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Metabolomic responses of juvenile pearl oyster *Pinctada maxima* to different growth performances

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ABSTRACT

Similar to other marine bivalves, *Pinctada maxima* shows unsynchronized growth, but the underlying metabolic mechanisms have not been studied. In this study, gold-lipped pearl oyster *P. maxima* from cultured stocks were selected to produce progeny stock. At 180 days, the stock was sorted by size, and fast-growing individuals and slow-growing individuals were separately sampled. Then, a metabolomic approach based on gas chromatography–mass spectroscopy was applied to assess the metabolite changes between the fast-growing and slow-growing groups of *P. maxima* and to understand the mechanism of their unsynchronized growth. In the metabolomics assay, among the 896 peaks isolated, 111 metabolites were revealed a spectral similarity value of > 700 by using mass spectrum matching, and 48 were considered as significantly different metabolites (SDMs; VIP > 1 and P < 0.1) between the fast-growing and slow-growing groups. Results revealed that pearl oyster *P. maxima* changes metabolic status with different growth performance. Pathway analysis indicated that these SDMs were involved in 11 pathways. Further integrated key metabolic pathway analysis showed that pearl oysters possessed different capabilities in valine, leucine, and isoleucine biosynthesis, glycine, serine, and threonine metabolism, pyrimidine metabolism, cysteine and methionine metabolism, and glutathione metabolism between fast-growing and slow-growing groups. This study is the first metabolomics study to identify the key pathways and crucial metabolites so as to understand the metabolic mechanism of unsynchronized growth of bivalves.

1. Introduction

The pearl oyster *Pinctada maxima* is naturally distributed in the central Indo-Pacific region from Myanmar to the Solomon Islands, such as Southeast Asia, the Philippines, South China Sea, and Australia (Southgate and Lucas, 2008). *P. maxima* is one of the most important components of molluscan mariculture in southern China and is primarily cultured for the high-value production of large-scale pearls. Since its successful hatchery production in 1970, the species has been cultured for approximately 40 years in China (Deng et al., 2013). Within the last decade, commercial pearl production from this species has developed slowly because of mass mortality and poor growth. Similar with other marine bivalves, *P. maxima* spat produced in a hatchery are transferred to either a sea- or land-based nurseries at approximately 2 mm in shell length for further growth. The period for successful transfer to the sea is limited by sudden changes in environmental factors, predation, fouling and boring organisms, and so on (Monteforte and Moralesmulia, 2000; Pit and Southgate, 2000; Fariborz et al., 2010). Cultivation sites for *P. maxima* in China are generally nearshore estuarine areas, where salinity fluctuates because of heavy rains and runoff in the summer months. However, the growth of this species is not synchronized; some oysters are fast growing, whereas some are slow growing. In the past decades, numerous researchers have reported the effects of environmental factors on the growth performance of *P. maxima* spat (Liang et al., 2011; Xie et al., 2011). However, the underlying metabolic mechanisms of their unsynchronized growth have not been studied. The limited knowledge of exogenous and endogenous regulation of development in different marine invertebrate species restricts rapid advancements in culture practices.

Metabolomics is the study of chemical processes involving metabolites. Metabolites comprise all compounds in a biological matrix that are typically smaller than 1 kDa in size (Beyoglu and Idle, 2013) and include small peptides, oligonucleotides, sugars, organic acids, ketones, aldehydes, amino acids, lipids, steroids, alkaloids, and xenobiotics. As

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Table 1
Variations in temperature, S, DO concentrations, and suspended matter in the cultured sea.

Date of sampling	WT	S	DOC	SOM
2017.04	25.1	31.2	5.07	19.8
2017.05	27.4	30.7	5.21	21.8
2017.06	29.4	30.2	5.34	24.6
2017.07	31.3	30.4	5.22	27.3
2017.08	31.5	31.3	5.24	29.8
2017.09	26.7	31.7	5.14	23.2
2017.10	25.3	31.3	5.11	18.6

WT, water temperature; S, salinity; DOC, dissolved oxygen concentration; SOM, suspended organic matter.

Table 2
Growth performance of the fast-growing and slow-growing groups of pearl oyster *P. maxima*.

	Fast-growing group	Slow-growing group
Shell length	40.97 ± 2.67 a	28.29 ± 2.06 b
Shell width	7.61 ± 0.46 a	5.98 ± 0.36 b
Shell height	40.75 ± 3.47 a	28.32 ± 2.21 b
Total weight	59.37 ± 13.01 a	23.58 ± 3.85 b

Within the line, means with the same letters are not significantly different ($P > 0.05$).

an emerging technological and analytical approach, metabolomics has been used to study the global metabolites in cells, tissues, and biofluids of living systems (Psychogios et al., 2011) and to understand the physiological and biochemical status of biosystems with further interpretation of biological principles. Metabolomics has been applied to the aquaculture industry, particularly in hatchery production (Young et al., 2015; 2016), nutrition and diet (Tuffnail et al., 2009; Wagner et al., 2014; Ma et al., 2017; Yang et al., 2018), disease and immunology (Guo et al., 2015; Li et al., 2016a; Zeng et al., 2016), and postharvest quality control (Villa et al., 2013; Castejón et al., 2016).

However, despite its wide applicability, the use of metabolomics in mollusk culture has not yet been realized. Here, we utilized GC–MS-based metabolomics to investigate the difference in metabolite profiles between the fast-growing and slow-growing juvenile *P. maxima* and to gain insight into the mechanisms underlying its unsynchronized growth.

2. Materials and methods

2.1. Experimental animals

We selected the gold-lipped pearl oyster from cultured stocks, which were developed by Xie et al. (2011). A total of 48 mature animals (female:male = 28:20) were used to produce the progeny stock. In April 2017, breeders were mass spawned in the hatchery. Larvae were reared following the techniques of Liang et al. (2016). Fertilized eggs were incubated in 200-L polyethylene tanks until the D stage, which occurred at 24 h after fertilization. Then, larvae were transferred to 1000-L polyethylene tanks. The density was maintained at 1 individual/mL. Water temperature (WT) was at 28 °C ± 1 °C, and salinity (S) was at 30 ± 1 ppt. Daily feeding consisted of *Isochrysis galbana* from day 2 to day 5 and a mixture of *I. galbana* and *I. zhanjiangensis* from day 6 to day 65. Feeding ration was increased with age. Every other day, 500 L of sea water was replaced in each tank. At day 25, plastic plates were provided as substrate for metamorphosis. At 65 days, large individuals 2–6 mm in size were removed from the plates, transferred to net cages (200 per net cage), and suspended in a commercial farm (20°25′N, 109°57′E) of Liusha Bay, Guangdong province, China. The shells were

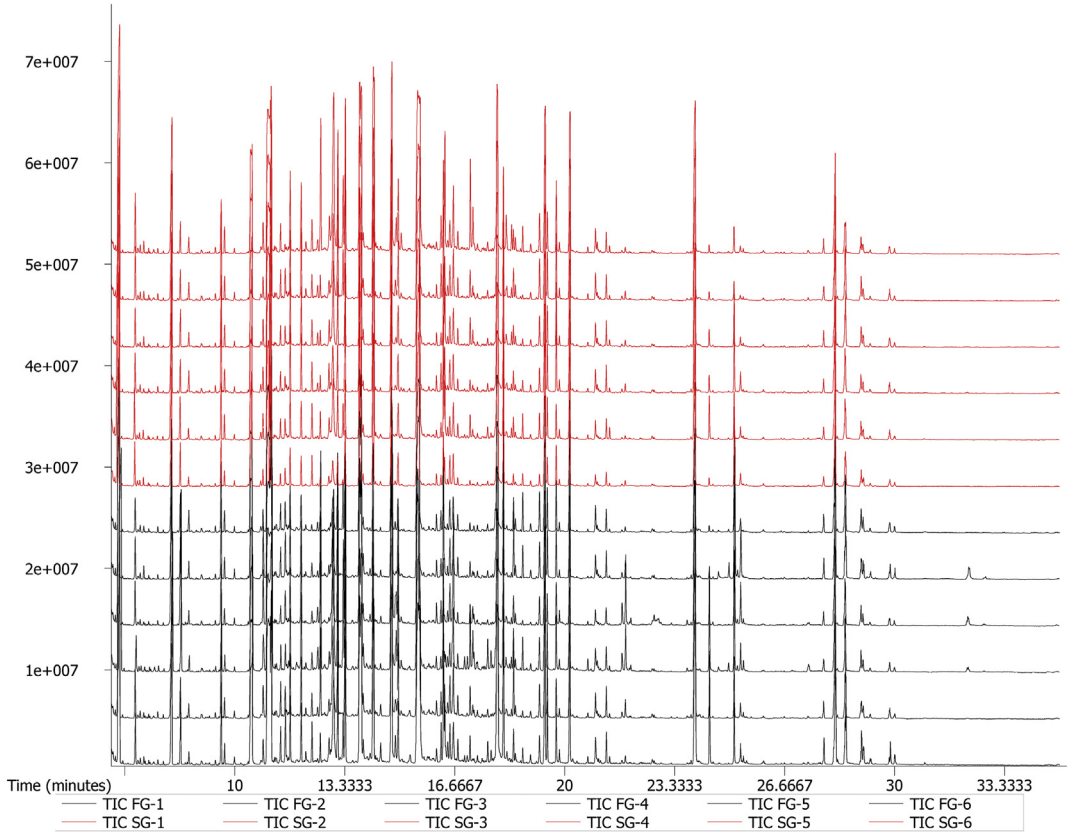

Fig. 1. Typical GC-TOF-MS TICs of *P. maxima* samples from the two groups. The ordinate shows the relative mass abundance, and the abscissa shows the retention time. The FG represents fast-growing group, and the SG represents slow-growing group.