MITOCHONDRIAL DNA VARIATION OF CULTURED AND WILD POPULATIONS OF ASIAN SEABASS (*Lates calcarifer*) IN THAILAND

Variasi DNA Mitokhondria dari Ikan kakap Putih Budidaya dan Dari Tangkapan Liar Di Thailand

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ABSTRAK

Kakap Putih (Lates calcarifer Bloch) adalah salah satu ikan ekonomis penting yang benihnya dapat diproduksi Thailand. Data genetik merupakan salah satu aspek penting dalam mengatur pemijahan induk untuk pemuliaan, namun hingga saat ini informasi terkait keragaman genetiknya masih minim. Penelitian ini bertujuan mengkaji keragaman dan perbedaan genetik kakap putih pada tiga populasi hatchery dan dua populasi alam di Thailand dengan menggunakan metode gabungan antara Restriction Fragment Length Polymorphisms dan Polymerase Chain Reaction (PCR-RFLP) pada D-loop control region di DNA mitokondria. Ditemukan tujuh potongan endonuklease dari 268 sampel yang diamati. Populasi alami Chantaburi (CH) memiliki keragaman haplotipe dan nukleotida tertinggi ($h = 0.6626$, $\pi = 0.0554$) dibandingkan populasi lainnya: tiga populasi hatcheri yaitu Rayong (RA), Chonburi (CB) dan Nakhon Si Thammarat (NK)) (h berkisar antara 0.2709 – 0.3227; π berkisar antara 0.0195 – 0.386) dan populasi alam Nakhon Si Thammarat (PN) ($h = 0.172$, $\pi = 0.0091$). Mismatch distribution analysis mengungkap kejadian bottleneck pada beberapa generasi sebelumnya di populasi alam PN dan seluruh populasi hatcheri. Analysis of Molecular Variance (AMOVA) menunjukkan 88.95% keragaman disebabkan perbedaan dalam populasi dan 11.05 % disebabkan perbedaan antar populasi. Perbedaan genetik yang signifikan terdapat pada perbedaan antar populasi alam ($\Phi_{ST} = 0.239$, $P<0.001$), namun perbedaan antar populasi hatchery tidak signifikan, menunjukkan terjadi pencampuran genetik sekerabat antar satu dengan hatcheri lainnya. Hal ini karena minimnya masukkan indukan baru dari alam. Kedua hal tersebut dapat menurunkan keragaman dan perbedaan genetik pada setiap populasi hatchery dan antar populasi hatchery. Data awal penelitian ini dapat digunakan untuk pengelolaan induk, upaya pemuliaan dan monitoring genetiknya di Thailand.

Kata kunci: keragaman dan perbedaan genetik, control region, DNA mitokondria, kakap Putih, Lates calcarifer.

INTRODUCTION

Asian seabass (*Lates calcarifer* Bloch) is an economically important coastal, estuaries and marine fish species in the world, especially in the Indo-pacific region. Thailand is an important producer and exporter of *L. calcarifer* fingerlings. After a long domestication period of this species (about 20 years), genetic data in Thailand are still lacking. Farmers started to observed undesirable symptoms, such as vulnerability to diseases, deformity of bone, and slow growth. This may be linked to the loss of genetic variation in hatchery populations. Genetic data is an important component to genetic management of broodstock. This study aimed to evaluate level of mitochondrial DNA variation within population and differentiation among populations of cultured broodstock of *L. calcarifer* in Thailand compared to wild populations using Restriction Fragment Length Polymorphisms combined with Polymerase Chain Reaction (PCR-RFLP) techniques. This information provides baseline data for genetic management program of broodstock as well as the development of a selective breeding program in Thailand.

METHODOLOGY

We analyzed the mtDNA variation of *L. calcarifer* populations along the Gulf of Thailand. Fin clips were collected from cultured and wild populations. Cultured populations included Rayong Coastal Fisheries Reseach and Development Center (RA), a private hatchery in Chonburi (CB), Nakhon Si Thammarat Coastal Fisheries Reseach and Development Center (NK), and the wild samples were from Chantaburi (CH) and Phak Nakhon district of Nakhon Si Thammarat (PN). Each sample consisted of 39 to 67 individuals (body weight = 4 to 7 kg). Small amount of caudal fin clips were cut and preserved in 95% ethanol.

Total DNA was extracted using salt-extraction method (Aljanabi and Martinez, 1997). PCR was used to amplify a 1 kb of the mtDNA control region using primer LN20 (5'-ACC ACT AGC ACC CAA AGC TA-3') and HN20 (5'-GTG TTA TGC TTT AGT TAA GC-3') (Bernatchez and Danzzman, 1993). A PCR reaction contained 1x PCR buffer, 0.4 mM $MgCl_2$, 2 mM dNTPs, 2 pmole each primers, 0.5 U Taq Polymerase, 15-50 ng DNA template and distilled water up to volume. PCR was performed in a thermal cycler for 30 cycles (92^oC, 60 s; 50^oC, 60 s; 72^oC, 90s) with a minute initial denaturing step at 92^oC and followed by a 10 minutes final extension at 72^oC. For RFLP, we used seven restriction enzymes: *Fau*I, *Hind*II, *Eco*RI, *Eco*RV, *Vne*I, *Bse1*I and *Bst*ENI to reveal composite of haplotypes. PCR and RFLP products were electrophoresed in 1.5 % Agarose.

Haplotype and nucleotide diversity were calculated according to Nei and Tajima (1981)'s methods using the Restriction Enzyme Analysis Package (REAP) (McElroy et al., 1992). To test the homogeneity of haplotype frequencies between samples, we perform aχ^2 analysis by the Monte Carlo procedure (Roff & Bentzen, 1989) using program MONTE implemented in the REAP with 1000 randomization. Population differentiation was calculated by Analysis of molecular variation (AMOVA) and global exact test. To detect an occurrence of bottleneck we used analysis of mismatch distribution. These three analyses were performed using Arlequin program (Excoffier et al., 2005) with 1000 randomization.

RESULTS AND DISCUSSION

Six composite haplotypes were obtained from seven restriction enzymes. The levels of haplotype diversity did not have a consistent pattern in wild versus hatchery populations (Table 1). Wild population CH had highest haplotype and nucleotide diversity (h = 0.6626 ± 0.04751; π = 0.0554) while the wild population PN had lowest haplotype and nucleotide diversity (h = 0.1742 ± 0.06531; π = 0.0091). The hatchery populations had moderate haplotye diversity ranging from 0.2709 ± 0.06876 to 0.3227 ± 0.07950.

One level AMOVA and global exact test indicated significant genetic differentiation among populations (Φ_{ST} = 0.11055, P<0.001 for AMOVA and P<0.05 for exact test, Table 1). For pair-wise population genetic differences, genetic differentiation between wild populations was highly significant (Φ_{ST} values = 0.239, P<0.001), while there was no significant differences among hatchery populations. The analysis of mismatch distribution indicated bottleneck event in three hatchery populations and wild PN population.

Table 1. Haplotype diversity(h, nucleotidediversity (π) at each population and AMOVA

Population	*H*		*Π*	Source of variation	d.f.	Percentage of variation
CH	*0.6626*	± *0.04751*	0.0554			
RA	*0.2709*	± *0.06876*	0.0195	Among Populations	4	11.05 %
CB	*0.3227*	± *0.07950*	0.0386	Within Populations	263	88.95 %
NK	*0.3212*	± *0.09186*	0.0279	Total	267	
PN	*0.1742*	± *0.06531*	0.0091	Fixation index	*0.11*	P<0.001

High level of genetic diversity of the wild CH population might reflect a larger historical population size of this population compared to other population studied. Slightly higher level of genetic diversity was observed in wild *L. calcarifer* populations in Northern Australia (Chenoweth et. al., 1998; Doupe et al., 1999). Larger population size will generally maintain higher level of genetic diversity in a population. The difference in the level of genetic diversity in the Australian and Thai populations may be due to larger population size of the Australian population compared to the Thai populations. Populations of *L. calcarifer* in Thailand are under much higher fishing pressure. Average annual catch during 1999-2005 in Gulf of Thailand was six times higher than in Northern Australia (Thailand Department of Fisheries Statistics; Newman et al., 2007). The unexpectedly low genetic diversity in the wild PN population and occurrence of recent bottleneck may be due to high fishing pressure in Nakhon Si Thammarat coastal areas. Based on fisheries statistics, Nakhon Si Thammarat province had higher numbers of fishing boats and fishermen during peak fishing season than in Chantaburi province. In addition, fishing may facilitate further loss of genetic variation by removing large individuals that are females from a population. *L. calcarifer* is protandrous hermaphrodites (individuals develop into males and then females as they get older and larger). Davis (1982) reported only few *L. calcarfer* females available in a population because only few individuals successfully reached older age and become females. Unequal sex ratio unequal family contribution to the successive generations can facilitate genetic drift and lead to loss of genetic diversity (Frost et al., 2007). Higher number of haplotypes

suggested that wild CH population is a potential founder source of *L. calcarifer* in Thailand.

Genetic diversity within RA, CB and NK hatcheries can be categorized as low compared to other finfish species, e.g., *L. calcarifer* in Singapore (Zhu et al., 2006), Japanese Flounder (Sekino et al., 2002), and common carp (Thai et al., 2006). Low level of genetic variation is typical for hatchery populations due to aquaculture practice such as mating limited number of broodstock, mass spawning with unequal number of females, and size grading (Frost et al., 2007). Bottleneck event detected in three hatcheries was probably due to insufficient number of founders (Hallerman, 2003). Mating large and equal number of parents collected from various population origins will prevent loss of genetic variation within hatcheries as suggested by Meejui et al. (2005) in *Beta splendens* and Sriphairoj et al. (2007) in *Pangasianodon gigas* in Thailand.

Population differentiation between two wild populations was highly significant, indicating strong population structure in Thailand. This pattern of population structure is concordant with life history and dispersal pattern of *L. calcarifer* that tend to migrates in closed river system (Salini & Shaklee, 1988; Chenoweth et. al., 1998; Doupe et al., 1999). Furthermore, hermaphroditic nature of *L. calcarifer* which males transformed into females in older ages (Moore, 1979), and limited number females available in a population (Davis, 1982) can contribute to genetic differentiation due to high level of genetic drift within each population.

CONCLUSIONS

Although the populations studied did not directly exchange their fish, most of them probably derived from similar ancestral populations with a population from National Institute for Coastal Aquaculture (NICA) being a major source as the institute developed the first domesticated stock of *L. calcarifer* in Thailand. Information this study provides baseline data for broodstock management, genetic improvement as well as genetic monitoring of *L. calcarifer* broodstock in Thailand.

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