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Original Article

ESTERASE ISOZYME BANDING PATTERN OF *Anguilla marmorata* FROM FOUR RIVERS OF KAUR DISTRICT, BENGKULU

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Abstract

The phylogenetic relationship of the tropical eel *Anguilla marmorata* from four rivers of Kaur District, Bengkulu Province, Indonesia was evaluated by esterase isozyme banding. Samples were collected from Padang Guci, Kinal, Luas and Nasal rivers from May to June, 2012. The isozyme esterase band of *Anguilla marmorata* was analyzed by electrophoresis. The relationship of esterase isozyme band among eels was determined by cluster analysis using the unweighted pair group method with arithmetic averages method. The results revealed that the pattern of isozyme esterase band of *Anguilla marmorata* caught from Padang Guci, Kinal and Nasal rivers was comparable, meanwhile, it obtained from Nasal and Luas rivers was similar and the last pattern was found from Nasal river. The relationship of esterase band profile of *Anguillla marmorata* between the first river group and the second was around 83.3% by cluster analysis; meanwhile, the esterase band profile of the last group revealed relationship with the first and second group was about 63%. In conclusion, *Anguilla marmorata* in Kaur District could be divided into three groups of subpopulations.

Keywords: *Anguilla marmorata*, cluster analysis, esterase isozyme, Kaur District

1. INTRODUCTION

*Anguilla marmorata* is cosmopolitan species which is distributed all over the tropical water in the world. Tropical eels are catadromous eels whose members enter freshwater and move upstream into a wide variety of marsh, stream, river, or lake habitats. The adult eel will do migration to the ocean for spawning and the juvenile will come back to the freshwater until they become adult stages. In Indonesian waters the genus *Anguilla* is suggested to distribute of about seven species, which is 18 species or subspecies recorded worldwide [1,2,3].

In Indonesian waters, some tropical eels appear to spawn after a relatively short migration to the deep water basin adjacent to their growth habitat [2]. Therefore, all the rivers which its mouth in coastal areas of southern Java and Bali, eastern Kalimantan, Sulawesi, and western Sumatra flow into the deep basin. For instance, a lot of rivers at Kaur District, such as Padang Guci, Kinal, Luas and Nasal river which flow into Indian Ocean and then become a good growth habitat of tropical eels [4].

Isozyme is an enzyme which is produced directly by a gene that consists of several active molecules which have a various chemistry structure, but it catalyzes the same reaction. Enzyme is any of a group of protein that act as catalyst and accelerate the rate of normally slow physiologically important reactions. This specific catalytic activity and its control by genetically ways [5]. Isozymes are multi form of an enzyme that often appears in different molecular forms in a species [6]. Therefore, if the enzyme bands of the two group of organism which is analyze were revealed the same genetic characters, it means that both of two group have the same cluster; and vice versa.

Isozyme is a good marker and a lot of used in an analyzing any gen, such as it able to identify biochemistry processes, which is previously never known [5,6,7].

Esterase isozymes are one of the lipid-hydrolyzing enzymes which have a great significance in the field of genetics [8] and can be separated by electrophoresis due to having different isoelectric points, so this isozyme is a very useful for identifying any mysterious species which it is very hard to identify using a morphologically method [7].

The esterase isozymes banding have been used to
investigate the genetic differentiation on carp [6,8], mosquito fish [9], tuna [10], sepat siam [11], tilapia [12], nile and blue tilapia [13], catfish [14] and sparid fish [15], respectively, meanwhile, the genetic diversity of \textit{A. marmorata} has been evaluated by RFLP-PCR analysis on cytochrome b of mitochondria DNA [3], cytochrome c of mitochondria DNA [16], 16S rRNA of mitochondria DNA [17] and 16S rRNA and cytochrome b of mitochondria DNA [18]. The aims of this study were to evaluate the genetic diversity of \textit{A. marmorata} from four rivers of Kaur District, Bengkulu Province, Indonesia by esterase isozyme banding.

2. MATERIALS AND METHODS

Samples of \textit{A. marmorata} were collected from May to June 2012 and the total length of specimens was around 14-24 cm. A total of 80 \textit{A. marmorata} individuals were caught from four rivers namely Padang Guci, Kinal, Luas and Nasal, Kaur District, Bengkulu Province, Indonesia (Figure 1). The experiment was conducted in the Laboratory Centre of Life Sciences, University of Brawijaya. Around 0.5 g of muscle was taken and crushed with quartz sand in 0.5 mL of extraction buffer.

The enzyme placed into the gel by load in a piece of filter paper of Whatman 0.5 centimeters into the eel flesh extract. Then the filter paper was repealed and cleaned by tissue paper. After that the filter tissue was filled into starch gel and punched it. For controlling the migration of enzyme, one of the hole of filter paper was already diluted in blue bromphenol. Then the gel was located into electrophoresis tray which was contained electrode buffer and connected to the electric field with the voltage of 100 volt and the electric current of about 18 mili Ampere for about 4 hours, then gel was colored by esterase staining. Finally, the gel was cleaned in the flow water and gave a fixation by using glycerol and ethanol (1: 1),and then photography and analyzing were executed [19]. Data were recorded as presence (1) or absence (0) of band products from the gel photograph. This data was then analyzed by SPSS ver. 16.0 software package in order to infer similarities and genetic distances.

3. RESULTS

The results showed that esterase isozyme banding of \textit{A. marmorata} from Padang Guci, Kinal, Nasal and Luas was detected (Figure 2). It appeared that isozyme band of phylogenetic relationship of \textit{A. marmorata} in Kaur can be devided into three groups. The first group was the eel comes from river waters Padang Guci, Kinal, and Nasal; the second group was the eel comes from river waters Nasal and Luas; and the third group was the eel comes only from Nasal river.

![Figure 2: Esterase isozyme band of \textit{A. marmorata} from Nasal (A, B, C), Kinal (D, E, F), Padang Guci (G, H, I) and Luas (J) river](image)

Based on the UPGMA cluster analysis on the esterase isozyme band, the genetic relationship of individual eels among three groups of \textit{A. marmorata} were revealed significantly very close. For examples, the individual eel within the first group and the second group have significantly very close relationship of about 100% and the third group of individual eel were only found at Nasal river, as it is shown in Figure 3. The first and the second groups of \textit{A. marmorata} in Kaur have significantly close genetic relationship of about 83.3%. However, the third group of \textit{Anguilla marmorata} compared to those of the first and the second groups of the eels were less close genetic relationship.

![Figure 3: Esterase isozyme dendrogram of \textit{Anguilla marmorata} from four rivers in Kaur](image)

4. DISCUSSION

The esterase isozyme band of \textit{A. marmorata} muscle from the four rivers of Kaur District was observed four bands and could stated as esterase 3. These bands were obtained similar on \textit{C. carpio} [6], \textit{H. molitrix} [8], \textit{G. affinis} [9] and \textit{C. brachyurus} and \textit{C. gariepinus} [10] and dominated majority on anterior muscle [10]. Figure 2 show that the intensity of the esterase isozyme band of \textit{A. marmorata} muscle from the four rivers of Kaur District was medium to strong. It means that the specimens are belonged to the fish in early development. This isozyme was observed medium to deep stained in the aforementioned muscle. The intensity of observed bands was positively with the esterase activity.
and the strongest of hand was obtained on early development stages of fish [8]. These facts are appeared that anguillid eels of A. marmorata from the four rivers of Kaur District derived from one subpopulation whose the individual eels spawned in Indian Ocean. Therefore, it might be suggested that all individual anguillid eels of A. marmorata can be expected that they do migration from a larva of leptocephalus in Indian Ocean until they undergo metamorphosis into a glass eel and elver stages as a juvenile group in the freshwaters habitats. They then continued to change their bodies to become the adult stages such as yellow eels and silver eels whose members move downstream into a deep basin at Indian Ocean for spawning [1]. This life history undergo continously to achieve the spawning activities as their final target, due to after spawning they will die [1,4]. Because their migration is relatively short, so that the spawning ground is not far from the mouth of rivers that they originally come to spawn. Therefore, it might be expected or suggested that the subpopulation of A. marmorata from the four rivers in Kaur will come back to their originally river as their home.

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