosome biogenesis in eukaryotes</u>	275 (0.97%)	ko03008
98 <u>Toll-like receptor signaling pathway</u>	273 (0.96%)	ko04620
99 Pancreatic cancer	265 (0.93%)	ko05212
100 Systemic lupus erythematosus	263 (0.93%)	ko05322
101 <u>Glioma</u>	261 (0.92%)	ko05214
102 <u>Colorectal cancer</u>	258 (0.91%)	ko05210
103 Antigen processing and presentation	258 (0.91%)	ko04612
104 <u>p53 signaling pathway</u>	250 (0.88%)	ko04115
105 <u>Glycerophospholipid metabolism</u>	248 (0.87%)	ko00564
106 <u>Ribosome</u>	244 (0.86%)	ko03010
107 <u>Retrograde endocannabinoid signaling</u>	243 (0.86%)	ko04723
108 <u>Peroxisome</u>	242 (0.85%)	ko04146
109 <u>Staphylococcus aureus infection</u>	240 (0.85%)	ko05150
110 <u>Legionellosis</u>	238 (0.84%)	ko05134
111 <u>RNA degradation</u>	236 (0.83%)	ko03018
112 mTOR signaling pathway	235 (0.83%)	ko04150
113 <u>PPAR signaling pathway</u>	232 (0.82%)	ko03320
114 Long-term depression	229 (0.81%)	ko04730
115 Parkinson's disease	224 (0.79%)	ko05012
116 Endometrial cancer	219 (0.77%)	ko05213

117 Epithelial cell signaling in Helicobacter pylori infection	217 (0.76%)	ko05120
118 Acute myeloid leukemia	215 (0.76%)	ko05221
119 <u>Rheumatoid arthritis</u>	212 (0.75%)	ko05323
120 GABAergic synapse	210 (0.74%)	ko04727
121 Amino sugar and nucleotide sugar metabolism	203 (0.72%)	ko00520
122 Morphine addiction	203 (0.72%)	ko05032
123 Non-small cell lung cancer	201 (0.71%)	ko05223
124 Basal cell carcinoma	197 (0.69%)	ko05217
125 <u>RIG-I-like receptor signaling pathway</u>	196 (0.69%)	ko04622
126 Prion diseases	194 (0.68%)	ko05020
127 Melanoma	192 (0.68%)	ko05218
128 Amphetamine addiction	192 (0.68%)	ko05031
129 <u>RNA polymerase</u>	188 (0.66%)	ko03020
130 ABC transporters	185 (0.65%)	ko02010
131 Oxidative phosphorylation	184 (0.65%)	ko00190
132 Mineral absorption	183 (0.64%)	ko04978
133 Autoimmune thyroid disease	183 (0.64%)	ko05320
134 <u>Hedgehog signaling pathway</u>	181 (0.64%)	ko04340
135 Endocrine and other factor-regulated calcium reabsorption	180 (0.63%)	ko04961
136 Allograft rejection	179 (0.63%)	ko05330
137 Glycolysis / Gluconeogenesis	179 (0.63%)	ko00010
138 Starch and sucrose metabolism	176 (0.62%)	ko00500
139 Fructose and mannose metabolism	176 (0.62%)	ko00051
140 <u>Type II diabetes mellitus</u>	174 (0.61%)	ko04930
141 Carbohydrate digestion and absorption	172 (0.61%)	ko04973
142 Aldosterone-regulated sodium reabsorption	169 (0.6%)	ko04960
143 <u>Type I diabetes mellitus</u>	168 (0.59%)	ko04940
144 Arginine and proline metabolism	166 (0.58%)	ko00330
145 Cytosolic DNA-sensing pathway	153 (0.54%)	ko04623
146 Graft-versus-host disease	153 (0.54%)	ko05332

147 <u>Glycerolipid metabolism</u>	152 (0.54%)	ko00561
148 Synaptic vesicle cycle	151 (0.53%)	ko04721
149 <u>Bladder cancer</u>	148 (0.52%)	ko05219
150 Pyruvate metabolism	146 (0.51%)	ko00620
151 Vasopressin-regulated water reabsorption	144 (0.51%)	ko04962
152 <u>Malaria</u>	142 (0.5%)	ko05144
153 <u>Thyroid cancer</u>	138 (0.49%)	ko05216
154 Basal transcription factors	137 (0.48%)	ko03022
155 African trypanosomiasis	135 (0.48%)	ko05143
156 Olfactory transduction	134 (0.47%)	ko04740
157 Arachidonic acid metabolism	133 (0.47%)	ko00590
158 Other types of O-glycan biosynthesis	132 (0.47%)	ko00514
159 Cysteine and methionine metabolism	130 (0.46%)	ko00270
160 Propanoate metabolism	129 (0.45%)	ko00640
161 Cocaine addiction	128 (0.45%)	ko05030
162 Intestinal immune network for IgA production	128 (0.45%)	ko04672
163 Dorso-ventral axis formation	127 (0.45%)	ko04320
164 <u>Sphingolipid metabolism</u>	127 (0.45%)	ko00600
165 Primary immunodeficiency	124 (0.44%)	ko05340
166 Fat digestion and absorption	123 (0.43%)	ko04975
167 N-Glycan biosynthesis	122 (0.43%)	ko00510
168 <u>Fanconi anemia pathway</u>	120 (0.42%)	ko03460
169 Nicotinate and nicotinamide metabolism	120 (0.42%)	ko00760
170 <u>Nucleotide excision repair</u>	117 (0.41%)	ko03420
171 Vitamin digestion and absorption	116 (0.41%)	ko04977
172 Fatty acid metabolism	114 (0.4%)	ko00071
173 Phototransduction - fly	114 (0.4%)	ko04745
174 <u>Circadian rhythm - mammal</u>	114 (0.4%)	ko04710
175 Drug metabolism - other enzymes	113 (0.4%)	ko00983
176 Aminoacyl-tRNA biosynthesis	111 (0.39%)	ko00970

177 <u>Glutathione metabolism</u>	110 (0.39%)	ko00480
178 Valine, leucine and isoleucine degradation	110 (0.39%)	ko00280
179 Pentose and glucuronate interconversions	109 (0.38%)	ko00040
180 <u>Tryptophan metabolism</u>	107 (0.38%)	ko00380
181 Metabolism of xenobiotics by cytochrome P450	106 (0.37%)	ko00980
182 <u>Retinol metabolism</u>	106 (0.37%)	ko00830
183 Steroid hormone biosynthesis	104 (0.37%)	ko00140
184 Proximal tubule bicarbonate reclamation	101 (0.36%)	ko04964
185 <u>Base excision repair</u>	99 (0.35%)	ko03410
186 Glycine, serine and threonine metabolism	97 (0.34%)	ko00260
187 Porphyrin and chlorophyll metabolism	96 (0.34%)	ko00860
188 Drug metabolism - cytochrome P450	95 (0.33%)	ko00982
189 Galactose metabolism	91 (0.32%)	ko00052
190 Ascorbate and aldarate metabolism	91 (0.32%)	ko00053
191 Ether lipid metabolism	90 (0.32%)	ko00565
192 Asthma	88 (0.31%)	ko05310
193 Alanine, aspartate and glutamate metabolism	87 (0.31%)	ko00250
194 <u>Proteasome</u>	87 (0.31%)	ko03050
195 <u>MAPK signaling pathway - fly</u>	84 (0.3%)	ko04013
196 DNA replication	83 (0.29%)	ko03030
197 <u>Citrate cycle (TCA cycle)</u>	78 (0.27%)	ko00020
198 Phototransduction	76 (0.27%)	ko04744
199 Linoleic acid metabolism	72 (0.25%)	ko00591
200 beta-Alanine metabolism	72 (0.25%)	ko00410
201 Collecting duct acid secretion	71 (0.25%)	ko04966
202 Pentose phosphate pathway	71 (0.25%)	ko00030
203 <u>Taste transduction</u>	66 (0.23%)	ko04742
204 Fatty acid elongation	65 (0.23%)	ko00062
205 Glyoxylate and dicarboxylate metabolism	64 (0.23%)	ko00630
206 Mismatch repair	64 (0.23%)	ko03430

207 <u>Circadian rhythm - fly</u>	64 (0.23%)	ko04711
208 Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	62 (0.22%)	ko00563
209 Homologous recombination	60 (0.21%)	ko03440
210 <u>Tyrosine metabolism</u>	60 (0.21%)	ko00350
211 Glycosaminoglycan biosynthesis - heparan sulfate	59 (0.21%)	ko00534
212 Biosynthesis of unsaturated fatty acids	58 (0.2%)	ko01040
213 <u>Regulation of autophagy</u>	57 (0.2%)	ko04140
214 SNARE interactions in vesicular transport	57 (0.2%)	ko04130
215 <u>Butanoate metabolism</u>	56 (0.2%)	ko00650
216 Fatty acid biosynthesis	55 (0.19%)	ko00061
217 <u>Histidine metabolism</u>	55 (0.19%)	ko00340
218 Mucin type O-Glycan biosynthesis	55 (0.19%)	ko00512
219 Other glycan degradation	53 (0.19%)	ko00511
220 Glycosaminoglycan biosynthesis - chondroitin sulfate	53 (0.19%)	ko00532
221 Non-homologous end-joining	53 (0.19%)	ko03450
222 <u>alpha-Linolenic acid metabolism</u>	49 (0.17%)	ko00592
223 Pantothenate and CoA biosynthesis	49 (0.17%)	ko00770
224 Primary bile acid biosynthesis	48 (0.17%)	ko00120
225 Glycosaminoglycan degradation	46 (0.16%)	ko00531
226 Glycosphingolipid biosynthesis - lacto and neolacto series	46 (0.16%)	ko00601
227 One carbon pool by folate	44 (0.16%)	ko00670
228 <u>Selenocompound metabolism</u>	43 (0.15%)	ko00450
229 Terpenoid backbone biosynthesis	39 (0.14%)	ko00900
230 <u>Renin-angiotensin system</u>	37 (0.13%)	ko04614
231 Maturity onset diabetes of the young	37 (0.13%)	ko04950
232 Phenylalanine metabolism	36 (0.13%)	ko00360
233 Steroid biosynthesis	36 (0.13%)	ko00100
234 Protein export	35 (0.12%)	ko03060
235 Glycosphingolipid biosynthesis - globo series	33 (0.12%)	ko00603
236 Glycosaminoglycan biosynthesis - keratan sulfate	33 (0.12%)	ko00533

237 <u>Sulfur relay system</u>	32 (0.11%)	ko04122
238 Glycosphingolipid biosynthesis - ganglio series	30 (0.11%)	ko00604
239 <u>Sulfur metabolism</u>	28 (0.1%)	ko00920
240 <u>Riboflavin metabolism</u>	27 (0.1%)	ko00740
241 Folate biosynthesis	27 (0.1%)	ko00790
242 Cyanoamino acid metabolism	27 (0.1%)	ko00460
243 Ubiquinone and other terpenoid-quinone biosynthesis	25 (0.09%)	ko00130
244 <u>Taurine and hypotaurine metabolism</u>	24 (0.08%)	ko00430
245 Nicotine addiction	19 (0.07%)	ko05033
246 Synthesis and degradation of ketone bodies	14 (0.05%)	ko00072
247 <u>Vitamin B6 metabolism</u>	13 (0.05%)	ko00750
248 <u>Thiamine metabolism</u>	12 (0.04%)	ko00730
249 D-Glutamine and D-glutamate metabolism	11 (0.04%)	ko00471
250 D-Arginine and D-ornithine metabolism	11 (0.04%)	ko00472
251 Phenylalanine, tyrosine and tryptophan biosynthesis	10 (0.04%)	ko00400
252 Butirosin and neomycin biosynthesis	10 (0.04%)	ko00524
253 Valine, leucine and isoleucine biosynthesis	8 (0.03%)	ko00290
254 <u>Caffeine metabolism</u>	8 (0.03%)	ko00232
255 Lipoic acid metabolism	7 (0.02%)	ko00785
256 Biotin metabolism	6 (0.02%)	ko00780
257 Polyketide sugar unit biosynthesis	4 (0.01%)	ko00523
258 Insect hormone biosynthesis	2 (0.01%)	ko00981
259 Lysine biosynthesis	2 (0.01%)	ko00300

Samples	Raw tags	Distinct tags	Clean tags	Distinct clean tags	Map to gene tags	Unique map to gene tags	Unknown tags
CG	3578555	322668	3362986	119479	2623920(78.02%)	2328453(69.24%)	739066(21.98%)
EG	3615650	316393	3404084	116890	2677430(78.65%)	2367148(69.54%)	726654(21.35%)

Table S4. Summary of the RNA-seq alignment statistics in the two libraries.

Gene Ontology term	Cluster frequency	Genome frequency of use	Corrected P-value	
Cellular Component	Cluster frequency (200)	Genome frequency of use (21131)		
extracellular region	33 (16.5%)	1267 (6.0%)	1.92E-05	
extracellular region part	20 (10.0%)	721 (3.4%)	0.0031	
extracellular matrix	14 (7.0%)	417 (2.0%)	0.00801	
hemoglobin complex	3 (1.5%)	9 (0.0%)	0.01163	
cytosolic part	10 (5.0%)	230 (1.1%)	0.01231	
cytosolic ribosome	6 (3.0%)	82 (0.4%)	0.02222	
MHC protein complex	5 (2.5%)	54 (0.3%)	0.02712	
proton-transporting two-sector ATPase complex	5 (2.5%)	58 (0.3%)	0.03811	
Molecular Function	Cluster frequency (210)	Genome frequency of use (22432)		
cytokine activity	6 (2.9%)	56 (0.2%)	0.00292	
oxygen transporter activity	3 (1.4%)	12 (0.1%)	0.03544	
structural molecule activity	18 (8.6%)	730 (3.3%)	0.03959	
monocarboxylic acid transmembrane transporter activity	4 (1.9%)	31 (0.1%)	0.04083	
Biological Process	Cluster frequency (214)	Genome frequency of use (21350)		
regulation of transcription involved in G1/S phase of mitotic cell cycle	3 (1.4%)	10 (0.0%)	0.010034	
response to bacterium	11 (5.1%)	278 (1.3%)	0.010739	
energy coupled proton transport, down electrochemical gradient	4 (1.9%)	32 (0.1%)	0.025118	
ATP synthesis coupled proton transport	4 (1.9%)	32 (0.1%)	0.025118	
prostaglandin transport	2 (0.9%)	3 (0.0%)	0.026434	
gas transport	3 (1.4%)	15 (0.1%)	0.036665	
oxygen transport	3 (1.4%)	15 (0.1%)	0.036665	
viral genome expression	5 (2.3%)	63 (0.3%)	0.037538	
viral transcription	5 (2.3%)	63 (0.3%)	0.037538	
translational termination	5 (2.3%)	65 (0.3%)	0.043404	
immune response	13 (6.1%)	440 (2.1%)	0.046614	
ATP biosynthetic process	7 (3.3%)	140 (0.7%)	0.047625	

Table S5. Gene ontology (GO) classification of the significantly differentially expressed genes.

Pathway	DEGs with pathway annotation (260)	Genes with pathway annotation (28386)	P-value	Q-value	Pathway ID
Rheumatoid arthritis	11 (4.23%)	212 (0.75%)	4.56E-06	0.000428	ko05323
Antigen processing and presentation	12 (4.62%)	258 (0.91%)	5.18E-06	0.000428	ko04612
Phagosome	16 (6.15%)	641 (2.26%)	0.00030861	0.012821	ko04145
Oxidative phosphorylation	8 (3.08%)	184 (0.65%)	0.00031082	0.012821	ko00190
Type I diabetes mellitus	7 (2.69%)	168 (0.59%)	0.00094356	0.026215	ko04940
Lysosome	11 (4.23%)	387 (1.36%)	0.00095326	0.026215	ko04142
Ribosome	8 (3.08%)	244 (0.86%)	0.00193558	0.045624	ko03010

Table S6. The significantly enriched KEGG pathways affected by A. hydrophila infection.

Note: Only the Q-values for pathways considered as significantly enriched (Q-value < 0.05) were shown in the table.

Gene names	Upstream primers	Downstream primers
IL-8	qIL8F: GCTGCTAGACTGTTTGCTCCAC	qIL8R: GAGGCAGGTCTGTCCTTGTTTC
MHC I	qMHCF: TGGAGCAGCGGCAAACTAC	qMHCR: TTGGGTAGAATCCTGTGGCA
CCR4	qCCRF: ATGGTAGGGAAACATCGCACT	qCCRR: GCAGAAATACAAGCGAGCGA
CXCL12	qCXCLF: GCTCAAGTTCCTCCACACACC	qCXCLR: GACGGTGGGCTGTCAGATTC
CCL25	qCCLF: TAACTAAACAGAGGAGGAGACATAAGC	qCCLR: CAGTTTTCATAGGAACCTTGTGC
ATP1A4	qATPF: GTTCTTCTTTGCCATTCGTTTG	qATPR: AGCACTTCATCCACATCATCACT
ELA2	qELAF: GTCAAGGAATCTATGGTCTGTGC	qELAR: GGACACACGGGTAAAGACAGTAG
RRM2	qRRMF: TTGGTGGAACGATTTACTCAGG	qRRMR: ACAAGGCATTGTCTCAATAGCA
PRSS	qPRSSF: ATGGTATTGTGTCCTGGGGTT	qPRSSR: TTAGTTGCTGCTTATGGTGTCG
PLA2	qPLAF: CCCTACAGCAATCCCACCTC	qPLAR: TTGGCTCTCACGCAATCTTC
CTSK	qCTSKF: TTACGGAGTCACACCGAAAGG	qCTSKR: TTACGAGCCATCAGGACATACC
RASA1	qRASAF: CAAACGCTTGTTGAATGTGGT	qRASAR: CAGATTGTGATTCAGCACTTTAGC
LASP1	qLASPF: CGTTTCTTCACCTTATTCCTTCG	qLASPR: CAGTATGGTCTCTTCTCAAAGCCTT
CD21	qCDF: CAATGCCACACTACTGTCCTACC	qCDR: GACCCATAGAGACGGTATCCACT
SERCA	qSERCAF: AGCGACAGCAACTCCAATAGC	qSERCAR: GATGCTGTCCCCGACCTTAG
IL-6	qIL6F: CAACGACATCAAACACGAGACC	qIL6R: TGTCCACCCTTCCTCTTGCT
IL-10	qIL10F: GGATGTTGCTGTGGATTGGA	qIL10R: TGCCTTATCCTACAGTATGTGGTT
IL10Ra	q10RAF: TCCGTGTGAAAGTGGAGATGAC	q10RAR: GGATTTCAGAACAGCAGGCAT

Table S7. The sequences of oligonucleotide primer pairs used in quantitative real-time PCR (qPCR).

IL10Rβ	q10RBF: ACTGACAGCAGCAGGGAGAAG	q10RBR: AGCAGAACCGAAAGAAAGCC
IL-12p40	qp40F: AGGAGCATCAATCTCACTCTGTTC	qp40R: GCCACTCAAATACATCATCTTCG
IL12Rβ2	q12RBF: CTCTTGAGGGATTTCAGACCATT	q12RBR: CACACATTAGAGGCATCTCCACA
IL-17R	qIL17RF: TCTCTGGCTGGACTCTTGGAT	qIL17RR: GAGGAGGATGTGAGAGGAGAAAC
IL2RG	qIL2RGF: GGTTGGAGAGGATGCTTTCAC	qIL2RGR: GCCTGTCCTGTGCGTGAGTA
IL-22	qIL22F: GTCGTGTTTCGTGGTCTTTGTC	qIL22R: TGTGCTGCTGGTGCTTTCTG
IL-17A/F1	qAF1F : GGACCTACACGTTTACGCATG	qAF1R: ACAGGTAATCCTCACTGCCATC
IL-17D	q17DF: ACCTGTCAATCAGCGTCTCG	q17DR: CTGGATCATGGGAGATTCTGTAC
IL23R	q23RF: GCCGATGATTCCAACTATTTACAC	q23RR: TGACCTGTGTTGAAGACAGACTCT
β-actin	actF: CCTTCTTGGGTATGGAGTCTTG	actR: AGAGTATTTACGCTCAGGTGGG

Notes: IL-8: interleukin-8; MHC I: major histocompatibility complex, class I; CCR4: chemokine (C-C motif) receptor 4; CXCL12: chemokine (C-X-C motif) ligand 12; CCL25: chemokine (C-C motif) ligand 25 β ; ATP1A4: Na⁺/K⁺ transporting ATPase subunit α -4; ELA2: pancreatic elastase II; RRM2: ribonucleoside-diphosphate reductase subunit M2; PRSS: trypsinogen; PLA2: phospholipase A2; CTSK: cathepsin K; RASA1: RasGTPase-activating protein 1; LASP1: LIM and SH3 protein 1; CD21: cluster of differentiation 21; SERCA: Sarco (endo) plasmic reticulum calcium ATPase; IL-6: interleukin-6; IL-10: interleukin-10; IL10RA: interleukin-10 receptor α ; IL10RB: interleukin-10 receptor β ; IL-12p40: interleukin-12 p40; IL12RB2: interleukin-12 receptor β -2; IL17R: interleukin-17 receptor; IL-17A/F1: interleukin-17A/F1; IL2RG: interleukin-2 receptor γ ; IL-22: interleukin-22; IL-17D: interleukin-17D; IL-23R: interleukin-23 receptor.



Figure S1. The size distribution of assembled contigs (A) and unigenes (B) from the grass carp transcriptome.



Figure S2. Distribution of total clean tags from DGE libraries.

The numbers (and the percentage in parentheses) indicate each tag class (including tags containing N, empty tags with the adaptor only, tags with a copy number < 2, and clean tags) number out of the total number of tags. Data from the experimental group (top) and control group (bottom) are shown.



Figure S3. The significantly DEG-enriched KEGG phagosome pathway.



Figure S4. The significantly DEG-enriched KEGG lysosome pathway.



Figure S5.The significantly DEG-enriched KEGG rheumatoid arthritis pathway.



Figure S6. The significantly DEG-enriched KEGG antigen processing and presentation pathway.



Figure S7. The significantly DEG-enriched KEGG oxidative phosphorylation pathway.



Figure S8. The significantly DEG-enriched KEGG type I diabetes mellitus pathway.



Figure S9. The significantly DEG-enriched KEGG ribosome pathway.