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Restriction of Enzyme In EEL MT DNA

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Abstract: *his study was conducted to determine the genetic diversity of eel from the estuary of the Cimandiri Palabuhanratu River. Research on the genetic diversity of eels is very important for maintaining genetic resources, species and eel fish ecosystems. Genetic resources are the basic stages in the effort to protect eel resources in Indonesia. From the results of the study, eel fish taken from the mouth of the Cimandiri River, Pelabuhan Ratu is from the genus Anguilla. The samples are then preserved with absolute ethanol solution (96%), followed by the extraction process, amplification of PCR (Polymerase Chain Reaction), electrophoresis, and limitation of DNA sequences by enzymes HaeIII, Hin6I, RsaI, TaqI and NdeII). From the results of PCR analysis that Sidat fish in the Cimandiri River have three species, namely bicolor bicolor with short fin, nebulosa nebulosa, and marmorata camouflage. namely white eel. MtDNA D-Loop eel sequence for nebulosa nebulosa bacillus PCR has a length of about 395 bp, the bicolor bicolor species has a length of 230 bp and A. marmorata bacillus PCR has a length of about 620 bp. The restriction enzymes used to cut DNA sequences are HaeIII, Hin6I, RsaI, TaqI and NdeII) all of which have cutting sites. Retention enzymes are enzymes that can cut DNA molecules, in the order of sugar phosphate without damaging the base pair. The main function of the rectric enzyme is to cut DNA sequences to study the regulation of gene functions and structures. Restriction enzymes that affect the cutting of mtDNA D-Loop sequences in Nebulose-Nebulose, namely Hin6I, RsaI, TaqI and NdeII from site sites 395 bp to 390 bp, 394 bp, 390 bp and 391 bp respectively. Restriction enzymes that affect the cutting of the D-Loop mtDNA sequence on bicolor bicolor, namely Hin6I, TaqI and NdeII from the site location of 230 bp to 235 bp, 232 bp and 233bp respectively. Restriction enzymes that affect the cutting of the D-Loop mtDNA sequence in amarmorata namely HaeIII, Hin6I, RSaI. and TaqI, each has a site of 620 bp, 630 bp, 600 bp, 625 bp, 610 bp.*

Key word : *eel, larvae, juvenils, Palabuhanratu, morphological, enzymes, restriction.*

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I. Introduction

Eel fish is a consumption fish that has very important value for local and foreign markets. Market demand for very high eels reaches 500,000 tons per year, mainly from Japan and Korea; The main suppliers of eels are China and Taiwan. Eels known as 'unagi' in Japan are very expensive because they have a high protein content of 16.4% and vitamin A (Haryono 2008).

Eels have katadrom life cycles that will lay eggs in the sea and will move to estuaries and stay in rivers since hatching to adulthood (Aoyama et al. 2009). After arriving at the beach, eel larvae from development become juvenile (glass eel) with a shape like a pipe (anguilik) and transparent. Teenage Sidat fish are then carried by tidal currents towards the river mouth and will live several days in the estuary to adapt quickly (Arai et al. 2007). During this migration, the larvae and juveniles of eels are captured from brackish water in rivers to be raised in enlargement ponds, fed intensively to adulthood and ready for sale to exporters. Most of the eel and juvenile larvae in Indonesia come from Java (Sukabumi, Cilacap, Jember) and the best seeds around Palabuhanratu, Sukabumi, West Java (Hakim et al. 2015).

II. Research methodology

The research procedure was started from taking samples of Eel (Anguilla spp.), From the mouth of the Cimandiri River, Pelabuhan Ratu Sukabumi using anco fishing gear and seser net. Samples were taken randomly from the river mouth up to 3 km to the river (Fahmi and Rina 2010). Samples are then collected and preserved. After that, the sample is analyzed for its molecular character.

Molecular analysis and restriction of enzyme in eel is as follows:

a. Samples were collected from net catches and sesame Anco. Then the tail fin is taken 5-10 gr and preserved in absolute ethanol (96%).

b. DNA isolation and purification is carried out in the laboratory. The DNA isolation and purification process uses the extraction kit method.
c. The extracted DNA was then amplified using the PCR (Polymerase Chain Reaction) method.
d. The PCR results are electrophoretic to ensure the quality of DNA amplification. electrophoresis can be seen and observed from a UV trans illuminator, and then sorted.
e. The sequence of mtDNA sequences is limited by enzymes.
f. Restriction of enzyme Hin6I, RsaI, TaqI and NdeII in mtDNA Eel fish

III. Results and Discussion

mtDNA D-Loop sequence in eel, for nebulose nebulose species that bacillus PCR has a length of about 395 bp, bicolor bicolor species has a bacillus with a length of 230 bp and A. marmorata has bacillus PCR with a length of about 620 bp. Of the five restriction enzymes used to cut DNA sequences (Haem, Hin6I, Rsal, Taqi and Ndell) all have impurity sites. Polymorphism of the cutting pattern is found in enzymes. Haeiii and Rsai. Cutting the mtDNA D-Loop sequence using the Haeiii and Rsal enzymes produces two types of patterns, while the Hin6I, Taql, and Ndell enzymes have only one pattern of restriction.

From the PCR measurements that the Cimandiri river located in Palabuhanratu has three species, namely A. bicolor bicolor namely Eel fish which have short fins, morphological characters from Anguilla bicolor bicolor including rounded elongated bodies, have perfect pectoral fins on the back of gill and back hats dorsal, interconnected caudal and anal fins, short dorsal fin (shortfin) equipped with soft fingers, eyes covered with membranes, nostrils channeled and located at the end of the face from the mouth, mouth horizontally to past the eye. Anguilla bicolor bicolor has golden yellow belly (yellow eel) or silver in silver eel, with a length of 15-42 cm and a weight range of 8 - 75 gr. The second species is A. Nebulosa Nebulosa, which is a camel eel, and A. marmorata. Namely White Eel fish. Phylogenetic analysis of amino acids and protein sequences will usually be an important area in the sequence analysis. Based on the analysis, the sequence that has proximity can be identified by occupying a branch adjacent to the tree. When family genes are found in organisms or groups of organisms, phylogenetic relationships between genes can predict the possibility that someone has an equivalent function. PCR identification of Sidat fish species can be seen in Figure 1.

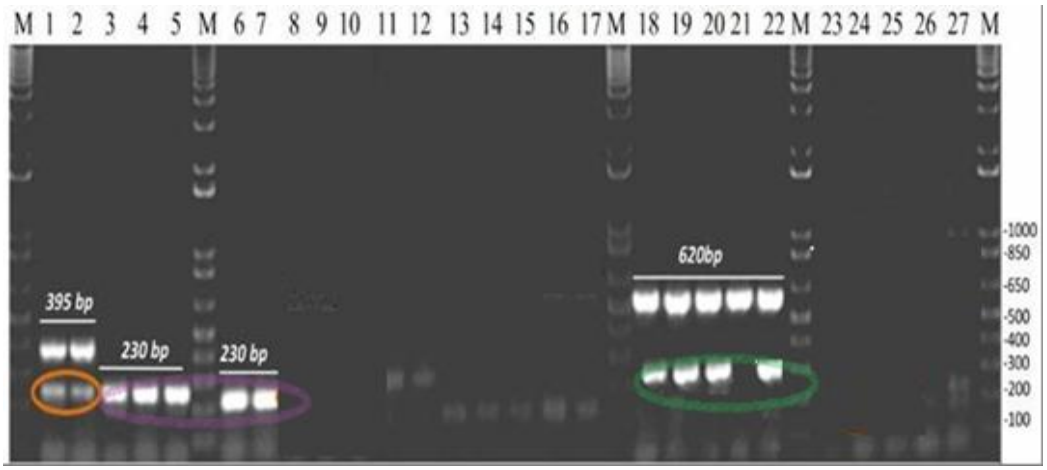

Figure 1. Identification of species with PCR, 1-2 is nebulosa nebulosa, 3-7 is bicolor bicolor, 18-22 is A. mamorata

tDNA D-Loop sequence in eel, from PCR analysis that for bacillus nebulose nebulose has a length of about 395 bp, the bicolor species has a length of 230 bp and A. marmorata bacillus has a length of about 620 bp. Of the five restriction enzymes used to cut DNA sequences (Haem, Hin6I, Rsal, Taqi and Ndell) all have impurity sites. Polymorphism of the cutting pattern is obtained in the enzyme. Haeiii and Rsai. Cutting the mtDNA D-Loop sequence using the Haeiii and Rsal enzymes produces two types of patterns, restriction site are Polymorphic. The Hin6I, Taql, and Ndell enzymes only have one pattern of restriction, restriction site are monomorphic (Table I). In general there are 4 composites known based on five types of restriction enzymes in the order of mtDNA nebulose-nebulose, bicolor bicolor, and A. marmorata with the following site forms:

Table 1. Results of the mtDNA sequence. Eel various restriction enzymes

No.	Type of enzyme	Restriction site	Location of site		
			Nebulosa-nebulosa	Bicolor bicolor	Amarmota
I.	*Hae* III	Polymorphic	395	230	630
2.	*Hin61*	Monomorphic	390	235	600
3.	*Rsa* I	Polymorphic	394	230	625
4.	*Taq* I	Monomorphic	390	232	610
5.	*Nde* II	Monomorphic	391	233	620

Enzyme restriction in mtDNA sequences in eel fish has an effect on the form of site restriction and the length of bacillus PCR from eel fish.

IV. Conclusion

Enzyme restriction in mtDNA sequences in eel fish has an effect on the form of site restriction and the length of bacillus PCR from eel fish. Cimandiri River has three species, namely A. bicolor bicolor short-finned A. nebulosa nebulosa colored camouflage, and A. marmorata. namely white eel.

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