Phylogenic relationship and toxic variations between Turkish Mediterranean and Japanese puffer fish species

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博士学位論文内容要旨
Abstract

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論文題目 Title	Phylogenic relationship and toxic variations between Turkish Mediterranean and Japanese puffer fish species（地中海に生息するフグ類の系統および毒性解明）		

Biological diversity has been negatively affected by invasive species and has become a global problem against protection efforts. The Mediterranean Sea and the Red Sea were connected after the opening of the canal Suez Canal in 1869. Therefore, both the Red Sea and Mediterranean Sea were exposed to the invasion of organism. However, the extensive majority of migrational movement has occurred from the Red Sea to the Mediterranean Sea and this bioraid is termed “Lessepsian migration”. The puffer fishes belonging to the family Tetraodontidae are highly abundant teleosts, with 184 species belonging to 27 genera and represented with six Lessepsian species in the Mediterranean coasts of Turkey. *Lagocephalus sceleratus*, *L. suezensis* and *L. spadiceus* have been detected as the most abundant puffer fish species among those Lessepsian puffer fishes and the economical and ecological effects in the eastern Mediterranean system are highly concerned. *Lagocephalus sceleratus* is regarded to be among the worst invasive species in the Mediterranean Sea with a significant impact on the surrounding ecosystem and on the fisheries sector.

L. sceleratus is considered to be a serious hazard to consumers since it contains a strong marine toxin, tetrodotoxin (TTX), which can be lethal to humans. Therefore, the studies in Chapter 2 focused on confirmation and comparison of toxicity of *L. sceleratus*. Firstly, TTX contents of twenty specimens caught from Marmaris and Iskenderun Bay, Turkey, and twelve specimens caught from Okinawa Island, Japan were determined by LC-MS/MS. Individual and

tissue depending TTX distribution were clarified. As a result, all of the specimens examined in this study contained detectable amount of TTX. Although testis, muscle and skin tissues relatively contained less toxicity comparing with the ovary and other internal organs, TTX contents were individual and tissue dependent. The results of the study in Chapter 2 indicates that *L. sceleratus* is a potential risk for human consumption and needed to be eliminate.

Whole mitogenome sequences from many teleost lineages have been determined and used for phylogenetic analyses. Thus, in Chapter 3, the complete mitochondrial DNA (mtDNA) of *L. sceleratus*, *L. suezensis* and *L. spadiceus* were determined according to primer-walking strategy and the gene structures were also analyzed. Afterwards, phylogeny within the family Tetraodontidae were clarified. Since these puffer fish species have migrated, it is needed to understand the zoogeographical and evolutional origin. Both the gene structure and phylogenetic trees showed that *L. sceleratus* and *L. suezensis* were closely related species, although morphological characteristics were unique of each species. In addition, the members of genus *Lagocephalus* can be distinguished by using molecular markers based on their mtDNA. Thus, molecular identification of three puffer fish species by polymerase chain reaction (PCR) were carried out in Chapter 4.

PCR-based identification methods have been widely used to identify and authenticate of fish species and seafood products. Species-specific PCR amplification was conducted based on the species-specific nucleotide sequences of mtDNA of three Lessepsian puffer fish species, *L. sceleratus*, *L. suezensis* and *L. spadiceus.* Analysis of the alignment of the eight reference sequences obtained from GenBank showed that the bases in 119 position of NADH dehydrogenase subunit 2 (ND2) region in mtDNA could differentiate *L. sceleratus*. On the other

hand, the bases in 123 and 138 position of cytochrome oxidase subunit 1 (COI) region in mtDNA could successfully differentiate *L. suezensis* and *L. spadiceus*, respectively. The method constructed in this Chapter is a useful tool for authentication and identification of *Lagocephalus* species. And also, the method can be used to verify the labeling issues of seafood products.

In Chapter 2, TTX contents of *L. sceleratus* were confirmed. However, the accumulation mechanism of TTX was not discussed. It has been recently reported that TTX binds with high molecular weight substances and the substances neutralize the lethal effects of TTX. Hence, in Chapter 5, the binding protein from the ovary of *L. sceleratus* were purified. The results of the analysis showed that TTX-binding ability was weak and destroyed during the gel filtration purification process. Therefore, the puffer fish saxitoxin and tetrodotoxin binding protein (PSTBP) was cloned from cDNA constructed from *L. sceleratus* liver. The characteristics of the protein (LsPSTBP) was highly similar with those of plasma binding proteins of *Takifugu pardalis*, which are known as PSTBP1 and PSTBP2. Moreover, recombinant LsPSTBP protein was expressed and purified in *E. coli*. The physicochemical properties of recombinant LsPSTBP were characterized by a combination of mass and circular dichroism and TTX binding ability assay was carried out. The binding ability of crude recombinant LsPSTBP was detected as 38% while purified LsPSTBP binding ability was approximately 10%. This result indicates that PSTBP might have an important role on TTX accumulation in genus *Lagocephalus* but not only *Takifugu*.

As a conclusion in the present, overall data collected from this study will be fundamental for the eastern Mediterranean fishery and the handling of the problems caused by Lessepsian puffer fish species. Furthermore, the identification method will give a great advantage to local authorities and consumers to eliminate these species and the PSTBP of *L. sceleratus* will be

guided to better understanding of the TTX accumulation mechanism in the puffer fish.