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- Note -

Genetic Differences between Wild and Cultured Populations in Olive Flounder in Korea Based on Mitochondrial DNA Analysis

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We sequenced a 522 bp fragment including the $tRNA^{Thr}$, $tRNA^{Pro}$ gene and the first half of the control region from 29 wild and cultured olive flounder specimens from Korea. Out of 522 nucleotide sites, 49 (9.4%) were variable, 23 haplotypes being found. Most haplotypes are unique in the wild population and only four were shared by cultured specimins. The nucleotide diversity and differences between wild and cultured populations were 0.025±0.013 and 0.015±0.008, and 12.94±6.00 and 7.83±3.75, respectively. Haplotype diversity was 0.98±0.02 and 0.49±0.09 in the wild and cultured populations, respectively. These results show that marked reductions of genetic variability in the hatchery strains were observed in the number of mitochondrial DNA haplotypes and haplotype diversity when compared to the wild populations. Furthermore, we detected significant population differentiation between both populations. The mtDNA sequencing technique used to evaluate the genetic variability of hatchery strains compared to that of the wild population is potential for genetic monitoring of olive flounder hatchery stocks.

Key words : Olive flounder, mitochondrial DNA, diversity, differentiation

Introduction

The olive flounder *Paralichthys olivaceus* is widely distributed throughout coastal areas of Korea, Japan and China. It is one of the commercially important species cultured in late 1980's in Korea. Recently, the Korean production exceeded approximately 3,000 tonnes, and millions of juveniles have been released every year for the resource enhancement. We are concerned with the potential genetic impact of the stocking practice on the wild fish stocks. Because the genetic variability in most hatchery stocks is lower than wild populations, and this may possibly result in the loss of disease resistance or in the reduction of population's capability to adapt to new environments [1]. Therefore, the investigation of the genetic diversity of local populations is required for stock management of the species.

Recently, many studies have been conducted on the genetic characteristics of marine fishes using mitochondrial DNA (mtDNA) analysis [2,9,11]. Especially, the control region in the mtDNA genome has been used in population studies because of its high evolutionary rate, as compared to many nuclear loci. In this study, we examined the genetic diversity of the wild population and the hatchery-raised stock using the sequences of the mtDNA control regions for the population structure analysis of olive flounder in Korea. This marker was appropriate to monitoring the reductions of genetic variability occurring in hatchery stocks. Our results will provide information for the production and management of hatchery flounder to maintain the genetic diversity in cultured fish.

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Materials and Methods

Sample collection and DNA extraction

The wild and cultured olive flounder were collected from Yangyang and the hatchery in Uljin in 2008, respectively. DNA extraction from the tissue samples was performed using the automated DNA extraction system MagExtractor MFX-2100 (TOYOBO) according to the manufacturers' recommendations.

Amplification and sequencing

We analyzed the control region of mtDNA from the 29 wild and cultured olive flounders. PCR was conducted in a reaction mixture containing 5.0 µl 10× reaction buffer, 4.0 µl dNTPs (2.5 mM for each of the four dNTPs), 2.5 µl of each primer (10 µM each), 5.0 µl DNA template, 0.25 µl ExTaq DNA polymerase (5 U/µl; Takara, Shiga, Japan), and distilled water up to 50 µl. PCR amplifications were con-

ducted using an RTC 200 machine (MJ-Research, Watertown, MA, USA).

The primers were deigned from a complete nucleotide sequence of mtDNA genome (GenBank accession AB028664) [5] to amplify approximately 600 bp segments as the following: the forward F (5′-ATG ACA GTG CAT TAG TAG CTC AGT-3′) and the reverse R (5′-GCT GGG TAA CGA GTC GTA TGT-3′). The amplification conditions used in this study were as follows; initial denaturation for 11 min at 95°C followed by 35 cycles of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C with a final 5 min extension step at 72°C.

PCR products were purified using PCR Purification Kits (Qiagen, Hilden, Germany) and sequenced on an ABI3100 Prism automatic DNA sequencer with the BigDye 3.1 Termination system (Applied Biosystems, Foster City, CA, USA).

Data analysis

Sequences were edited and aligned with the SeqMan (DNASTAR Inc., Madison, WI, USA) and MEGA 4.0 programs [8]. The DNASIS software (version 2.5; Hitachi, Tokyo, Japan) was utilized in other to determine the haplotype of the control region from the obtained sequence data. Molecular diversity indices such as number of transitions, transversions and indels, were obtained using the program Arlequin (Ver. 3.1) [6]. Haplotype diversity, nucleotide diversity and their corresponding variances were performed with the software package DnaSP (Ver. 4.5) [4] and Arlequin. In addition, overall *F*-statistics [10] was estimated based on mtDNA sequences using Arlequin. The significance of the pairwise comparisons of the F_{ST} was tested by 10,000 permutations according to a Kimura's two- parameters model [3].

Results

A total of 522 bp of the fragments included the $tRNA^{thr}$ (22 bp) and entire $tRNA^{pro}$ genes (71 bp) and the first portion of the control region (429 bp) were sequenced for 29 wild and hatchery populations, respectively. Haplotype 3 (13% in wild population) and Haplotype 5 (68% in cultural population), respectively were the most common one in each population. Only Haplotype 1, 3, 5 of the 23 were shared by both populations. The others were wild population specific haplotypes. Forty-nine variable sites (9.4%) were observed in 23 haplotypes(Fig. 1) defined among all samples and their frequencies were shown Table 1. Haplotype diversity, nucleotide diversity and other specific diversity indices were shown in Table 2. Number of haplotype, polymorphic sites and observed transitions and transversions in wild population were more than cultured one. A pattern in the base substitutions showed dominant transitions both populations. Indels were detected only wild population. Haplotype diversity was 0.98±0.02 in the wild population and 0.49±0.09 in the cultured one. Pairwise sequence differences in the wild population (12.94%) were also higher than cultured one (7.83%), reflecting the abundance of variable site. The FST

Table 1. The haplotype frequencies of control region in olive flounder

Haplotype	Haplotype frequency		
	Wild (29)	Cultured (29)	total
H1	1	4	5
H2	1	0	1
H3	4	5	9
H4	1	0	1
H5	2	20	22
H6	1	0	1
H7	1	0	1
H8	1	0	1
H9	1	0	1
H10	1	0	1
H11	1	0	1
H12	1	0	1
H13	1	0	1
H14	1	0	1
H15	1	0	1
H16	1	0	1
H17	1	0	1
H18	2	0	2
H19	1	0	1
H20	2	0	2
H21	1	0	1
H22	1	0	1
H23	1	0	1

Table 2. Summary of genetic diversity indices of olive flounder

Genetic diversity indices	Wild	Cultured
No. of haplotypes	23	3
No. of polymorphic sites	49	18
No. of observed transitions	43	14
No. of observed transversions	6	4
No. of observed indels	3	0
Hd	0.98±0.02	0.49±0.09
π	0.025±0.012	0.015±0.008
κ	11.97	7.83

Hd, haplotype diversity; π, nucleotide diversity, κ, average No. of nucleotide differences

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          11111111 2222222222 2222222333 3333333333 344444445
          1204567799 0001123444 4455678022 2455566666 700235590
          9075725646 1681458012 3757231101 9915912468 017062376
H1        GTTTACAGAT AACATGAACA TTACAAGCGT TTTC-AA-CC TTTGTTATT
H2        .....A.A.A .G.G...GT. .A....A.A. ...T...... ..CA.C.CC
H3        .......... .......... .......... .......... ......G..
H4        ..CCG....A G......... ........A. .CCTA..... .........
H5        .....A.A.A .GT.....TG .A.T..AGA. ...T...... ..CA.C.C.
H6        ....G....A .......... ...T..A.AA .C.T.G.... .........
H7        .....A.A.A .G.G....TG .A.T..A.A. ...T....T. ..CA.C.CC
H8        ....G...GA ........T. .......... .......... .........
H9        .....A.A.A .G......T. .A....A.A. ...TA.G... ..CACC.C.
H10       .....A.A.A ..T..A..TG .A.T..A.A. ...T....T. ..CA.C.C.
H11       ..CCG....A .......... C...GG..A. .CCTA....T .........
H12       .....A.A.A .G.G....TG .A....A.A. ...T...A.. ..CA.C.C.
H13       .....A.A.A .G.G....TG .A....A.A. ...T...... ..CA.C.C.
H14       .....A...A .G.GC...T. .A....A.A. ...T.....T .CCA.C.C.
H15       ..CCG....A .....CG... ......A.A. .CCTA..... .........
H16       ..CCGTG..A .......... ....G.A.A. .CCTA..... .........
H17       ..CCG....A .......... C...GG..A. .CCTA..... .........
H18       ....G....A .......... ..G.....A. .CCTA..... .........
H19       ..CC.....A .......... C...GG..A. .CCTA..... .........
H20       ....G....- .......... ..G.....A. .CCTA..... .........
H21       ..CCG....A .......... C...GG..A. .CCTA..... C........
H22       AC.......A ....C..... ......A.A. C......... .........
H23       .....A.A.A .G......TG .A.T..A.A. ...T...... ..CA.C.C.
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Fig. 1. Aligned mitochondrial control region and adjacent threonine and proline transfer RNA gene sequences from 59 olive flounders, showing variable sites and deletion event (-). Dots (.) represent the same base with those of the haplotype N1.

value (0.214) showed significant differentiation between populations ($p<0.05$).

Discussion

The analysis of mtDNA control region has been conducted to detect the genetic diversity and population structure in various marine fish. We sequenced a fragment included the control region to analyze the genetic variation between wild and cultured olive flounders in Korea. Only four haplotypes in wild population were shared between two or four individuals and the others were unique. All haplotypes in the cultured population was genetically originated from the wild population. Haplotype diversity of the same segment of the mitochondrial D-loop in other marine fish were 1.00 in Japanese flounder [7], 0.98 in yellow croaker [11] and 0.97 in big eyed herring [9]. Our result also showed high haplotype diversity of 0.98. The number of mtDNA haplotypes identified in the hatchery population was reduced by 83% as compared with ones in the wild population. These results indicated that gene diversity level of wild population was higher than cultured population. Furthermore, high F_{ST} values estimated for between the wild and cultured populations demonstrate a noticeable genetic population differentiation between them.

Artificially raised juvenile Japanese flounder have been released around Korea for a long time. There are the concerns about loss of the genetic variability in the cultured stocks after long periods of hatchery production. Artificially raised stocks were very inferior to wild stocks in their genetic variability because of the probably limited number of parents. They may be harmful effect on several traits such as fecundity, survival and growth. Accordingly, monitoring of genetic variability should be performed.

The sequence of the mtDNA control region in this study might be a powerful approach to monitor genetic conditions in hatchery stains of olive flounder. The stocking practice with considerations of genetic impact on wild population should be attended. The only way to minimize the genetic impacts is to improve the genetic management for all hatchery stains through monitoring the genetic variability and estimating precise effective population size.

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초록 : 미토콘드리아 DNA분석에 의한 자연산 및 양식산 넙치 집단의 유전적 다양성 변화

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우리나라의 주요 양식 대상 종이며, 년간 총 생산량 1위를 점하고 있는 넙치를 모델로 하여 양식 집단의 유전적 다양성의 변화를 확인하였다. 이를 위해, 한국에서 서식하고 있는 자연산 및 양식산 넙치 각 29개체를 사용하여, hypervariation 영역으로 알려진 tRNA ($tRNA^{Thr}$, $tRNA^{Pro}$) 영역과 control region의 앞부분까지의 522 bp에 대한 염기서열의 특성을 분석하였다. 23개의 haplotype에서 522 bp의 염기 중 49곳(9.4%) 에서 변이가 나타났다. 대부분의 haplotype은 자연집단에서 유일하게 나타났으며, 오직 4개의 haplotype만이 양식집단에서 나타났다. 또한, 두 집단 사이에서는 유전적으로 유의한 집단분화가 발생하였다는 사실도 확인할 수 있었다. 따라서 미토콘드리아 DNA 염기서열 분석 기법은 집단의 유전적 다양성을 평가뿐만 아니라 양식집단의 유전적 모니터링에 사용 가능 할 것으로 판단된다.