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Non-Targeted Metabolomics Reveals the Metabolic Alterations in Response to Artificial Selective Breeding in the Fast-Growing Strains of Pacific Oyster

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Abstract Pacific oyster (*Crassostrea gigas*) is one of the most important mollusks cultured all around the world. Selective breeding programs of Pacific oysters in China is initiated since 2006 and developed the genetically improved strain with fast-growing trait. However, little is known about the metabolic signatures of the fast-growing trait. In the present study, the non-targeted metabolomics was performed to analyze the metabolic signatures of adductor muscle tissue in one-year old Pacific oysters from fast-growing strain and the wild population. A total of 7767 and 10174 valid peaks were extracted and quantified in ESI^+ and ESI^- modes, resulting in 399 and 381 annotated metabolites, respectively. PCA and OPLS-DA revealed that considerable separation among samples from fast-growing strain and wild population, suggesting the differences in metabolic signatures. Meanwhile, 81 significantly different metabolites (SDMs) were identified in the comparisons between fast-growing strain and wild population, based on the strict thresholds. It was found that there were highly correlation and conserved coordination among these SDMs. KEGG enrichment analysis indicated that the SDMs were tightly related to pantothenate and CoA biosynthesis, steroid hormone biosynthesis, riboflavin metabolism, and arginine and proline metabolism. Of them, the CoA biosynthesis and metabolism, affected by pantetheine and pantothenic acid, might be important for the growth of Pacific oysters under artificial selective breeding. The study provides the comprehensive views of metabolic signatures in response to artificially selective breeding, and is helpful to better understand the molecular mechanism of fast-growing traits in Pacific oysters.

Key words metabolic signature; Pacific oyster; artificial selection; fast-growing trait

1 Introduction

Pacific oyster (*Crassostrea gigas*), native in the Pacific coast of Northeast Asia, has been introduced to many countries during the 20th century and becomes main oyster species farmed all around the world (Ruesink *et al.*, 2005; Petton *et al.*, 2021; Martínez-García *et al.*, 2022). As the statistics of Food and Agriculture Organization, the global production of Pacific oyster reaches over 5.45 million tons in 2020, representing one of the most widely cultivated and commercially important mollusks all around the world (FAO, 2022). Given its considerable importance in economics, several countries have initiated the selective breeding programs of Pacific oyster and developed a few genetically improved strains in the last decades, such as Australia (Ward *et al.*, 2000), America (Langdon *et al.*, 2003), France (Dégremont *et al.*, 2007) and England (Adams *et al.*, 2008). As the production leader of Pacific oysters, the selective breeding program in China was initiated in 2006. The breeding base populations of Pacific oysters consisted of wild individuals collected from Rushan (China), Miyagi (Japan) and Busan (South Korea) (Li *et al*., 2011). After six-generation selection, the Pacific oysters from Rushan have achieved superior growth performance and become the first breeding stain of oysters in China named 'Haida No. 1' (Li *et al*., 2011).

At present, the fast-growing strain of Pacific oysters has been widely cultivated in the north regions of China. Meanwhile, it provides excellent materials to investigate the molecular mechanisms concerning fast-growing trait in aquatic animals. Whole-genome re-sequencing analysis revealed the distinct genetic structures and genomic signatures between these Pacific oysters from the fast-growing strains and their corresponding wild populations (Jiao *et al.*, 2021; Hu *et al.*, 2022a). Moreover, it was found that 33 candidate genes potentially under selection act as key regulators of cell cycle, which could be responsible for the growth differences (Hu *et al.*, 2022a). Artificial selection has re-shaped the expression patterns of Pacific oyster, resulting in 1303 differentially expressed genes (DEGs) between fast-growing individuals and wild controls (Zhang *et al.*, 2019). Of these genes, DEGs that associate with microtubule motor acti-

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vity and biosynthesis of nucleotides and proteins might be important for the growth of Pacific oyster under selective breeding (Zhang *et al.*, 2019). In addition, emerging evidence suggested that tyrosine hydroxylase could directly affect the synthesis of dopamine and release of insulin-like peptides, and participate in the growth regulation in fast-growing strain of Pacific oysters (Li *et al.*, 2021a, 2021b). Although several effective approaches have been performed to uncover the genetic basis, expression patterns and key genes associated with fast-growing trait, the information about metabolic signatures remained largely unexplored.

Metabolites consist of all the compounds in biological matrix with size typically smaller than 1 kb (Beyoğlu and Idle, 2013). It provides information halfway between genomes and phenotypes that can be used to study complex traits of interest. It is well known that artificial selective breeding can affect the metabolite signatures of organisms (Hao *et al.*, 2019). Therefore, it is of great significance to characterize the metabolic signatures among breeding strains and wild controls to investigate the molecular mechanism of the complex traits. Metabolomics is considered to be an effective technique to detect the global metabolite levels and provide the comprehensive metabolic signatures (Cevallos-Cevallos *et al.*, 2009; Wolfender *et al.*, 2009). In the last years, metabolomics has increasingly been applied to determine the significant differences in metabolites caused by artificial selective breeding. For example, a total of 30 metabolites display significantly abundance between fast- and slow-growing groups of pearl oyster (*Pinctada fucata martensii*), and the most relevant metabolic pathways are glutathione metabolism, sulfur metabolism, and valine, leucine and isoleucine biosyntheses (Cevallos-Cevallos *et al.*, 2009). The altered metabolites of glycerophospholipids, fatty acyls, and steroids are observed in the whole soft body and the mantle tissues of black-shelled and white-shelled Pacific oyster (Chen *et al.*, 2021). Carbohydrate, lipid, amino acid, and energy metabolism have been reported to be associated with condition index differences in Pacific oyster (He *et al.*, 2022).

In this study, the non-targeted metabolomics was used to analyze the metabolic signatures and determine the differences in the abundance of metabolites in adductor muscle tissue of Pacific oysters from fast-growing strain 'Haida No.1' and their corresponding wild population. The study will provide valuable information on the metabolic changes in response to artificially selective breeding, and help us better understand the molecular mechanism of fast-growing traits in Pacific oysters.

2 Materials and Methods

2.1 Ethics Statement

Animal experiments were conducted in accordance with the guidelines and approval of the respective Animal Research and Ethics Committees of Ocean University of China (Permit number: 20141201). No endangered or protected species was involved in the experiments.

2.2 Sample Collection

Pacific oysters used in the present study contained the individuals from fast-growing strain and their corresponding wild population, namely ZF and ZY groups, respectively. The fast-growing strain of Pacific oysters were produced by the selective breeding program for genetic improvement of growth since 2006. In 2017, the tenth generation of fast-growing strain 'Haida No.1' of Pacific oyster have been produced. In the present study, Pacific oysters from fast-growing strain and their corresponding wild population were cultivated by the same rearing procedures and under the same environmental conditions for one year. Then 6 individuals were randomly selected from the fast-growing strain and wild population respectively, and were immediately dissected to collect the adductor muscle tissues. The samples were frozen in liquid nitrogen and stored at −80℃ until the following metabolomics analysis.

2.3 Metabolites Extraction and Detection

Equal quality of freeze-dried adductor muscle tissues (50 g) from each sample were placed into 2 mL EP tubes and mixed with 800 L extraction solvent (methanol:water = 1:1, v/v). The mixed samples were homogenized in a ball mill for 4 min at 45 Hz, and ultrasound-treated for 5 min in ice. Ice incubation was performed for 1 h to precipitate the proteins. The samples were centrifuged at 12000 r min^{-1} for 15 min at 4℃. The metabolite extractions of each sample were repeated for three times. Equal amount of supernatant (250 L) from the repeated extractions was merged and subsequently transferred to a fresh glass vial for the preparation of quality control (QC) samples and LC-MS/MS analysis. Then, 50 μL of each merged supernatant was pooled as quality control (QC) samples that would be used to evaluate the stability and reliability of the LC-MS/MS platform and system (Tian *et al*., 2021; Zhang *et al*., 2021). At the end, a total of 15 samples (6 × ZF samples, 6 × ZY samples and 3 × QC samples) were obtained for LC-MS/MS analysis.

2.4 LC-MS/MS Analysis

2.4.1 Chromatographic separation

ZF and ZY samples were randomly placed in an automatic sampler at 4℃, and QC samples were inserted into the queue of experimental samples to evaluate the stability of LC-MS/MS system and the reliability of experimental data. Chromatographic separation was performed using a Vanquish UHPLC ultrahigh liquid chromatography system (Thermo Fisher) with a Hypersil GOLD column (C 18, 100 mm × 2.1 mm, 1.9 μm, Thermo Fisher). The column temperature was maintained at 40℃, the flow rate was set as 0.2 mL min^{-1}, and the injection volume was 1 μL. The mobile phase consisted of (A) water with 0.1% formic acid and (B) methanol in positive-ion mode (ESI^+), as well as (A) 5 mmol L^{-1} ammonium acetate (pH 9.0) and (B) methanol in negative-ion mode (ESI^-) with following elution gradient: 0–1.5 min, 98% A; 1.6–12 min, 98% A; 12–14 min, 0% A; 14.1–17 min, 98% A.

2.4.2 Mass spectrometry acquisition

Separated metabolites by UHPLC were further analyzed using a Q ExactiveTM HF mass spectrometer (Thermo Fisher) in both ESI^+ and ESI^- modes, respectively. The scanning range was set as 100−1050 *m*/*z*. In addition, the specific parameters of electrospray ionization (ESI) were as follows: spray voltage at 3.5 kV, curtain gas at 35 Psi, capillary temperature at 320°C, auxiliary gas flow rate 10 L min^{-1}, sheath and aux gas flow rate, 40 arb and 10 arb, respectively.

2.5 Data Analysis

Compound Discoverer 3.1 software is developed and released by Thermo Scientific for the data analysis of both targeted and untargeted metabolomics. In the present study, Compound Discoverer software was performed to identify the metabolites in adductor muscle tissues of Pacific oysters by an automated data processing workflow, containing peak alignment, peak feature extraction, peak identification, compound grouping across all samples, and differential analysis. In brief, raw files of non-targeted metabolomics data (chromatograms and mass spectra) were loaded into software. Data was trimmed and filtered with the limited *m*/*z* range from 50 to 700 by setting a threshold of peak intensity at a signal-to-noise (*S*/*N*) ratio of 1.5. For chromatographic alignment, the mass tolerance error was set as 5 mg L^{-1} and the maximum retention time shift was limited at 0.3 min. Then, peak extraction was performed with a few parameters of the minimum peak intensity of 10000, the maximum peak width of 0.5 min as well as the minimum of 5 scans per peak. The metabolites were identified and annotated by comparing the exact masses of compounds and MS/MS ion fragmentation spectra with available metabolomics database, including mzCloud, mzVault, ChemSpider, HMDB, KEGG and LIPID MAPS databases.

The three-dimensional data, including peak number, sample name, and normalized peak area, were inputted into SIMCA software v14.1 package (MKS Data Analytics Solutions, Umea, Sweden) for both principal component analysis (PCA) and orthogonal projections to latent structures-discriminate analysis (OPLS-DA). The results of PCA indicated the distribution of original data, while supervised OPLS-DA represented the high-level group separation and the variables responsible for classification. Meanwhile, variable importance in projection (VIP), defined by OPLS-DA, was used to explain metabolite expression patterns among groups. Sevenfold cross validation was used to estimate the model robustness and prediction ability.

2.6 Significantly Different Metabolites (SDMs) and Enrichment Analyses

The VIP values >1 together with $2 \leq FC \leq 0.5$ and *P*-value < 0.05 were set as the thresholds of significantly different metabolites (SDMs) between the comparisons of ZF and ZY groups. The correlations among the abundance of SDMs were determined by the calculation of Pearson correlation coefficients in samples. In addition, functional enrichment analysis was performed by the integrated web-based platform MetaboAnalyst 5.0 (http://www.metaboanalyst.ca) with high-quality metabolic pathway database, and the results were further visualized using ggplot2 R packages.

3 Results

3.1 Metabolic Signatures in Adductor Muscles of Pacific Oysters

In the present study, a total of 15 samples were performed for the non-targeted metabolomics analysis to investigate the metabolic signatures of adductor muscle in Pacific oysters selected from fast-growing strain and wild population. After filtering, 7767 and 10174 valid peaks were extracted and quantified in ESI^+ and ESI^- modes, respectively. Of them, 399 and 381 peaks were further identified as metabolites in ESI^+ and ESI^- modes by mass spectrum matching with a spectral similarity ≥600.

The signatures of metabolites were firstly monitored by an unsupervised pattern recognition method of PCA. It not only reduces the data dimensionality, but also reveals the intrinsic variation within data of metabolites. In the score scatter plot of PCA (Figs.1A, B), there were obvious separations between samples from ZF and ZY groups. The R^2 values in PCA model were 0.951 (ESI^+) and 0.959 (ESI^-), and Q^2 values were 0.682 (ESI^+) and 0.674 (ESI^-), respectively. The total amount of variation explained by PC1 and PC2 were 38.5% (ESI^+) and 39.1% (ESI^-), representing the effective information of differences in the metabolic signatures. To maximize the discrimination between groups, supervised OPLS-DA was performed to explain the differences in metabolic signatures between Pacific oysters from fast-growing strain and wild population. As showed in Figs. 1C and D, there were considerable separation between ZF and ZY groups. The parameters considered for classification were $R^2=0.949$, $Q^2=0.643$ (ESI^+) and $R^2=0.978$, $Q^2=0.575$ (ESI^-). It provided strong evidence for the low risk of over-fitting and excellent stability of established OPLS-DA model, making it suitable to be exploited in subsequent analyses.

3.2 Identification of SDMs Between Fast-Growing Strain and Wild Population

Based on the strict thresholds, a total of 81 SDMs were determined in the comparisons between ZF and ZY groups, which were considered the most relevant metabolites for explaining the differences caused by artificial selection (Fig.2). Compared with individuals from wild population, the abundance of 38 SDMs was significantly increased after artificial selection, whereas the abundance of 43 SDMs was found to be significantly repressed in fast-growing strains. More specifically, there were 81 and 93 SDMs identified in ESI^+ and ESI^- modes, respectively. Of them, ESI^+ mode revealed 38 up-regulated and 41 down-regulated SDMs in the comparison of ZF *vs*. ZY. And 43 up-regulated and 49 down-regulated SDMs were detected in ESI^- mode. As shown in Figs.2C and D, heatmap plots were constructed to visualize the abundance patterns of SDMs in

ESI^+ and ESI^- modes. It was obvious that the metabolic signatures were dramatically changed by artificial selection.

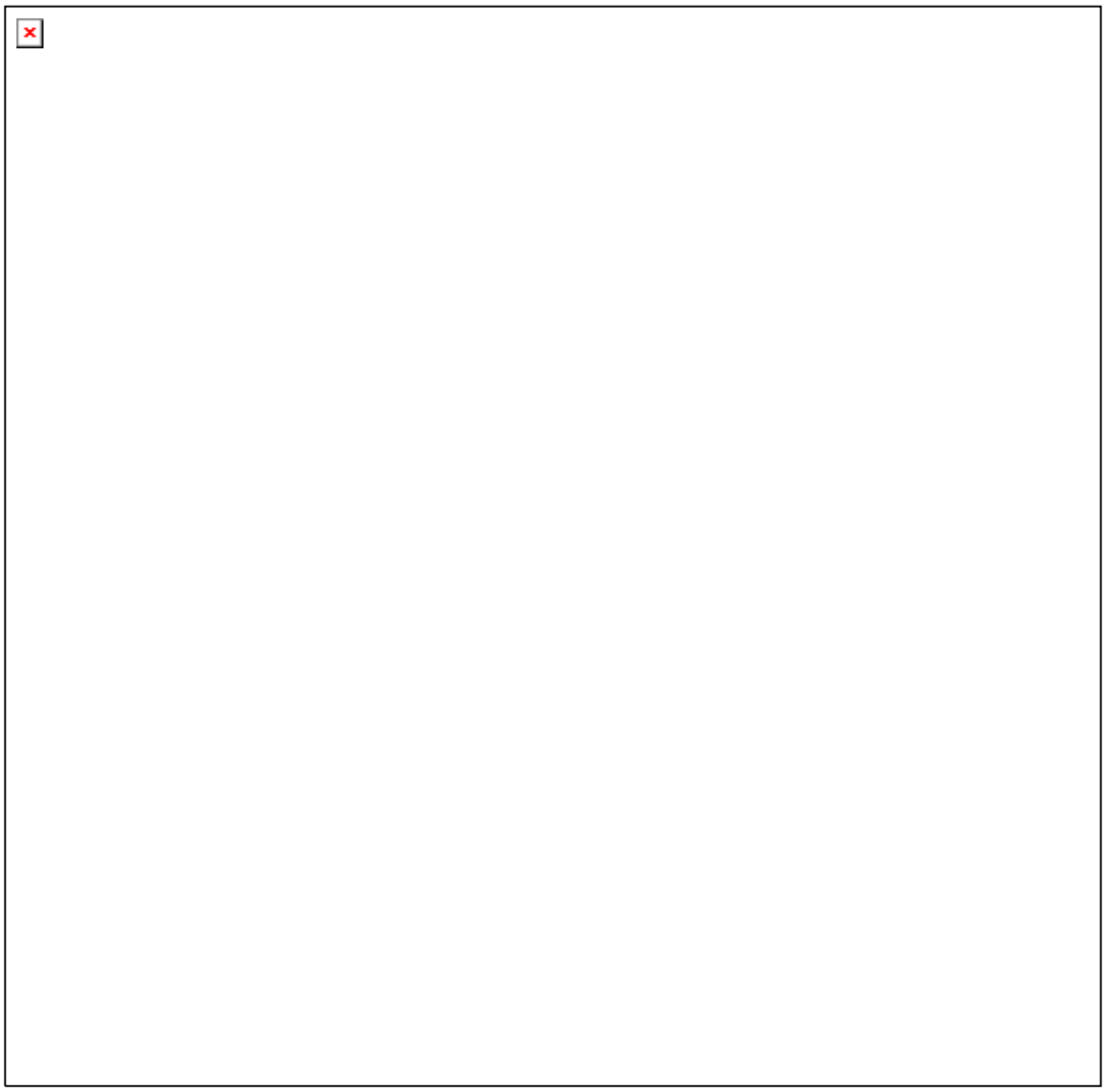

Fig.1 Principal component analysis (PCA) and partial least squares discriminant analysis (OPLS-DA) of metabolites identified in ESI^+ and ESI^- modes by LC-MS/MS analysis. The samples of Pacific oysters from fast-growing strain (ZF) and wild population (ZY) are represented with green and blue colors, respectively. QC samples are marked with red color.

In the HMDB database, 81 SDMs in ESI^+ mode were found to be closely associated with organic acids and derivatives, lipids and lipid-like molecules, organoheterocyclic compounds, organic nitrogen compounds, phenylpropanoids and polyketides, benzenoids, alkaloids and derivatives, nucleosides and analogues (Fig.3A). In addition, 92 SDMs in ESI^- mode were tightly related to lipids and lipid-like molecules, organic acids and derivatives, organoheterocyclic compounds, nucleosides and analogues, phenylpropanoids and polyketides, organic oxygen compounds, benzenoids, and organic nitrogen compounds (Fig.3B).

3.3 SDM-SDM Correlation Analysis

Pearson correlation coefficients were calculated to reveal the positive and negative correlation relationships among SDMs in the comparisons between fast-growing strain (ZF) and wild population (ZY). At the thresholds of Pearson correlation coefficients greater than 0.50 or less than −0.50, there were 1571 pairs of positive correlations and 2270 pairs of negative correlations, respectively. In ESI^+ mode, it was obvious that 3-hydroxyropivacaine and lysoPC 17:0 were positive correlated to 3-(3-pyridinyl) propanoic acid, 4-methylpyridine-3-sulfonic acid, docosapentaenoic acid, N-Oleoyl dopamine, while their abundance were negatively correlated to DMH (Fig.4A). N-acetyl-L-leucine, monocrotaline, APK and estriol were positive correlated to RLK, 7α-hydroxytestosterone, and L-carnitine. In ESI^- mode, some SDMs with highly positive correlations were observed in Ip7G, N-acetyl-L-glutamine and riboflavin. Additionally, Bz-RS-iSer(3-Ph)-OMe, LPC 13:0, and 19-nortestosterone displayed the highly negative association. The results indicated the differences in the conserved and highly coordinated interplay in the metabolic signatures of Pacific oysters.

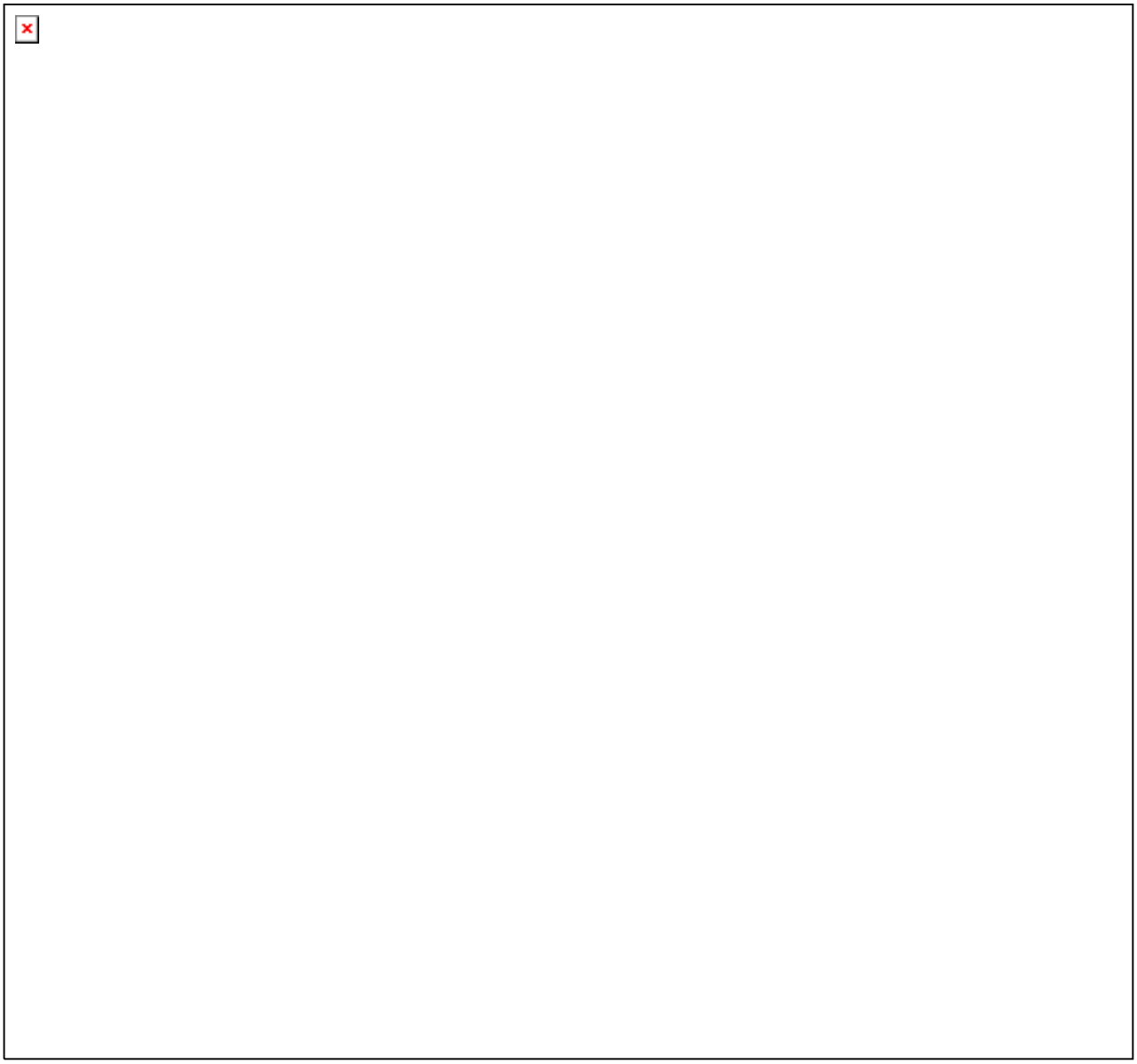

Fig.2 Volcano and heatmap plots showing the SDMs identified in ESI^+ and ESI^- modes in adductor muscle of Pacific oysters from fast-growing strain (ZF) and wild population (ZY). The SDMs were determined based on the strict thresholds of VIP>1, $2 \leq FC \leq 0.5$ and *P*-value<0.05. In volcano plots, the up- and down-regulated SDMs were represented by red and blue pots, respectively, The VIP values were showed by the size of these dots. In heatmaps, red and blue colors represented the highest and lowest abundance of SDMs in ESI^+ and ESI^- modes in adductor muscle of Pacific oysters.

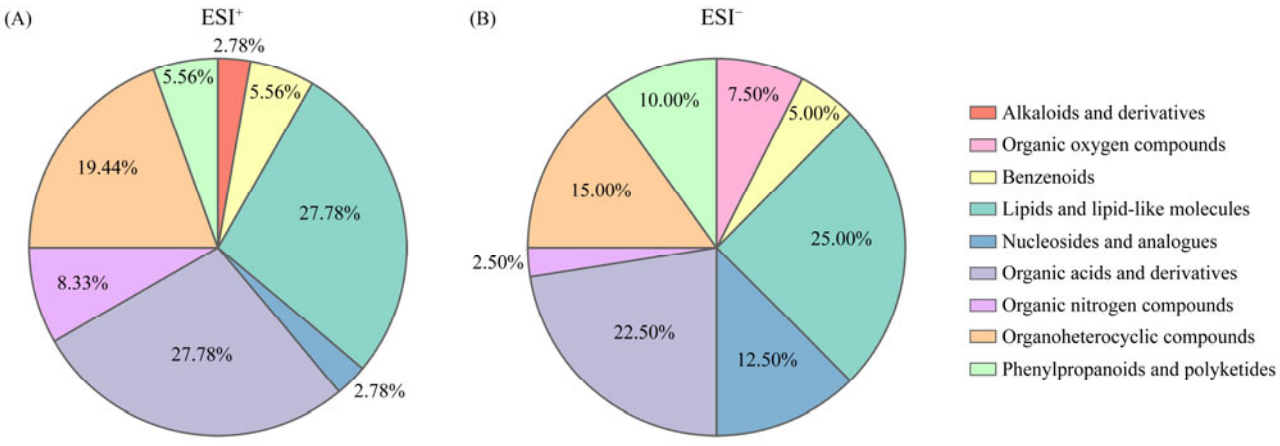

Fig.3 Annotation and classification of SDMs detected in ESI^+ (A) and ESI^- (B) modes based on the HMDB database.

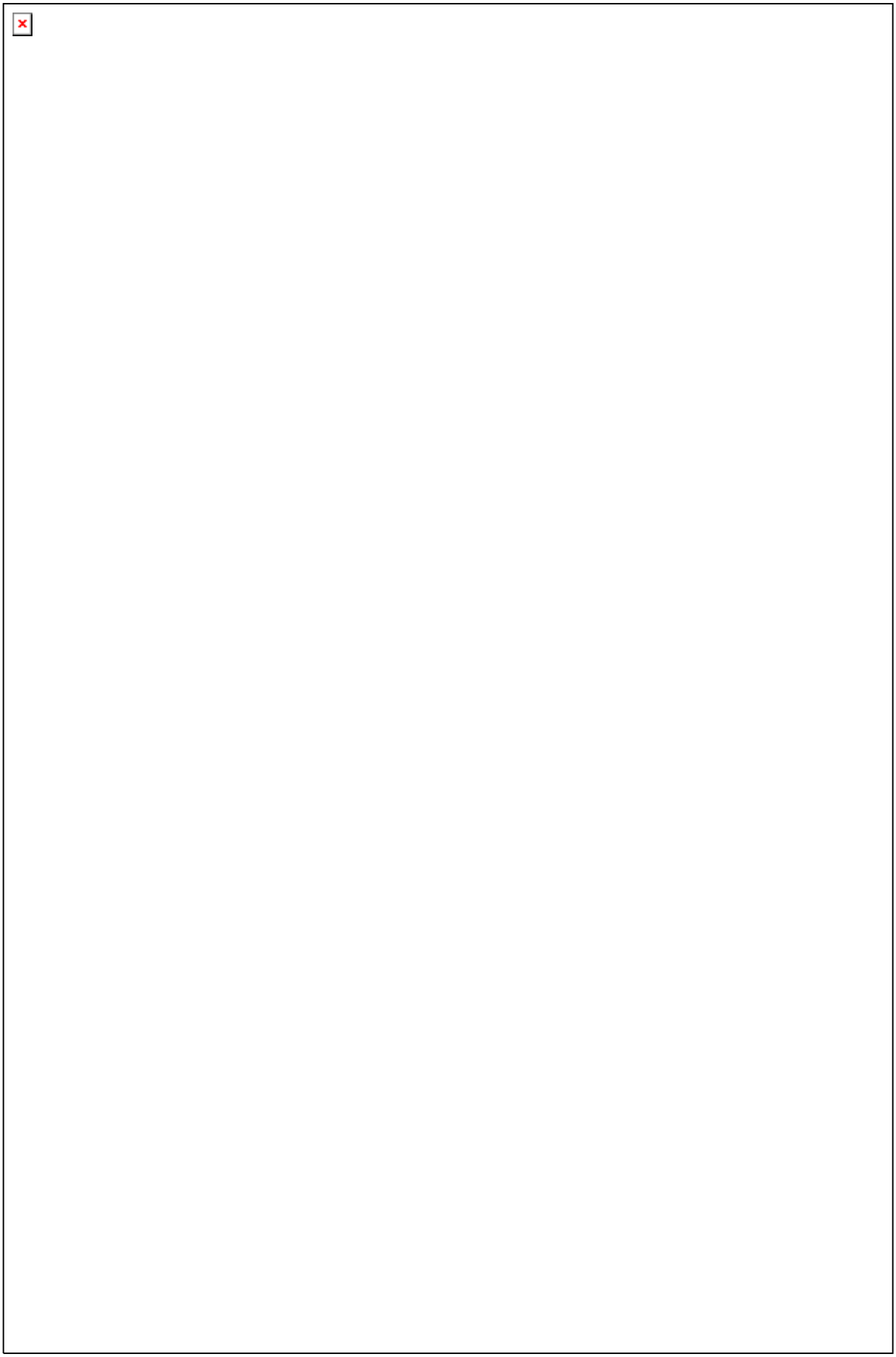

Fig.4 Correlation maps of SDMs identified in both ESI^+ (A) and ESI^- (B) modes. Pearson correlation coefficients are calculated and showed with continuous colors ranging from blue (−1) to red (1).

3.4 KEGG Enrichment Analysis

To explore the potential metabolic pathways affected by artificial selection, these SDMs were imported into MetbraAnalyst 5.0 platform for further analysis. A total of 19 relevant metabolic pathways were identified based on the thresholds of both −ln(*P*-value) and impact scores (Fig.5A). The top enriched pathways consisted of pantothenate and CoA biosynthesis (Ko00770), steroid hormone biosynthesis (Ko00140), riboflavin metabolism (Ko00740), arginine and proline metabolism (Ko00330), pyrimidine metabolism (Ko00240) and histidine metabolism (Ko00340). It was found that the abundance of pantetheine, pantothenic acid and vitamin B2 were significantly increased in adductor muscle tissues of Pacific oysters from fast-growing strain (ZF), while the abundance of estrone, thymine and thymidine were decreased in individuals from ZF (Fig.5B). It indicates that CoA biosynthesis and metabolism, affected by pantetheine and pantothenic acid, might be important for the growth of Pacific oysters under artificial selection.

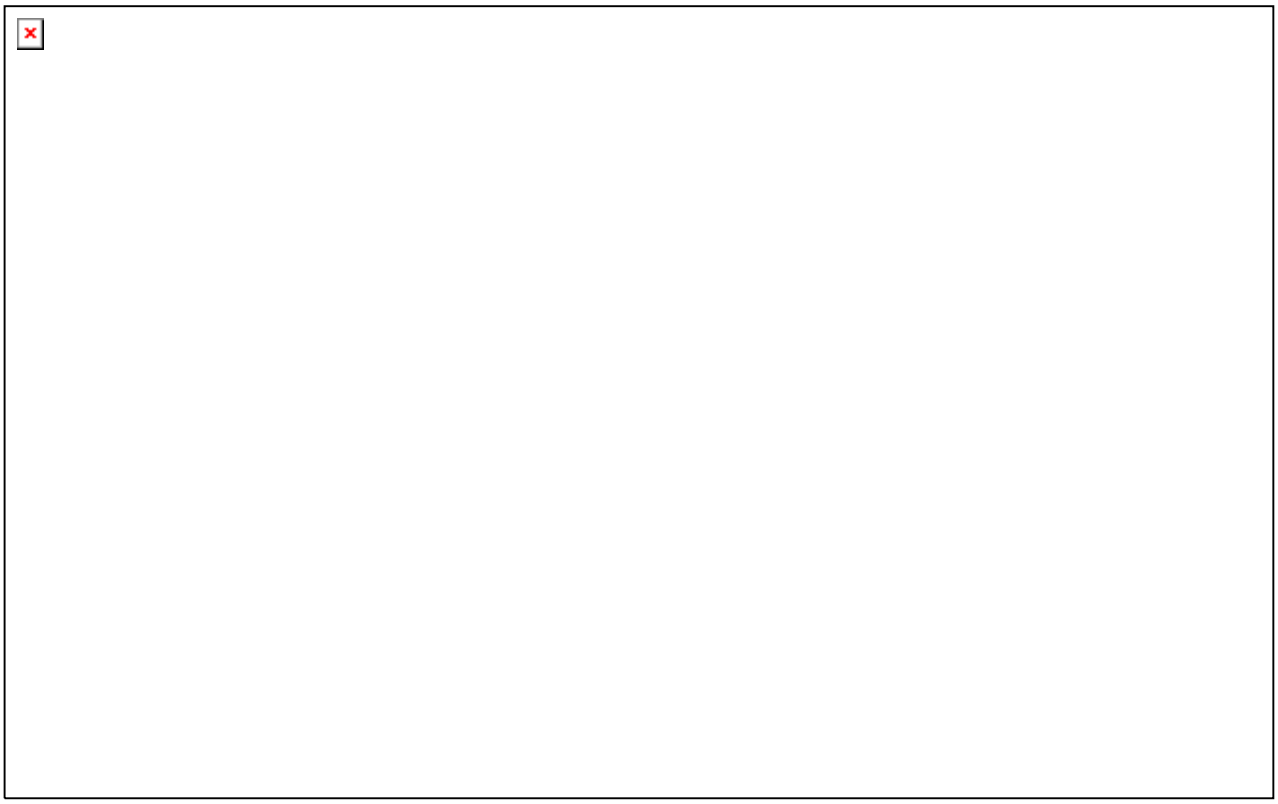

Fig.5 Enriched KEGG pathways of SDMs (A) and their relative abundance (B) in adductor muscle tissues of Pacific oysters from fast-growing strain (ZF) and wild population (ZY). In these enriched KEGG pathways, the sizes of dots represent the number of SDMs, and signatures were marked by red color. Additionally, the abundance of SDMs in adductor muscle tissues is showed in box plot.

4 Discussion

Improving growth performance has been considered the major focus of breeding programs for most organisms (Hu *et al.*, 2022a). To explore the metabolic signatures and molecular mechanisms of excellent growth performance, Pacific oysters from fast-growing strain (ZF) and their corresponding wild population (ZY) were selected for the metabolomics analyses in this study. Adductor muscle is the main muscular system in bivalve mollusks and controls the open or close states of their shells (Poulet *et al.*, 2003; Zheng *et al.*, 2016). Moreover, accumulating documents have revealed that many function genes associated with growth and development display relatively high expression levels in adductor muscle tissues of bivalve mollusks (Sun *et al.*, 2014; Wang *et al.*, 2018b). Therefore, the targeted adductor muscle tissues were selected and performed for metabolomics analysis.

After one-year cultivation by the same rearing procedures and under the same environmental conditions, individuals under artificial selection exhibited excellent growth performance (Guo *et al.*, 2016; Zhong *et al.*, 2016). In addition, it has been found that there were numerous SDMs between these individuals from fast-growing strain (ZF) and wild population (ZY), suggesting the metabolic signatures were reshaped by artificial selection. Based on the HMDB database, these SDMs were mainly annotated and classified into organic acids and derivatives, and lipids and lipid-like molecules. Organic acids, widely presenting in nature, are crucial for the metabolic processes of plants and animals (Cherrington *et al.*, 1991; Li *et al.*, 2018; Ben and Smaoui, 2021). As reported, acetic, propionic, phosphoric and citric acid are beneficial to the growth performance, gut microbiota and digestive function of newly weaned pigs (Namkung *et al.*, 2004; Tugnoli *et al.*, 2020). It is also proved that organic acids and derivatives could greatly influence the

growth performance, nutrient utilization and disease resistance in several commercially important farmed aquatic animals, such as Nile tilapia (*Oreochromis niloticus*) (Lim *et al.*, 2010), rainbow trout (*Oncorhynchus mykiss*) (Pandey and Satoh, 2008) and shrimp (*Litopenaeus vannamei*) (Su *et al.*, 2014; Ng and Koh, 2017). However, the studies on organic acids and their derivatives remain limited in mollusks.

Additionally, lipids and lipid-like molecules generally play multiple roles in animals, which are known as efficient energy reserves, important metabolism intermediates, cellular signal elements and major cell components (Montero-Torreiro *et al.*, 1998; Roy *et al.*, 1999; Castell *et al.*, 2004; Hu *et al.*, 2009; Dadina *et al.*, 2021). Recently, it has been documented that the metabolism of lipids and lipid-like molecules are tightly related to the growth performance and physiological states of aquatic animals, such as yellow catfish (*Pelteobagrus fulvidraco*), Pacific abalone (*Haliotis discus hannai*) (Shen *et al.*, 2021) and its hybrids (*H. discus hannai* ♀×*H. fulgens* ♂) (Hu *et al.*, 2022b). It was found that there are different fatty acid compositions among the wild individuals of Japanese scallop (*Patinopecten yessoensis*) and the breeding families, the artificial selection of which is primarily focused on growth performance, survival rate and shell color (Xie *et al*., 2019). Additionally, the hybridization of Pacific oysters and Portuguese oysters (*C. angulate*) alters their original fatty acid compositions in gonads (Tan *et al*., 2019). These results imply that the metabolism of lipids and lipid-like molecules, especially fatty acids, are able to be greatly shaped by either artificial selection or hybridization. In the present study, it was observed that artificially selective breeding also changed the metabolism of lipids and lipid-like molecules in the adductor muscles of Pacific oysters. It might lead to the excellent growth performance in Pacific oysters from the fast-growing strains.

KEGG enrichment analysis was performed to further investigate the potential functions of these SDMs. As a result, these SDMs are believed to be mainly involved in pantothenate and CoA biosyntheses, riboflavin metabolism, steroid hormone biosynthesis, and pyrimidine metabolism. Both pantetheine and pantothenic acid serve as an advanced precursor for CoA synthesis, which is a ubiquitous and essential cofactor in all living organisms (Spry *et al.*, 2008; Butman *et al.*, 2020). CoA functions as a carrier for activated acyl groups in numerous reactions and central metabolic processes, especially ATP production and energy supply through the tricarboxylic acid (TCA) cycle (Jackowski and Rock, 1981; Balibar *et al.*, 2011; Brunetti *et al.*, 2014). In the present study, the abundance of pantetheine and pantothenic acid was significantly increased in Pacific oysters from fast-growing strain. It suggested the more active biosynthesis of CoA and energy metabolism, which made a great contribution to the excellent growth performance of Pacific oysters under artificial selection. In agreement with this observation, the CoA metabolism is also altered in domestic pigs, because of the intense selection pressures on *acetyl-CoA carboxylase alpha* gene, an important regulator of CoA metabolism (Wang *et al.*, 2018a). In aquatic animals, the abundance of CoA has been thought to be closely associated with the growth performance in Japanese seabass (*Lateolabrax japonicus*) (Xie *et al.*, 2021), large yellow croaker (*Larimichthys crocea*) (Ding *et al.*, 2020), blunt snout bream (*Megalobrama amblycephala*) (Qian *et al.*, 2015), Chinese mitten crab (*Eriocheir sinensis*) (Wei *et al.*, 2018). Additionally, SDMs between fast-growing individuals and slow-growing individuals of pearl oyster (*Pinctada maxima*) are also found to be significantly enriched in pantothenate and CoA biosyntheses, which provides evidence for the importance of pantetheine and CoA metabolism in growth performance (Hao *et al*., 2018). Moreover, recent study suggests that *Acyl-CoA desaturase* and *3-hydroxy-3-methylglutaryl-coenzyme A reductase* genes, tightly related to CoA metabolism, are differentially expressed between fast-growing strains and wild population of Pacific oysters (Li *et al*., 2023). Hence, pantothenate and CoA metabolism could be associated with the excellent growth performance of Pacific oysters from fast-growing strains.

Oxidative stress is defined as a disturbance in the balance between the generation of reactive oxygen species and the ability to deactivate them (Betteridge, 2000; Ashoori and Saedisomeolia, 2014). Riboflavin, also named water-soluble vitamin B2, is one of the neglected antioxidant nutrients that play important roles in protecting the organisms from oxidative stress caused by the glutathione redox cycle (Mazzotta *et al.*, 2014; Pallotta, 2019). It has been proven that dietary riboflavin can remarkably enhance immunity and anti-oxidative status against multiple abiotic stresses in a series of fishes, such as Nile tilapia, striped catfish (*Pangasianodon hypophthalmus*) and snakehead (*Channa punctatus*) (Zehra and Khan, 2017; Kumar, 2021; Li *et al*., 2022). However, the researches of riboflavin are limited in mollusks until now. In the present study, the relatively higher abundance of riboflavin was observed in Pacific oysters from wild populations, rather than those from fast-growing strains. It suggested that artificial selection has affected the metabolism of riboflavin, which may result in weak antioxidant system of Pacific oysters in response to stresses. Similar result has been reported in mammals, such as dog. The total antioxidant capacity and glutathione peroxidase activity in wild dogs are gradually increased with ages, while increased lipid damage is observed in domestic dogs, highlighting that artificial selection may have decreased antioxidant capacity (Jimenez *et al*., 2020). Estrogen is an important steroid hormone in females, responseble for the sexual development and sexual characteristics formation (Grow, 2002; Lance, 2009). The present study revealed significantly higher abundance of estrogen in individuals from wild population. It may be attributed by the differences in genders of selected Pacific oyster samples for metabolomic analysis. Low estrogen implied the repressed gonad development in Pacific oyster from fast-growing strains, more energy of which may be saved and used for the development of muscle. However, the related mechanism required to be further studied in the future.

5 Conclusions

In the present study, a total of 15 samples were performed for the non-targeted metabolomics analysis to investigate the metabolic signatures of adductor muscle in Pacific oysters from fast-growing strain (ZF) and wild population (ZY). A total of 81 SDMs were determined in the comparisons between ZF and ZY groups, indicating that the artificial selection greatly changed the metabolic signatures in adductor muscle tissues of Pacific oysters. Among them, numerous SDMs could be classified into organic acids and derivatives, and lipids and lipid-like molecules. Additionally, KEGG enrichment analysis revealed that these SDMs were enriched in pantothenate and CoA biosyntheses, steroid hormone biosynthesis, riboflavin metabolism, and arginine and proline metabolism. It was noted that the active biosynthesis of CoA and energy metabolism in Pacific oysters of fast-growing strains may produce more ATP and provide much energy for growth and development. Riboflavin metabolism was active in Pacific oysters from wild populations rather than those from fast-growing strains, suggesting that artificial selection may result in weak antioxidant system of Pacific oysters in response to stresses. The study provided comprehensive views of metabolic changes in response to artificially selective breeding, and helped us better understand the molecular mechanism of fastgrowing traits in Pacific oysters.

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