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Microsatellite DNA Markers for Control Fish Diseases

Yuniar Mulyani[1], Dian Yuni Pratiwi[1]
[*1]*Department of Fisheries, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Indonesia*
Corresponding Author: Yuniar Mulyani

Abstract
The challenging problem to be resolved in aquaculture is fish disease. It can make abnormalities in fish, fish death, and economic loss. So fish disease control is needed. Common methods to control the fish disease are the use of antibiotics and other chemicals and the selection of disease-resistant fish through crosses. However, the continuous use of antibiotics and other chemicals will make pollution. The alternative method that can be used to control fish disease is Microsatellite DNA Markers for control fish diseases. this article aims to describe the application of Microsatellite DNA markers for control fish diseases.

Keywords: *Microsatellite DNA markers, fish disease, economic loss, fish abnormalities, fish death*

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I. INTRODUCTION

Fish diseases are one of the problems that must be faced in fish farming. They can disrupt cultivated fish and even cause death and economic losses. Chemicals and antibiotics have been widely used in the prevention of diseases in fish. However, the continuous use of chemicals will have negative effects for both the environment, fish, and consumers and in the long run, can cause resistance and fish residue in nature [1].

DNA markers are a piece of DNA that using certain techniques can be seen and its inheritance can be followed (Taryono, 2016). DNA markers are further divided into three groups based on the underlying molecular techniques, namely: (1) DNA markers based on hybridization techniques such as RFLP (Restriction Fragment Length Polymorphism) and VNTR (Variable Number pf Tandem Repeats), (2) DNA marking is based on PCR techniques such as RAPD (Random Amplified Polymorphic DNA) which can be converted into SCAR (Sequence Characterized Amplified Regions), AFLP (Amplified Fragment Length).

Polymorphism), microsatellites or SSR (Simple Sequence Repeat), and ISA (Intersimple Sequence Repeat Application), CAPS (Cleavage Amplified Polymorphic Sequences), and (3) DNA markers based on DNA sequencing techniques such as SNPs (Single Nucleotide Polymorphisms) [2]

II. MOLECULAR APPROACH TO CONTROL FISH DISEASES

2.1 Selection of Disease Resistant Fish

The common freshwater fish breeding techniques are selection techniques by exploiting the potential and genetic characters, particularly the variety of additive genes (VA). VA is a function of an allele that will be passed down through haploid gametes from generation to generation. VA is that each gene adds certain traits to a trait, in other words, each allele together with different abilities to form a variety of phenotypes (VP). Selection does not create new genes, but VA exploitation will change the frequency of genes to improve genetic quality qualitatively and quantitatively with the ultimate goal of obtaining superior parents as parents. Improvement of genetic quality (genetic gain) in the parent through selection will change the average population of the offspring for the better. It is estimated that selection in each generation will increase genetic quality by 10% - 15% [3].

The conventional application of selection has been done in making Krasnodar carp (*Cyprinus carpio*) resistant to dropsy. However, the time required is relatively long. Goldfish resistance to dropsy was acquired in generation 9 [4]. Another approach that can be used to save time is a marker-assisted selection (MAS). This method has been used successfully to make goldfish resistant to KHV virus infection. The molecular marker used is Cyca-DAB1*05 which is related to the immune system and belongs to the major histocompatibility complex class II (MHC II) gene group. MHC II plays a role in the activation of phagocyte cells to produce antibodies and activate the immune system which is involved in eliminating parasites, bacteria and neutralizing viruses. Another MHC group involved in the immune system is MHC I [5].

2.2 Fish Disease Detection

a Fish disease is a physical condition, morphology, and function that changes from normal conditions caused by internal and external factors. Fish are easily infected with diseases through water media in the cultivation area. Water is not only a place to live but also as an intermediary for pathogens. Fish diseases can be caused by biological and non-biological agents. Diseases caused by biological agents are also known as infectious diseases. Infectious diseases can be transferred to other individual animals in various ways.

2.3 Application of Microsatellite DNA Markers for Control Fish Diseases

A Microsatellites are repeating DNA sequences with a repeat size of 1-6 bp. The number of repeats of microsatellites is usually less than 100 bp [6]. Microsatellites are DNA sequences with repeating lengths of several nucleotide bases. Microsatellite genetic markers are a very effective method for DNA fingerprinting in mammals and eukaryotic microorganisms such as pathogenic fungi, this method is discriminate, reproducible, easy to perform and the results remain stable in each generation.

Microsatellite markers have a high degree of polymorphism, visible banding pattern profiles that can be interpreted easily as alleles in a locus, allele codominance, and very accurate because the allele size can be distinguished up to one base pair (1 bp). The high polymorphic level of this marker is an important feature so that it can be used to identify individuals between and within populations. Microsatellites have a high degree of variation in animal and plant species [6].

Various molecular markers, protein, or DNA (mitochondrial DNA or core DNA such as minisatellites, microsatellites, transcribed sequences, anonymous cDNA, or RAPD) can be used for aquaculture. The combination of molecular markers with statistical development can be used to explain the differences and similarities between stock and individuals, in the original population of each fish. This is a new invention and can be applied to fisheries and fish farming processing [2]. Research on the apparent traits that are regulated by genetic factors is important in the development of cross selection strategies to improve the quality of cultured yields for the future [3].

Genetic markers based on DNA are frequently used in aquaculture applications such as Tandemly repeated DNA (mini- and microsatellites), RAPD, and AFLPs [6]. In 1996, the Canadian halibut fish industry was still in its infancy, requiring a relatively long time (5-7 years) for the fish to mature as a key factor in the realization of development program for selection of broodstock. Another problem lies in the cost of feed and crosses between families.

Microsatellite markers consist of several copies of simple sequence repeats (SSRs) arranged in an orderly manner ranging from 1 to 6 base pairs. Its abundant distribution in the genome, small locus size, high polymorphism, and the Mendelian model of inheritance provide its convenience in various new studies [7]. Microsatellites can be found in all chromosomes, microsatellites are present in the coding region, introns, and non-gene sequences. Microsatellites are composed of repeated nucleotides, ranging from a few nucleotides to several hundred repetitions; however, relatively small loci are frequently used because they are important for the genotype and can be applied and facilitated by PCR. Polymorphisms in microsatellites are caused by the variation in the number of repeating units contained by alleles at a particular locus. Microsatellite mutations caused by polymerase enzyme activity during DNA replication resulted in differences in the number of repeat units [8] [9]. The main advantage of microsatellites is that they are Mendelian and codominant. This opens up opportunities for genome mapping studies, heredity, kinship, and stock determination. One of the studies related to disease control, among others, increased disease resistance in rainbow trout against infection with hematopoietic necrosis (IHNV) which causes a highly contagious disease called infectious hematopoietic necrosis (IHN) in important species such as salmon and trout can be achieved with the help of microsatellite markers.

Rodriguez et al. [10] have carried out reverse cross-sectional trials between rainbow trout and disease-resistant steelhead trout resulting in a link map for broodstock and identified the relationship between molecular markers and QTL(quantitative trait locus) affecting resistance to IHNV. IHNV-resistant rainbow trout varieties were also successfully obtained by cross-crossing with highly resistant Yellowstone mackerel (*Oncorhynchus clarki*) [11]. Three regions of the genome were detected and differed between species, which were related to survival after the challenge test without affecting body mass and length [10]. A microsatellite allele, Poli9-8TUF, associated with lymphocytic disease resistance (LD-R) in Japanese flounder was also identified by Fuji et al. [12]. This allele has a dominant effect on a single major locus. Selection with the aid of successful markers and subsequent agricultural trials confirmed the feasibility of the selection process [13]. QTL for IHNV resistance was identified in rainbow trout and yellowstone cutthroat-assisted crossbreeds and selected AFLP in the hybrid genome [11]. Studies finding QTL for infectious salmon anemia (ISA) in Atlantic salmon have also been identified by microsatellites [14]. The QTL associated with an infection for pancreatic necrosis in Atlantic salmon has also been identified with the aid of microsatellite markers [15]. Microsatellite markers can also be used to detect QTL for *A. salmonicida* resistance in four Turbot (*Scophthalmus maximus*) families [16].

Microsatellite markers associated with *Gyrodactylus salaris* resistance in Atlantic salmon have also been studied [17].

III. CONCLUSION

In conclusion, Fish disease is one problem that needs to be resolved. Microsatellite DNA Markers can be used for fish resistant selection and plays a role in controlling fish diseases. The advantages of Microsatellite DNA Markers are discriminating, reproducible, easy to do and the results remain stable in each generation.

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