Supplementary Material

**1 Supplementary Figures**



**Fig. S1.** Hierarchical cluster analysis (HCA) of whole genes expression. Gi represents *Crassostrea gigas*, and An represents *C. angulata*. (**A**) Result before deleting Gi-1 and Gi-7. (**B**) Result after deleting Gi-1 and Gi-7.



**Fig. S2.** Principal component analysis (PCA) and hierarchical cluster analysis (HCA) plots from the LC-QTOF-MS metabolite profiles of *Crassostrea gigas* and *C. angulata*. (**A**, **B**) Metabolic profiles of *C. gigas* and *C. angulata* in the positive and negative ionization modes, visualized by PCA. The points represent the scores of biological replicates. (**C**, **D**) Heat map of all identifiable metabolites in the positive and negative ionization modes comparing *C. gigas*, and *C. angulata*. Columns represent biological replicates (n = 10 per group), and rows represent individual metabolites. The dendrograms denote the overall similarity of the metabolite expression profiles (y-axis, left). The dendrograms at the top of the x-axis denote the similarity of samples. Gi represents *C. gigas*, and An represents *C. angulata*.



**Fig. S3**. Co-enrichment pathway of differentially abundant metabolites and differentially expressed genes.

**2 Supplementary Tables**

|  |  |  |
| --- | --- | --- |
| Abbreviation | Gene name | Primer sequence(5′-3′) |
| GPX | Glutathione peroxidase | F: TGCTCCTTCTCCCCTTCCTC |
| R: CCAAATCCACAGTTTCCAAGTT |
| CAT | Catalase | F: AGAATGGTCCTGAACAGAAATC |
| R: AATGGACAGTTGACGGGAAT |
| GST | Glutathione S-Transferase | F: TTACGAAAACGGAAGAACAAGC |
| R: AAGAGGTCCCCACTAGGTAGCC |
| SOD | Superoxide Dismutase | F: ATCCACGAGTACGGAGATATG |
| R: AAGTCCTGGTAGCATTCACAT |
| XDH | Xanthine Dehydrogenase | F: TAGCGGCGGATCAAGTTAGG |
| R: CAGGCAAAGATGTAGGGTCA |
| EF-1α  (*C.gigas*) | Eukaryotic Translation Elongation Factor 1 Alpha | F: AGTCACCAAGGCTGCACAGAAAG |
| R: TCCGACGTATTTCTTTGCGATGT |
| EF-1α (*C.angulata*) | Eukaryotic Translation Elongation Factor 1 Alpha | F: TTCCCAGCAAGCCTATGT |
| R: GCTCAGCCTTCTCAACCTC |

**Table S1.** Abbreviations of genes and primer sequences in *Crassostrea gigas* and *C. angulata*.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | Sample | Clean Reads | Q30(%) | GC content(%) | Mapped Reads(%) |
| Crassostrea gigas | 1 | 51880130 | 93.28 | 42.38 | 73.48 |
| 2 | 40939952 | 93.75 | 42.25 | 72.81 |
| 3 | 40757922 | 92.80 | 42.03 | 71.90 |
| 4 | 41830586 | 94.13 | 42.79 | 71.18 |
| 5 | 43502566 | 93.73 | 41.95 | 72.05 |
| 6 | 44033982 | 93.58 | 42.41 | 73.38 |
| 7 | 41973848 | 93.24 | 42.44 | 72.61 |
| 8 | 41600998 | 93.67 | 42.64 | 73.59 |
| Crassostrea angulata | 1 | 53744468 | 93.79 | 42.62 | 70.98 |
| 2 | 46390630 | 93.60 | 42.42 | 70.30 |
| 3 | 42003262 | 93.82 | 42.55 | 70.80 |
| 4 | 45825614 | 92.83 | 42.93 | 71.03 |
| 5 | 42723230 | 93.40 | 42.14 | 67.12 |
| 6 | 41578148 | 93.48 | 41.83 | 68.75 |
| 7 | 51798384 | 93.34 | 42.21 | 69.98 |
| 8 | 51127002 | 93.14 | 42.16 | 69.69 |

**Table S2.** Statistics of the transcriptome sequencing data

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Abbreviation | Gene\_ID | Gene name | Gi\_fpkm | An\_fpkm | log2(FC) | FDR |
| HK | Cg24577 | Hexokinases | 8.8498 | 26.5162 | -1.4813 | 1.32E-08 |
| GPI | Cg06526 | Glucose-6-Phosphate Isomerase | 0.8160 | 3.1084 | -1.7842 | 8.60E-13 |
| EHHADH | Cg29727 | Peroxisomal Bifunctional Enzyme | 1.4879 | 4.5770 | -1.4835 | 2.13E-07 |
| FASN | Cg18495 | Fatty Acid Synthase | 8.1931 | 24.9377 | -1.1917 | 0.009641899 |
| BHMT | Cg10929 | Betaine-homocysteine S-methyltransferase | 0.3427 | 2.2249 | -1.3885 | 0.007226192 |
| PIPOX | Cg26789 | Sarcosine oxidase | 0.1554 | 1.4594 | -1.8049 | 0.000245791 |
| CHDH | Cg08575 | Choline dehydrogenase | 0.9400 | 12.2884 | -1.5727 | 0.002115109 |
| ASPB | Cg21019 | Aspartate aminotransferase | 1.3917 | 10.6426 | -2.7437 | 7.41E-34 |
| DDO | Cg06694 | D-aspartate oxidase | 0.4533 | 1.2465 | -1.2786 | 0.000142482 |
| CTH | Cg19223 | Cystathionine gamma-lyase | 44.2832 | 21.2743 | 1.0051 | 1.07E-13 |
| PAH | Cg07773 | Phenylalanine-4-hydroxylase | 1.4016 | 4.5678 | -1.4769 | 2.25E-05 |
| GGT | Cg17027 | Gamma-glutamyl transpeptidase | 1.1361 | 14.3276 | -2.1010 | 1.25E-05 |
| ANPEP | Cg09618 | Aminopeptidase N | 2.3797 | 10.2718 | -2.0147 | 1.90E-21 |
| RPS19 | Cg14612 | 40S ribosomal protein S19 | 871.2604 | 394.8536 | 1.0563 | 1.01E-06 |
| RPLP1 | Cg21254 | 60S acidic ribosomal protein P1 | 56.1176 | 414.4956 | -2.0721 | 2.18E-06 |
| CHIT | Cg05227 | Chitinase | 8.0946 | 2.2277 | 1.5894 | 9.24E-07 |
| SCD | Cg17019 | Stearoyl-CoA desaturase | 4.2601 | 12.0837 | -1.3331 | 6.34E-05 |
| GST | Cg08544 | Glutathione S-transferase | 9.3374 | 2.3289 | 1.8914 | 1.08E-24 |
| GPX | Cg19453 | Glutathione peroxidase | 64.5947 | 16.6284 | 1.6491 | 1.93E-06 |
| AMIE | Cg10211 | Amidase | 45.3839 | 19.7986 | 1.1272 | 9.67E-11 |
| SMOX | Cg21952 | Spermine oxidase | 4.8183 | 1.0944 | 1.3435 | 0.007212415 |
| DDC | Cg14520 | Aromatic-L-amino-acid decarboxylase | 7.3668 | 1.1775 | 2.3762 | 1.12E-19 |
| COMT | Cg27340 | Catechol O-methyltransferase | 16.1196 | 3.5350 | 1.5475 | 0.000706496 |

**Table S4.** Expression levels and annotation of the genes referred to in this article.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Term** | **ID** | **Input number** | **Background number** | **P-Value** | **Qvalue** |
| Peroxisome | ko04146 | 23 | 154 | 1.45E-05 | 0.001966653 |
| Notch signaling pathway | ko04330 | 24 | 171 | 2.75E-05 | 0.001966653 |
| Pyrimidine metabolism | ko00240 | 13 | 83 | 0.000673431 | 0.024286866 |
| Dopaminergic synapse | ko04728 | 3 | 4 | 0.000678604 | 0.024286866 |
| Amino sugar and nucleotide sugar metabolism | ko00520 | 19 | 162 | 0.00179284 | 0.051331832 |
| Glutathione metabolism | ko00480 | 14 | 107 | 0.002554275 | 0.060944117 |
| Glycine, serine and threonine metabolism | ko00260 | 10 | 76 | 0.009721731 | 0.198820364 |
| Nitrogen metabolism | ko00910 | 6 | 37 | 0.016130894 | 0.279581227 |
| Glycosphingolipid biosynthesis - globo and isoglobo series | ko00603 | 7 | 49 | 0.018824411 | 0.279581227 |
| Phenylalanine metabolism | ko00360 | 4 | 19 | 0.019529571 | 0.279581227 |
| Glycosphingolipid biosynthesis - lacto and neolacto series | ko00601 | 9 | 77 | 0.028113076 | 0.326562954 |
| Toll and Imd signaling pathway | ko04624 | 6 | 42 | 0.028786393 | 0.326562954 |
| Alanine, aspartate and glutamate metabolism | ko00250 | 6 | 43 | 0.0319271 | 0.326562954 |
| Drug metabolism - other enzymes | ko00983 | 9 | 79 | 0.032532547 | 0.326562954 |
| Arachidonic acid metabolism | ko00590 | 13 | 132 | 0.034217074 | 0.326562954 |
| Purine metabolism | ko00230 | 17 | 189 | 0.036957065 | 0.330668477 |
| Phenylalanine, tyrosine and tryptophan biosynthesis | ko00400 | 2 | 6 | 0.040760727 | 0.343248224 |

**Table S5.** KEGG pathways enriched from the transcriptome analysis.

**Positive ionization mode**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Description** | **MetaboliteRatio** | **BgRatio** | **Enrich\_factor** | ***P*-value** | **Qvalue** |
| **ko00650** | **Butanoate metabolism** | **3.54%** | **1.64%** | **2.16** | **0.044687249** | **0.702477436** |
| **ko00760** | **Nicotinate and nicotinamide metabolism** | **3.54%** | **1.64%** | **2.16** | **0.044687249** | **0.702477436** |
| **ko01200** | **Carbon metabolism** | **6.19%** | **3.69%** | **1.68** | **0.055228049** | **0.702477436** |
| **ko00630** | **Glyoxylate and dicarboxylate metabolism** | **4.42%** | **2.46%** | **1.8** | **0.075916905** | **0.702477436** |
| **ko04216** | **Ferroptosis** | **4.42%** | **2.46%** | **1.8** | **0.075916905** | **0.702477436** |
| **ko00020** | **Citrate cycle (TCA cycle)** | **2.65%** | **1.23%** | **2.16** | **0.0979057** | **0.702477436** |
| **ko00430** | **Taurine and hypotaurine metabolism** | **2.65%** | **1.23%** | **2.16** | **0.0979057** | **0.702477436** |
| **ko00620** | **Pyruvate metabolism** | **2.65%** | **1.23%** | **2.16** | **0.0979057** | **0.702477436** |
| **ko04922** | **Glucagon signaling pathway** | **2.65%** | **1.23%** | **2.16** | **0.0979057** | **0.702477436** |
| **ko00130** | **Ubiquinone and other terpenoid-quinone**  **biosynthesis** | **3.54%** | **2.05%** | **1.73** | **0.14225441** | **0.702477436** |
| **ko04913** | **Ovarian steroidogenesis** | **4.42%** | **2.87%** | **1.54** | **0.167061292** | **0.702477436** |
| **ko05230** | **Central carbon metabolism in cancer** | **4.42%** | **2.87%** | **1.54** | **0.167061292** | **0.702477436** |
| **ko05200** | **Pathways in cancer** | **5.31%** | **3.69%** | **1.44** | **0.182364788** | **0.702477436** |
| **ko00230** | **Purine metabolism** | **6.19%** | **4.51%** | **1.37** | **0.192278633** | **0.702477436** |
| **ko00250** | **Alanine, aspartate and glutamate metabolism** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko00472** | **D-Arginine and D-ornithine metabolism** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko00562** | **Inositol phosphate metabolism** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko00920** | **Sulfur metabolism** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko01523** | **Antifolate resistance** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko04664** | **Fc epsilon RI signaling pathway** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko04727** | **GABAergic synapse** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko04931** | **Insulin resistance** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko04975** | **Fat digestion and absorption** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko04979** | **Cholesterol metabolism** | **1.77%** | **0.82%** | **2.16** | **0.213452068** | **0.702477436** |
| **ko00100** | **Steroid biosynthesis** | **2.65%** | **1.64%** | **1.62** | **0.257561054** | **0.702477436** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ko00740** | **Riboflavin metabolism** | **2.65%** | **1.64%** | **1.62** | **0.257561054** | **0.702477436** |
| **ko00980** | **Metabolism of xenobiotics by cytochrome P450** | **2.65%** | **1.64%** | **1.62** | **0.257561054** | **0.702477436** |
| **ko01212** | **Fatty acid metabolism** | **2.65%** | **1.64%** | **1.62** | **0.257561054** | **0.702477436** |
| **ko04714** | **Thermogenesis** | **2.65%** | **1.64%** | **1.62** | **0.257561054** | **0.702477436** |
| **ko04723** | **Retrograde endocannabinoid signaling** | **2.65%** | **1.64%** | **1.62** | **0.257561054** | **0.702477436** |
| **ko05215** | **Prostate cancer** | **2.65%** | **1.64%** | **1.62** | **0.257561054** | **0.702477436** |
| **ko00310** | **Lysine degradation** | **4.42%** | **3.28%** | **1.35** | **0.282818509** | **0.702477436** |
| **ko00590** | **Arachidonic acid metabolism** | **7.08%** | **6.15%** | **1.15** | **0.382207367** | **0.702477436** |
| **ko00240** | **Pyrimidine metabolism** | **5.31%** | **4.51%** | **1.18** | **0.398989702** | **0.702477436** |
| **ko00380** | **Tryptophan metabolism** | **3.54%** | **2.87%** | **1.23** | **0.418753589** | **0.702477436** |
| **ko00480** | **Glutathione metabolism** | **3.54%** | **2.87%** | **1.23** | **0.418753589** | **0.702477436** |
| **ko00061** | **Fatty acid biosynthesis** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko00270** | **Cysteine and methionine metabolism** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko00280** | **Valine, leucine and isoleucine degradation** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko00330** | **Arginine and proline metabolism** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko00340** | **Histidine metabolism** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko00790** | **Folate biosynthesis** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko01522** | **Endocrine resistance** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko04750** | **Inflammatory mediator regulation of TRP**  **channels** | **2.65%** | **2.05%** | **1.3** | **0.430521021** | **0.702477436** |
| **ko00040** | **Pentose and glucuronate interconversions** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko00062** | **Fatty acid elongation** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko00190** | **Oxidative phosphorylation** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko00360** | **Phenylalanine metabolism** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko00531** | **Glycosaminoglycan degradation** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko00565** | **Ether lipid metabolism** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko00640** | **Propanoate metabolism** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko04071** | **Sphingolipid signaling pathway** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko04742** | **Taste transduction** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ko04918** | **Thyroid hormone synthesis** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko04929** | **GnRH secretion** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko05204** | **Chemical carcinogenesis** | **1.77%** | **1.23%** | **1.44** | **0.444544802** | **0.702477436** |
| **ko00053** | **Ascorbate and aldarate metabolism** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00072** | **Synthesis and degradation of ketone bodies** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00220** | **Arginine biosynthesis** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00232** | **Caffeine metabolism** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00261** | **Monobactam biosynthesis** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00311** | **Penicillin and cephalosporin biosynthesis** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00500** | **Starch and sucrose metabolism** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00523** | **Polyketide sugar unit biosynthesis** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00561** | **Glycerolipid metabolism** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko00670** | **One carbon pool by folate** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04066** | **HIF-1 signaling pathway** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04070** | **Phosphatidylinositol signaling system** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04150** | **mTOR signaling pathway** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04211** | **Longevity regulating pathway** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04212** | **Longevity regulating pathway - worm** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04217** | **Necroptosis** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04218** | **Cellular senescence** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04611** | **Platelet activation** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04722** | **Neurotrophin signaling pathway** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04920** | **Adipocytokine signaling pathway** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04925** | **Aldosterone synthesis and secretion** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04927** | **Cortisol synthesis and secretion** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04933** | **AGE-RAGE signaling pathway in diabetic**  **complications** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04934** | **Cushing syndrome** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04964** | **Proximal tubule bicarbonate reclamation** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ko05014** | **Amyotrophic lateral sclerosis** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko05032** | **Morphine addiction** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko05033** | **Nicotine addiction** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko05142** | **Chagas disease** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko05211** | **Renal cell carcinoma** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko05217** | **Basal cell carcinoma** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko05225** | **Hepatocellular carcinoma** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko05310** | **Asthma** | **0.88%** | **0.41%** | **2.16** | **0.463114754** | **0.702477436** |
| **ko04976** | **Bile secretion** | **10.62%** | **10.25%** | **1.04** | **0.511431803** | **0.767147704** |
| **ko04080** | **Neuroactive ligand-receptor interaction** | **3.54%** | **3.28%** | **1.08** | **0.554688669** | **0.822889784** |
| **ko00520** | **Amino sugar and nucleotide sugar metabolism** | **2.65%** | **2.46%** | **1.08** | **0.586112623** | **0.832896885** |
| **ko00600** | **Sphingolipid metabolism** | **2.65%** | **2.46%** | **1.08** | **0.586112623** | **0.832896885** |
| **ko00970** | **Aminoacyl-tRNA biosynthesis** | **2.65%** | **2.46%** | **1.08** | **0.586112623** | **0.832896885** |
| **ko04726** | **Serotonergic synapse** | **2.65%** | **2.46%** | **1.08** | **0.586112623** | **0.832896885** |
| **ko00071** | **Fatty acid degradation** | **1.77%** | **1.64%** | **1.08** | **0.63152855** | **0.836738734** |
| **ko00770** | **Pantothenate and CoA biosynthesis** | **1.77%** | **1.64%** | **1.08** | **0.63152855** | **0.836738734** |
| **ko00983** | **Drug metabolism - other enzymes** | **1.77%** | **1.64%** | **1.08** | **0.63152855** | **0.836738734** |
| **ko04024** | **cAMP signaling pathway** | **1.77%** | **1.64%** | **1.08** | **0.63152855** | **0.836738734** |
| **ko04917** | **Prolactin signaling pathway** | **1.77%** | **1.64%** | **1.08** | **0.63152855** | **0.836738734** |
| **ko02010** | **ABC transporters** | **8.85%** | **9.43%** | **0.94** | **0.691962049** | **0.836738734** |
| **ko00120** | **Primary bile acid biosynthesis** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko00290** | **Valine, leucine and isoleucine biosynthesis** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko00300** | **Lysine biosynthesis** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko00350** | **Tyrosine metabolism** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko00730** | **Thiamine metabolism** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko00981** | **Insect hormone biosynthesis** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko04122** | **Sulfur relay system** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko04152** | **AMPK signaling pathway** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko04361** | **Axon regeneration** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ko04721** | **Synaptic vesicle cycle** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko04725** | **Cholinergic synapse** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko04915** | **Estrogen signaling pathway** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko05144** | **Malaria** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko05205** | **Proteoglycans in cancer** | **0.88%** | **0.82%** | **1.08** | **0.71277744** | **0.836738734** |
| **ko04977** | **Vitamin digestion and absorption** | **4.42%** | **4.92%** | **0.9** | **0.732566361** | **0.852555679** |
| **ko00410** | **beta-Alanine metabolism** | **1.77%** | **2.05%** | **0.86** | **0.765533569** | **0.883307965** |
| **ko05231** | **Choline metabolism in cancer** | **7.08%** | **8.20%** | **0.86** | **0.794459882** | **0.908915966** |
| **ko01040** | **Biosynthesis of unsaturated fatty acids** | **3.54%** | **4.51%** | **0.79** | **0.837697595** | **0.929517541** |
| **ko00010** | **Glycolysis / Gluconeogenesis** | **0.88%** | **1.23%** | **0.72** | **0.84689376** | **0.929517541** |
| **ko00052** | **Galactose metabolism** | **0.88%** | **1.23%** | **0.72** | **0.84689376** | **0.929517541** |
| **ko00524** | **Neomycin, kanamycin and gentamicin**  **biosynthesis** | **0.88%** | **1.23%** | **0.72** | **0.84689376** | **0.929517541** |
| **ko00900** | **Terpenoid backbone biosynthesis** | **0.88%** | **1.23%** | **0.72** | **0.84689376** | **0.929517541** |
| **ko04974** | **Protein digestion and absorption** | **1.77%** | **2.46%** | **0.72** | **0.855244042** | **0.931112466** |
| **ko00140** | **Steroid hormone biosynthesis** | **6.19%** | **7.79%** | **0.8** | **0.865033527** | **0.934236209** |
| **ko00591** | **Linoleic acid metabolism** | **1.77%** | **2.87%** | **0.62** | **0.9126889** | **0.946733527** |
| **ko01230** | **Biosynthesis of amino acids** | **1.77%** | **2.87%** | **0.62** | **0.9126889** | **0.946733527** |
| **ko00564** | **Glycerophospholipid metabolism** | **7.08%** | **9.43%** | **0.75** | **0.918142254** | **0.946733527** |
| **ko00592** | **alpha-Linolenic acid metabolism** | **2.65%** | **4.10%** | **0.65** | **0.918674712** | **0.946733527** |
| **ko05140** | **Leishmaniasis** | **0.88%** | **1.64%** | **0.54** | **0.918682163** | **0.946733527** |
| **ko05146** | **Amoebiasis** | **0.88%** | **1.64%** | **0.54** | **0.918682163** | **0.946733527** |
| **ko00260** | **Glycine, serine and threonine metabolism** | **0.88%** | **2.05%** | **0.43** | **0.956969311** | **0.964110873** |
| **ko00780** | **Biotin metabolism** | **0.88%** | **2.05%** | **0.43** | **0.956969311** | **0.964110873** |
| **ko00860** | **Porphyrin and chlorophyll metabolism** | **0.88%** | **2.05%** | **0.43** | **0.956969311** | **0.964110873** |
| **ko00982** | **Drug metabolism - cytochrome P450** | **0.88%** | **2.46%** | **0.36** | **0.977314365** | **0.977314365** |

**Negative ionization modes**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Description** | **MetaboliteRatio** | **BgRatio** | **Enrich\_factor** | ***P*-value** | **Qvalue** |
| **ko04976** | **Bile secretion** | **17.14%** | **7.69%** | **2.23** | **0.016677** | **0.740177** |
| **ko04918** | **Thyroid hormone synthesis** | **8.57%** | **2.88%** | **2.97** | **0.035941** | **0.740177** |
| **ko00592** | **alpha-Linolenic acid metabolism** | **8.57%** | **3.85%** | **2.23** | **0.109602** | **0.740177** |
| **ko04216** | **Ferroptosis** | **8.57%** | **3.85%** | **2.23** | **0.109602** | **0.740177** |
| **ko04979** | **Cholesterol metabolism** | **5.71%** | **1.92%** | **2.97** | **0.11109** | **0.740177** |
| **ko00590** | **Arachidonic acid metabolism** | **17.14%** | **10.58%** | **1.62** | **0.114179** | **0.740177** |
| **ko00480** | **Glutathione metabolism** | **8.57%** | **4.81%** | **1.78** | **0.209782** | **0.740177** |
| **ko00591** | **Linoleic acid metabolism** | **5.71%** | **2.88%** | **1.98** | **0.261389** | **0.740177** |
| **ko01040** | **Biosynthesis of unsaturated fatty acids** | **5.71%** | **2.88%** | **1.98** | **0.261389** | **0.740177** |
| **ko00230** | **Purine metabolism** | **8.57%** | **5.77%** | **1.49** | **0.322778** | **0.740177** |
| **ko00061** | **Fatty acid biosynthesis** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko00240** | **Pyrimidine metabolism** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko00532** | **Glycosaminoglycan biosynthesis - chondroitin sulfate /**  **dermatan sulfate** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko00740** | **Riboflavin metabolism** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko04022** | **cGMP-PKG signaling pathway** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko04212** | **Longevity regulating pathway - worm** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko04614** | **Renin-angiotensin system** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko04740** | **Olfactory transduction** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko04744** | **Phototransduction** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko04921** | **Oxytocin signaling pathway** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko05205** | **Proteoglycans in cancer** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko05211** | **Renal cell carcinoma** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko05418** | **Fluid shear stress and atherosclerosis** | **2.86%** | **0.96%** | **2.97** | **0.336538** | **0.740177** |
| **ko00120** | **Primary bile acid biosynthesis** | **5.71%** | **3.85%** | **1.49** | **0.413176** | **0.740177** |
| **ko04723** | **Retrograde endocannabinoid signaling** | **5.71%** | **3.85%** | **1.49** | **0.413176** | **0.740177** |
| **ko04726** | **Serotonergic synapse** | **5.71%** | **3.85%** | **1.49** | **0.413176** | **0.740177** |
| **ko04742** | **Taste transduction** | **5.71%** | **3.85%** | **1.49** | **0.413176** | **0.740177** |
| **ko05231** | **Choline metabolism in cancer** | **20%** | **19.23%** | **1.04** | **0.541077** | **0.740177** |
| **ko00564** | **Glycerophospholipid metabolism** | **22.86%** | **22.12%** | **1.03** | **0.541596** | **0.740177** |
| **ko02010** | **ABC transporters** | **5.71%** | **4.81%** | **1.19** | **0.548772** | **0.740177** |
| **ko00051** | **Fructose and mannose metabolism** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko00270** | **Cysteine and methionine metabolism** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko00380** | **Tryptophan metabolism** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko00531** | **Glycosaminoglycan degradation** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko01523** | **Antifolate resistance** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko04072** | **Phospholipase D signaling pathway** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko04750** | **Inflammatory mediator regulation of TRP channels** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko04913** | **Ovarian steroidogenesis** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ko04964** | **Proximal tubule bicarbonate reclamation** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko04977** | **Vitamin digestion and absorption** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko05200** | **Pathways in cancer** | **2.86%** | **1.92%** | **1.49** | **0.561987** | **0.740177** |
| **ko04080** | **Neuroactive ligand-receptor interaction** | **5.71%** | **5.77%** | **0.99** | **0.661769** | **0.784967** |
| **ko00020** | **Citrate cycle (TCA cycle)** | **2.86%** | **2.88%** | **0.99** | **0.712285** | **0.784967** |
| **ko00260** | **Glycine, serine and threonine metabolism** | **2.86%** | **2.88%** | **0.99** | **0.712285** | **0.784967** |
| **ko00430** | **Taurine and hypotaurine metabolism** | **2.86%** | **2.88%** | **0.99** | **0.712285** | **0.784967** |
| **ko00620** | **Pyruvate metabolism** | **2.86%** | **2.88%** | **0.99** | **0.712285** | **0.784967** |
| **ko05140** | **Leishmaniasis** | **2.86%** | **2.88%** | **0.99** | **0.712285** | **0.784967** |
| **ko05146** | **Amoebiasis** | **2.86%** | **2.88%** | **0.99** | **0.712285** | **0.784967** |
| **ko05322** | **Systemic lupus erythematosus** | **2.86%** | **2.88%** | **0.99** | **0.712285** | **0.784967** |
| **ko00140** | **Steroid hormone biosynthesis** | **2.86%** | **4.81%** | **0.59** | **0.877792** | **0.894355** |
| **ko01200** | **Carbon metabolism** | **2.86%** | **4.81%** | **0.59** | **0.877792** | **0.894355** |
| **ko04922** | **Glucagon signaling pathway** | **2.86%** | **4.81%** | **0.59** | **0.877792** | **0.894355** |
| **ko05230** | **Central carbon metabolism in cancer** | **2.86%** | **4.81%** | **0.59** | **0.877792** | **0.894355** |
| **ko00630** | **Glyoxylate and dicarboxylate metabolism** | **2.86%** | **5.77%** | **0.5** | **0.920997** | **0.920997** |

**Table S7.** Results from metabolomics pathway analyses in *Crassostrea gigas* and *Crassostrea angulata*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Shell length | Shell width | Shell height | Wet weight |
| *C.gigas* | 47.74±14.54 | 33.18±20.08 | 55.65±10.01 | 31.21±15.74 |
| *C.angulata* | 44.39±9.18 | 34.25±14.26\*\* | 61.39±6.31\*\* | 36.23±13.61\*\* |

**Table S8.** Growth trait comparisons of *Crassostrea gigas* and *C. angulata*. *C.gigas* represents *Crassostrea gigas*. *C.angulata* represents *Crassostrea angulata*. “\*\*” indicates the significant difference among the growth traits in the two groups (P < 0.01).

|  |  |  |
| --- | --- | --- |
|  | Glycogen (mg/g) | Crude fat (%) |
| *C.gigas* | 3.506±1.156 | 0.088±0.009 |
| *C.angulata* | 1.149±0.473 | 0.069±0.007 |

**Table S9.** Glycogen and crude fat contents of *Crassostrea gigas* and *C. angulata*.

|  |  |  |
| --- | --- | --- |
|  | Oxygen consumption rate (mg/ (g · h)) | Total antioxidant capacity (μmol/ml) |
| *C.gigas* | 0.205884267±0.037202048 | 0.0647±0.009 |
| *C.angulata* | 0.250682667±0.051523304 | 0.0473±0.0039 |

**Table S10.** Oxygen consumption rate and total antioxidant capacity of Crassostrea gigas and C.angulata.