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Characterization of the Tyrosinase Gene of Aquarium-Maintained Nyalian Fish (Rasbora sp.)

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A B S T R A C T

Rasbora fish is a local Indonesian fish whose population has decreased. In the Province of Bali this fish is called the Nyalian fish and no one has cultivated this fish. Nyalian fish in Bali Province are used as consumption fish and have potential as freshwater ornamental fish. Catching Nyalian fish continuously can cause this fish decrease in population and can cause extinction. Nyalian fish conservation can be done by domestication and adapting fish caught from their natural habitat to aquaculture aquarium, then used in aquaculture activities and ensuring continuity of numbers and avoiding extinction. During the domestication process, the Nyalian fish will experience stress due to changes in their habitat. Stress in fish can be shown by a drastically darker body condition. Parts of the body that blacken or show a darker color are due to the activity of melanin as an active role in it. The Tyrosinase gene is one of the genes responsible for coding pigment patterns. The TYR gene has an important role in instructing the formation of the enzyme Tyrosinase.

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INTRODUCTION

Nyalian fish or often called Wader Pari is one of the consumption fish in the river flow and is a source of biodiversity for fish resources in the area. This fish has a fairly wide distribution and is a native fish in fresh waters such as rivers. Their body size is relatively small and they generally live on the banks of rivers where the current is not fast and they are omnivores . Ecologically, Wader Pari or in Bali it is called Nyalian fish which is a biological constituent component in rivers and serves as a niche filler for plankton eaters. Wader fish is one of the consumption fish so that it can be a source of protein for the community. Nyalian fish has not been widely cultivated in Bali Province and is still being caught in nature for consumption needs.

Keeping Nyalian fish in aquariums can increase mortality on a large scale is something cultivators must be aware of, internal and external factors that cause mortality to be aware of. Stress is one of the causes of death in Nyalian fish. Stressful fish show different features depending on the factors that cause the stress condition. Stressful fish caused by poor pool water conditions, fish will show characteristics such as, fish always swimming on the surface of the water, reduced appetite, and often staying at the corner of the pond or alone.

Stress in fish can be demonstrated by drastically darker body conditions. The environmental factors that disturb fish conditions are the main factors that cause fish to experience darker color changes as a characteristic of stress. In blackened parts of the body or showing darker colors, melanin activity plays an active role in it. Melanin is a product of melanocytes that play a role in the formation of brown or black pigments. Not only does melanin play a role in black or brown pigmentation, it has another function: as skin protection from ultraviolet (UV) light. Melanin has varying quantities in the skin depending on the body's response condition. In animals, melanin has an additional function: as a camouflage aspect and characteristic of the animal's body .Melanocytes are located in the basal stratum region, having a function in the formation of melanin that is assisted by the work of the enzyme tyrosinase group. Tyrosine is converted to 3.4 dihydroxyenylalanine (DOPA) and subsequently converted to doppaquinone after several stages of conversion to melanin.

Several studies have shown that environmental factors also affect pigmentation in fish, which suggests a positive correlation to the migration of clownfish pigment patterns in maintenance experiments under two different conditions: outdoor and indoor.

Polymerase Chain Reaction (PCR) is an in-vitro DNA amplification technique to obtain the required number of target DNA segments. Nested PCR (nPCR) is a type of PCR method in which the process uses two PCR reactions using two different primer sets. In the nPCR process, the first reaction uses the outer-primer to start the amplification process, then in the second reaction, the inner-primer is used to minimize unwanted amplification products.

THEORETICAL REVIEW

The activity of the tyrosinase group of enzymes affects the speed of the melanogenesis process, where the Tyrosinase-Related Protein-1 (TYRP1) gene plays an active role in instructing the manufacture of the enzyme tyrosinase related protein-1. TYRP1 is a gene that plays a role in the process of melanin synthesis. TYRP1 functions to instruct the manufacture of the enzyme tyrosinase-related protein-1, where the enzyme is located in melanocyte cells, namely cells that produce melanin pigment. Melanin gives color to the skin, eyes and other tissues that are sensitive to light. Currently, it is not known whether there is a link between TYRP1 gene expression and changes in skin color in Nyalian fish that experience stress. This is due to the lack of genetic information related to gene expression. The documented TYRP1 gene sequence in Nyalian fish is known to have very little information on the GenBank website (http://ncbi.nlm.nih.gov). In identifying a gene whose sequence is unknown or with minimal information regarding the gene, researchers generally design degenerate primer pairs. The existence of degenerate bases is unique in a primary sequence. Defined as a sequence of primers representing more than one nucleotide base, degenerate primers have a tendency to represent four nucleotide bases in a single sequence. Researchers often use Coding DNA Sequence (CDS) regions in designing degenerate primers. CDS is a conserved area on the exon, where the area is a specific area in a sequence. In identifying a gene using a degenerate primer pair, it is necessary to know the CDS area, so that the designed primer is a specific primer for a particular gene. In designing degenerate primer pairs for gene analysis with minimal information related to the organism, related sequences that have good genetic information are used to support the findings of the target organism. According to Edwin Chargaff's chromatographic method it was concluded that the bases that make up the DNA chain of closely related species have the same base composition.

METHODOLOGY

In this study, samples of Nyalian fish were collected from those already maintained in aquariums and taking from nature in Bali Province. The Polymerase Chain Reaction stages include:

a. Extraction

Sample extraction was carried out with 10% Chelex solution. The sample tissue was taken + 2 mm using tweezers and put into a tube containing Chelex solution. Before and after the tissue is taken, the tweezers are dipped in 95% ethanol and burned with a Bunsen flame. The chelex solution in which sample tissue has been included, vortexed and centrifuged for + 20 seconds, then heated in a heating block with a temperature of 95oC for + 45 minutes. After being heated, the tube is again vortexed and centrifuged for + 20 seconds. The extraction solution is ready to be used for amplification.

b. DNA Amplification

DNA amplification was performed using the PCR (polymerase chain reaction) method. The extracted samples were amplified at the TYR (Tyrosinase) locus using the Hotstart method. The parameters used in this method are as follows: denaturation at 94^{o}C for 30 seconds, annealing at 50^{o}C for 30 seconds, and

extension at 72°C for 30 seconds, and the PCR process is repeated for 38 cycles [18]. In this method, two primers are used, namely the forward primer Tyr F with the following nucleotide sequence ATGCTCTCTCTGGTTGTTATAGTCTTTTTCGC and the reverse primer Tyr R with the following nucleotide sequence TCACGGCATCATGGTTTTGGTAAGA

c. Sequencing

Samples that have been amplified by the PCR method, followed by sequencing at a sequencing service facility to obtain the nucleotide sequence, using the Sanger sequencing method.

d. Data Analysis

The data was analyzed according to the band profile confirmation that appeared. polymorphism is scored based on the pattern of genetic variation that arises from each individual in each population. Furthermore, UPGMA clustering was carried out to determine the closeness relationship between each population using the TFPGA (Tools for Population Genetic Analysis) software.

RESULTS AND DISCUSSION

The PCR results showed that nearly 50% of the Nyalian fish kept in aquariums had the same color performance as those in nature, while 50% had a different performance from those in nature. The results of the analysis of similarity and nuleotide differences show that the number of nucleotides encoding the Tyr gene between Nyalian fish in aquariums is largely different. The range of nucleotide differences between the parents and their seeds was 14 – 29% of the total 133 nucleotides analyzed. This causes differences in color patterns between those kept in aquariums and in nature.

The regulation of a pigment is genetically controlled which is encoded by a gene that has an interspecific nucleotide arrangement that matches the pattern of the pigment formed [20]. The tyrosinase gene is one of the genes responsible for coding pigment patterns. The results of isolated genomic DNA amplification with Tyr (F) and Tyr (R) primers. Tyr gene amplification with Tyr (F) and Tyr (R) primers produced a product of 70 bp in size.

The color performance similarity coefficient shows an average value of 50 percent, this means that the color pattern is actually only 50 percent similar to nature, as a result the phenotype of the color pattern that appears is different. From the results of the similarity analysis, it turned out that there was a difference of 19 nucleotides from the 46 nucleotides encoding the Tyr gene in fish kept in aquariums, as well as in the Puntius fish there was a difference of 21 nucleotides out of 45 nucleotides. The difference in the number of nucleotides between Nyalian fish kept in aquariums and those in nature is thought to be due to the recombination process during meiosis as crossing over of chromosomes between paired chromosomes and this is also known as the law of segregation. This process causes the seeds to have different gene combinations from their parents, and can produce new chimeric alleles. In sexual populations, genes are recombined in each generation, resulting in new genotypes. Most offspring of sexual species inherit half of their genes from the female parent and half from the male parent, thus differing genetically from both parents or from other individuals in the population.

Through a genetic approach, a phenotypic character will always be passed on by parents to their offspring, but in reality there are various things that cause deviations in phenotypic characters, this is what creates genetic diversity in a population. This phenotypic character deviation can occur due to the interaction of several factors such as mutation, migration, recombination, selection, and genetic drift. Mutation, migration, and recombination of genes will increase diversity in natural populations, while selection and genetic drift tend to reduce variation. Genetic diversity in a population of organisms is mainly produced by three mechanisms, namely mutation, free allele pairing or recombination, and migration of genes from one place to another.

In addition to gene factors as controlling pigment patterns, feed and the environment also affect the physiology of pigment cells which encourage changes in the formation of pigment patterns that appear. Administration of copepod nauplii gave cod larvae a yellower color pigment than treatment (copoda and rotifer enrichment), rotifer enrichment, and Chlorella rotifers. Copepods are a natural food commonly found in native fish reared in floating net cages. The pigmentation expression of beta-carotene contained in fish meat or skin is a biological component of the red color of fish. Environmental factors also affect pigmentation in fish, there is a positive correlation with the migration of clown fish pigment patterns in rearing experiments with two different conditions, namely outdoor and indoor. The maintenance environment can affect the coloration of fish. Maintained fish in bright conditions will give a different color reaction to maintained fish in the dark because of the reaction of melanosomes containing melanophore pigments to existing light stimuli. Therefore, the color patterns in the breeding clones are not only influenced by genetic factors, but also by feed, environmental factors or genotype interactions with the environment.

CONCLUSIONS AND RECOMMENDATIONS

The total partial DNA sequence area of the Nyalian TYRP1 gene obtained was 525 bp which was conserved over the exon region 489 bp long and the intron region 36 bp long as a result of sequence alignment results. The construction of the TYRP1 gene phylogenetic tree was designed from the nucleotide sequence of the CDS area and the amino acids translated from the nucleotide sequence of the CDS area and the result was that the TYRP1 gene in gouramy grouped with other fish groups in the ingroup. This shows that the design of degenerate primers from the Teleostei infraclass fish family sequences is able to amplify the TYRP1 gene in the Nyalian fish genomic DNA well. A pair of Tyr primers encoding pigment control gene sequences can be used to analyze genetic color degradation in Nyalian fish. The coefficient of color similarity between fish kept in aquariums and in nature in Nyalian fish genotypically shows that half of the genes are influenced by the environment.

FURTHER STUDY

Plans for future research could involve additional, more specific steps to explore certain aspects of tyrosinase gene characterization in Nyalian fish (Rasbora sp.).

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