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Fatty acid profiles in muscle of large yellow croakers (*Larimichthys crocea*) can be used to distinguish between the samples of Dai-qu stock and Min-yuedong stock

Xi-Quan Shen, Xiao-Jun Yan*, Ji-Lin Xu

Key Laboratory of the Ministry of Education for Applied Marine Biotechnology, School of Marine Science, Ningbo University, Ningbo 315211, China

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ABSTRACT

Large yellow croaker (*Larimichthys crocea*) is a type of economic marine fishes mainly living in coastal area of China. In the present study, we investigated the fatty acid (FA) compositions in the muscles of Dai-qu stock and Min-yuedong stock large yellow croakers using gas chromatography-mass spectrometry (GC-MS). The results showed that the contents of some FAs were quite different between these two populations. Therefore, the FA composition could be used as a potential indicator for the identification of Dai-qu stock and Min-yuedong stock large yellow croakers. Specifically, the large yellow croakers with the FA ratios of [C18:1/(C20:1 + C22:1)]>5.0 and/or C20:5/C20:4 < 4.0 could be classified to Min-yuedong stock, while those with the FA ratios of [C18:1/(C20:1 + C22:1)] <5.0 and/or C20:5/C20:4 > 4.0 were classified to Dai-qu stock. The differences in the FA ratio between Dai-qu stock and Min-yuedong stock could be attributed to their primitive parents, which live in different sea areas with different temperatures. The cold-resistant genes in Dai-qu stock could be used as promising indicators for natural selection in the low-temperature environment, which mainly contributed to the differences of biochemical substances between Dai-qu stock and Min-yuedong stock.

1. Introduction

As an economically important marine fish in China, large yellow croaker (*Larimichthys crocea*) belongs to Perciformes, Sciaenidae, *Larimichthys*. Traditionally, large yellow croaker can be divided into three geographical populations as follows: Dai-qu stock, Min-yuedong stock and Nao-zhou stock. The majority of large yellow croakers farmed in Ningbo area are Min-yuedong stock, but its growth rate and cold tolerance are not as good as those of the native Dai-qu stock large yellow croakers (Xu et al., 2006). Additionally, the native Dai-qu stock large yellow croaker have better meat quality and flavor compared with Min-yuedong stock (Li et al., 2009). Despite these fascinating advantages, the Dai-qu stock is costly and facing extinction because of overfishing (Liu and De Mitcheson, 2008). Therefore, the local government has launched a series of projects to farm wild Dai-qu stock large yellow croakers, such as harvesting, domestication, breeding and germplasm repository. So far, a large number of Dai-qu stock large yellow croakers with good quality have been bred.

Due to their similarity in the morphological features, it is quite difficult to distinguish the Dai-qu stock and Min-yuedong stock large yellow croakers by the traditional observation method. Several methods have been developed for the identification of Dai-qu stock and Min-yuedong stock large yellow croakers. However, these methods are associated with some problems, greatly limiting their practical applications. For example, Ding et al. (2009) have established the identification formula to identify the difference of four families of large yellow croakers through the analysis of 10 morphological variables with high contribution rate, but its comprehensive discrimination rate only reaches 92.5%. Ding et al. (2006)have used the randomly amplified polymorphic DNA (RAPD) amplification spectrum to distinguish Dai-qu stock and Min-yuedong stock large yellow croakers. However, the RAPD technique requires high quantity of DNA template, amplification condition and system characters. Moreover, the repeatability of RAPD method is not good. In our previous work (Shen et al., 2013), we have comprehensively analyzed the different loci in D-loop and ND4 region between Dai-qu stock and Min-yuedong stock by classification of operational taxonomic units (OTU). The

* Corresponding author.
E-mail address: yanxiaojun@nbu.edu.cn (X.-J. Yan).

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repeatability of this method is good with relatively high accuracy, but it still has minor probability of error. Therefore, it is important to develop a more objective identification method, which would be greatly helpful for the protection of germplasm resource, seed selection and marketing of Dai-qu stock large yellow croakers.

Recently, the method, using fatty acid (FA) profiles as fingerprints to distinguish different populations, has been developed and widely applied in the identification of *Salmo salar* (Peter et al., 2009; Olsen et al., 2013), *Ictalurus punctatus* (Randals et al., 2006), *Diplodus puntazzo* (Rueda et al., 2001), *Oncorhynchus mykiss* (OZ and Dikel, 2015) and so on. In the present study, we aimed to distinguish Dai-qu stock and Min-yuedong stock large yellow croakers by analyzing the FA compositions of these two populations.

2. Materials and methods

2.1. Sample collection

The samples of large yellow croakers were collected in July, 2010 from the Breeding Base of Ningbo Academy of Ocean and Fishery in Xiangshan Bay, Zhejiang Province of China. Three batches of Min-yuedong stock of 4 months old (marked as M-S1~3), 16 months old (M-M1~3) and 28 months old (M-L1~3) were analyzed, respectively. Three batches of Dai-qu stock of 4 months old (marked as D-S1~3) as well as two batches of 16 months old (D-M1~2) and 48 months old (D-L1~2) were analyzed, respectively. Additionally, two batches of wild large yellow croakers were collected from Daiquyang sea area (marked as DQS1 and DQS2). Morphological characteristics of these large yellow croakers were summarized in Table S1.

Eight Dai-qu stock large yellow croakers were captured from Daiquyang sea area between 2007 and 2008, and then they were domesticated at the Breeding Base of Ningbo Academy of Ocean and Fishery in Xiangshan Bay. Two of these eight large yellow croakers were denoted as the samples D-L1~2. D-M1~2 and D-S1~3 were F1 generation bred by the parents of domesticated Dai-qu stock. In the fry stage, the temperature of seawater was basically identical (about 21 °C) through artificial control, and the annual average salinity of seawater of Ningde and Ningbo sea area were about 26‰ and 28‰, respectively. After about 45 days, all the fries were transferred to the farm in Xiangshan Bay, and fed with the same source of fresh frozen trash fish (TF). Since then, the seawater temperature and salinity for the two populations were the same.

2.2. GC-MS analysis

Back muscles in the side position were dissected and collected. Specifically, total lipids were extracted from 0.1 g of freeze-dried sample with $CHCl_3/CH_3OH/H_2O$ (1:2:0.8, v/v/v) containing 0.05% BHT using a previously described method (Bligh and Dyer, 1959).

FA analysis was performed according to the method described by Xu et al. (2012a) with minor modifications. Briefly, 2 mL methanol/water (4:1, v/v) containing 6% potassium hydroxide was added to the sample and incubated at 60 °C for 2 h. After cooling, 1 mL of saturated NaCl solution was added, and the pH of the solution was adjusted to be lower than 1 using HCl/H_2O (4:1, v/v). The saponated FAs were extracted with 6 mL of chloroform/hexane (v/v, 1:4) for three times. All the extractions were pooled, and 2 mL water was added, yielding a solution separation. The supernatant was collected and dried under a gentle stream of N_2. The dried FAs were resuspended in 0.5 mL 14% BF_3-CH_3OH and heated to 60 °C for 1 h in order to form fatty acid methyl esters (FAMEs). After cooling, the FAME mixture was extracted by adding 1 mL saturated NaCl solution, followed by sequential addition of 2 mL hexane (two times) and 3 mL chloroform/hexane (v/v, 1:4). All the extractions were collected and dried with 3 g sodium sulfate for 5 h, and the upper solution was evaporated with N_2 and dissolved in hexane for GC-MS analysis.

GC-MS analysis was performed on the SPB-50 fused capillary silica column (30 m × 0.25 mm, 0.25 μm Supelo, USA) using the Shimadzu QP2010 gas chromatography mass spectrometer. The injection temperature was 250 °C, and high-purity helium (≥99.999%) was used as carrier gas at a flow rate of 0.81 mL min^{-1} and a pre-column pressure of 73.0 kPa. After injection, the column temperature was maintained at 150 °C for 3.5 min, and then it was increased to 200 °C at a rate of 20 °C/min and kept for 5 min. Subsequently, the column temperature was increased to a final temperature of 280 °C at a rate of 5 °C/min and kept for 37 min. The injection volume was 1 μL with a split ratio of 50:1. Mass spectrometry was operated under the electron impact mode with electron energy of 70 eV. The ion source temperature and interface temperature were set at 200 °C and 250 °C, respectively. The scanning ranged from m/z 40 to 600, and the solvent cut off time was set at 3.5 min. The identity of FAs was determined by comparing the retention time and fragmentation ions with the FAME standards. The National Institute of Standards and Technology (NIST) 92 Spectral Database, Wiley7 and those related to the previous analysis of commercially available pure references were also used for these comparisons. The relative content of each FA was calculated and normalized by peak area normalization method.

2.3. Data processing

GC-MS data were analyzed by the principal component analysis (PCA), the projection to latent structures-discriminant analysis (PLS-DA) and the orthogonal projection to latent structures-discriminant analysis (OPLS-DA) using the SIMCA-P^+ software package (V.12.0, Umetrics AB, Umea, Sweden). The PLS-DA models were validated with the cross-validation function of SIMCA with 200 as the permutation number. The statistical analysis (one-way ANOVA) was performed to confirm the differences among treatments using the SPSS 13.5 version (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Composition analysis of FAs

Table 1 shows the good similarity in the types of FAs between Dai-qu stock and Min-yuedong stock large yellow croakers. The amounts of total SFAs and MUFAs were higher than those of total polyunsaturated fatty acids (PUFAs). Additionally, Table 1 also illustrates that C16:0 was dominant in both Dai-qu stock and Min-yuedong stock large yellow croakers, and the relative contents of other FAs could be ranked in a descending order as follows: C18:1n-9>C22:6n-3>C20:5n-3>C16:1n-9. The relative contents of C16:0, C20:1n-9, C20:5n-3 and C22:1n-9 in Dai-qu stock large yellow croakers were higher than those in Min-yuedong stock, while the relative contents of C18:0, C18:1n-9 and C20:4n-6 in Dai-qu stock large yellow croakers were lower than those in Min-yuedong stock. The relative contents of C16:0, C16:1n-9, C18:1n-9 and C22:5n-3 were increased with the size of large yellow croakers, while the relative contents of C18:0, C18:2n-6, C20:5n-3, C22:1n-9, C22:6n-3 and C24:1n-9 were decreased with the size.