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Application of Microsatellite in Fish Biotechnology: Prospects and Drawback - *Review*

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Abstract

Fisheries and aquaculture has evolved and metamorphosed into so many stages, however, compared with the constant rise in population. hence the quest to meet the ever growing demand of fish in the world gave rise to the advent of the application of biotechnology in fish; molecular markers are identifiable DNA sequence and they have found application in different parts which includes breeding, population genetics, and environmental management. They produce more accurate information, microsatellite markers has become indispensable among other markers because of its unique characteristics such as co-dominance, high polymorphism, short stretches and it is widely distributed in the genome of organisms. Microsatellite has wide variety of application which includes parental and pedigree analysis, population genetics, conservation of stocks, assessment of wild and cultured population, marker assisted selection and breeding, however high cost of developing species specific markers has been one of the major challenges of microsatellite but multiplexing is effective in reducing its cost production. The unique attributes of microsatellite marker has made it an indispensable marker over all other dominant markers.

Keywords

Biotechnology, Fish, Markers, Microsatellites, Polymorphism

1. Introduction

1.1. Background

The supply of fish around the globe have exceeded or close to maximum sustainable yield as a result of over-fishing as a result of ever growing population hence demand outpace supply, [1]. Global production of capture fisheries and aquaculture combined has risen tremendously reaching 46.8 percent in 2016, up from 25.7 percent in 2000 which leaves aquaculture as the only remedy to providing enough fish to meet up the demand being the cheapest source of animal protein, [2].

Aquaculture on the other hand has experienced a tremendous increase most importantly the freshwater species making it a potential sustainable means to meeting ever increasing demand for fish, [3]

Biotechnology which involves techniques that make use of living organisms or their parts in the modification of production processes, [4]) has found a wide application in fisheries and aquaculture, [5] Aquaculture Biotechnology is saddled with the responsibility to improve aquaculture stock, preserve genetic resources, improve fish health, and control of microbial/micro-algal genetic engineering, [6]. It also has wide potential to boost agriculture, food and feed production, increase growth rate in farmed species, conserve and manage of wild and hatchery stocks and the technology is beneficial to both the aquaculturists and the consumers, [7] Biotechnological application such as fertilization of ponds to increase feed availability are crude with age long application while others are advanced taking advantage of increasing knowledge of molecular biology and genetics, [8]

The field of genetic biotechnology similarly ranges from simple techniques such as hybridization, to advance processes such as the transfer of specific genes between species to create genetically modified organismsand the technology is growing in leaps and bounds, [7, 18].

Molecular markers are important in genetic analysis and the measurement of genetic variation is achieved via the use

of molecular markers which are based on DNA sequence polymorphisms. According to current state of science, [9] molecular markers are classified into three; Nucleic acid hybridization based on complementary bases, e.g., restriction fragment length polymorphisms (RFLPs), Polymerase Chain Reaction (PCR) based on DNA amplification, e.g., random amplification of polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLP), microsatellites or simple sequence repeats (SSRs) and single Nucleotide Polymorphisms (SNPs) [10]'.

Molecular markers can be sub-grouped under broad heading as type I and type II markers. Type I when with genes of known function, while type II markers when it possess unnamed genomic regions [11]', subject to this grouping, allozymes markers are regarded as type I markers because the protein they encode has known function. RAPD markers are type II markers because RAPD bands are amplified from anonymous genomic regions via the polymerase chain reaction (PCR) [12]

Microsatellite markers are also type II markers unless they are associated with genes of known function. Generally, type II markers such as RAPDs, microsatellites, and AFLPs are regarded as non-coding and neutral markers [13]'.

Allozymes markers are cheap, rapid and gives an unbiased variation range within a population without an in depth morphological and quantitative survey, [14] But it exhibit occasional heterozygote deficiencies as a result of enzymatically dormant alleles and tissue samples quality as well as lower level of noticeable variation due to masked DNA sequence at protein level. It use is restricted to population structure and phylogenetic studies and primitive in aquaculture genetics, [15].

RAPD aside been inexpensive and simple has high polymorphism and requires minute quantity of DNA for molecular hybridization [16]'. but it uses is limited because of its low reproducibility and insensitivity to only large scale mutation which makes underestimation of variation probable [13]. AFLP possess the quality of both RAPD and RLFP, large number of polymorphisms can be score in a single polyacrylamide gel without the necessity for any prior research and development [17]. it also allow for more loci analysis within the genome in a short time but the drawback is that they are dominant markers and difficulty occur in analyzing procedures as a result of enormous unrelated fragments visible on the gel along with the polymorphic fragment [16] Single Nucleotide Polymorphisms (SNPs) in fisheries serves as genomic markers and diagnostics markers for disease [10].

Microsatellites or simple sequence repeats (SSR) [19] or "short tandem repeat" (STR) DNA, [20] they are molecular genetic markers with short stretches of about 1-6 base pairs in length, [21]. DNA is composed of di-, tri- or tetra-nucleotide repeats arrayed in tandem. They are highly abundant, small and co-dominant with frequencies of 10^3 to 10^5 copies, 20 and 100 bases and are widely distributed in the genome of eukaryotes and they have multiple allele in them [22]. They are hyper-variable, nuclear encoded genetic markers which can be amplified by polymerase chain reaction or DNA sequencing markers [23-24]. Its application is vast cutting across variety of field which includes forensic, medicine, biology, parasitological amidst others [25]. In fisheries and aquaculture, microsatellites are used for characterization of genetic stock, brood-stock selection, constructing dense linkage map, marker assisted selection quantitative trait mapping [26].

Microsatellites have been classified into various types according to the type of repeated sequence present. They include; perfect, when showing only perfect repetitions, e.g., (AT) 20, imperfect repeats, when the repeated sequence is interrupted by different nucleotides that are not repeated, e.g., $(AT)_{12}$ GC $(AT)_8$ and composite, when there are two or more different motifs in tandem, e.g., $(AT)_7$ $(GC)_6$ [27]). The composite repeats can be perfect or imperfect. The sequences of di-, tri- and tetra- nucleotide repeats are the most common choices for molecular genetic studies, based on the length of repeat motif, [28]): microsatellites can be of two types namely; class I microsatellites- perfect SSRs of >20 nucleotides in length, class II microsatellites- perfect SSRs of >12 nucleotides and <20 nucleotides in length [9].

Microsatellites have provided lots of vital information in fish population such as detection of parentage and pedigree analysis, genome, conservation genetics, detection of demographic bottlenecks, identification of genetic variability between and within stocks, monitoring genetic changes in stocks (gene tags), in detection of quantitative trait loci (QTL) [29-31]

Hence, this review will examine the function, applications, prospects and limitation of microsatellites.

1.2. Characteristics and Functions of Microsatellite Markers

Microsatellites were detected in eukaryote genomes almost thirty years ago and they are the most promising PCR-based markers they are found at high frequency in the nuclear genomes of most taxa [32-33]. Microsatellites are ubiquitously distributed in many prokaryotes; eukaryotes and they are mostly referred to as junk DNA [34-36]. It allows for cross-species amplification as an alternative strategy for genetic characterization of a species using primers developed from other closely related species though shows evolutionary conservation [37-38]. They are referred to as neutral markers and they contribute a great deal in complex activities such as chromatin organization, transcription and translation, gene expression, DNA structure as well as cell cycle dynamics.

Microsatellite marker can be used to select desirable allele when gene of interest is known; and has become an extremely popular marker type in a wide variety of genetic investigations. SSR plays a major role in the chromosomal structure organization [26] some of its sequences such as GT, CA, CT, GA amidst others has a contributory effects on DNA recombination directly through the structure, [39]

In addition; microsatellites have a wide distribution in the genome and can be efficiently identified, which is essential in studies about genetic variability of populations [40]

2. Application of Microsatellite

Species Identification and parental linkage.

It is important to note that identification of fish species is highly essential in the developing countries where variety of fish species exist. Species identification is important for a comprehensive knowledge of genetic materials or biological potentials of individual and microsatellite is suitable in the analysis and identification [25].

Microsatellite is an important tool in the assignment of parent as there is always an inheritance of allele by each progeny from both parents, they are single- locus DNA markers which are co-dominant in nature, with the use of a multiple panel microsatellite marker and comprehensive genotypic information on the character of tested individual can be obtained [10, 40-41].

Microsatellite markers have been used for determination of paternity and relatedness analysis of natural populations, hatchery brood-stock and trade control of fish products which includes those from aquaculture. It is also suitable for precise acquisition of pedigree information [42-43] Microsatellite loci though widely distributed in the genome of organisms has its remains usually in the bone remnants and dental tissue as well as fish scale; hence this has given a clearer picture of the demographic declines in abundance which historically led to the collapse of Lake trouts population in the Great Lakes of North America over 40 years ago [44-45].

A number of studies have verified the use microsatellite loci to reconstruct pedigrees in fish populations with families mixed from hatching [46- 49]. SSR has gained it fame due to its high variability amidst individuals of the same strain.

2.1. Genetic Variation and Population Structure Study of Natural Populations

The study of genetic variability evaluates the variation that exists between and within population and it is useful in the selection of stock for breeding programs, estimation of gene contributions to stock and management of species for conservation [50-52]. Varying methods of genetic relatedness assessment of species exist which includes the morphometric methods, protein profiling and molecular markers.

The morphometric methods utilize information gained from the physical characteristics such as height, weight, size, lengths amidst others to assess genetic diversity but it is subjective to environmental influences [53-55]. Protein profiling in genetic diversity assessment is preliminary interestingly, molecular markers such as microsatellite markers have been more reliable in the assessment of genetic variation among species and populations since they utilize information directly from the DNA and adapted towards exploring the genetic diversity within and between populations [56].

2.2. Comparison Between Wild and Hatchery Populations

It is important to examine the genetic composition and make comparisons between hatchery strains with their wild populations as well as within strains from hatcheries as well as within wild strains [9]. Reasons such as low effective number of parents and has been attributed to the loss of genetic variation in hatchery stocks especially in salmonids [57, 58].

Although, DNA fingerprinting has been used in stock identification and monitoring potential changes in brood stock [29, 60-61] using hatchery, and wild populations of Japanese flounder (*Paralichthysolivaceus*) by means of microsatellite and mtDNA sequencing analysis with French and Czech strains of hatchery stocks of common carp (*Cyprinuscarpio*) using allozymes and microsatellites to both examine the genetic divergence detected a wide discrimination between the strains of the two countries by the microsatellite markers.

Farm-raised fish on escape from aquaculture facilities to the wild on mating can result to a decrease in the genetic diversity, Competition for food, habitat and mate aside disease and parasite which can be introduced to the wild counterparts, Beside the intraspecific hybridization, interspecific gene exchanges also occurs [26].

Microsatellites have important applications in monitoring inbreeding depression by locating specific chromosomal regions responsible for inbreeding depression [48]. Although, it would be most feasible with cultured species where parents and their progeny can be managed and traced within a closed system. The polymorphism obtained with microsatellite markers have provided strong information utilized in the management of fish stocks [62].

2.3. Quantitative Trait Locus Mapping

Quantitative traits are measurable and observable characters of an organism (phenotypic), though multi-factorial but they are dependent on the cumulative actions of various actions of gene and the environment. Examples include: height, weight and blood pressure amidst others. In other words, traits that can be quantified numerically and are variable among individuals.

Quantitative trait locus (QTL) is the location of gene having correlation with variation of a quantitative trait in the phenotype of population of the organism. The production of mono-sex population has influenced multiple research have been conducted research sex determination and sex linkage in fish using microsatellites. Sex linked inheritance in fish was first reported by [63] in medaka. In *Oreochromisniloticus*, quantitative traits loci for sex and colour have been mapped [64], while microsatellite accounted for 7.5% of the variance in thermal tolerance in unselected population of rainbow trouts [65].

Microsatellite has proven effective in the determination of sex chromosome region in channel catfish, identification of several chromosome regions containing putative quantitative traits loci genes that affect resistance to infectious pancreatic necrosis (IPN) in rainbow trout as well as identification of correlation with stress-related plasma cortisol levels and basal plasma glucose levels in common carp [65-67].

2.4. Population and Conservation Genetics

Stock identification and assessment has become a major area of concern, hence the need to protect biodiversity, conservation and fisheries genetics on the other hand gives priority to effects of inbreeding, demography, contemporary genetic structuring and adaptation to long-term survival of fish species [68].

Genetic variability and evolutionary studies of wide varieties of fish can be conducted using microsatellite markers. SSR are more sensitive than allozymes for evaluation of the dynamics of population which includes demographic bottlenecks [26]. They remain an effective tool in the analysis of recent and contemporary event as the change in the genetic composition is 10^2 -10^3 times faster than single copy nuclear DNA [70].

Microsatellite has been used to enumerate intervals and mechanisms of decline in population of brown trouts in Denmark [71]. Their high mutation rate the microsatellite loci is a plus in their use in genetic analysis of population dynamics.

2.5. Marker Assisted Selection

Marker assisted selection is an important phenomenon to breeders; this is done when there is a correlation between phenotype of interest and genotypic information. Traits such as growth rate, stress response, disease resistance, sex determination and development rate are paramount to breeders [26]. Marker assisted selection programs is important to reduce inbreeding through the production of genetically improved stock.

In fish culture, microsatellite marker is useful in the selection of parent stock for further crossing and characterization of subsequent offspring, specific markers linked to the quantitative traits loci are used for this purpose and this will help in precision in gene selection microsatellites markers have been used in selective breeding for different species of fish [72] Channel catfish [73] and Salmonids [29].

3. Prospects of Microsatellite Marker

Microsatellite markers are very beneficial and they are becoming indispensable in fish biotechnology because they pose some unique characteristics such as highly polymorphism, reproducibility, co-dominance, strong discriminatory power specific PCR-based assay, wide distribution in the genome and exceedingly large allelic variation [25, 40]. It has variety of functions in fish biotechnology which comprises of genetic fingerprinting [75].

Furthermore, these markers are more closely connected with genes of known function [76]. Mutations in the motifs and flanking sequences as well as distribution of SSRs in the genome of a species are exploited to reveal genetic variation and varietal identity [77].

Microsatellite markers have been said to become marker of choice in fish biotechnology providing genetic framework for other markers, cost reduction can as well be achieved by multiplexing which entails the agglomeration of polymerase chain reaction amplification products from multiple microsatellites into a single lane of an Electrophoretic gel of each microsatellite locus has to be identified and its flanking region sequenced to design of PCR primers [77].

Large scale marker assisted selection programs will give room for cloning of targeted economically important traits or direct injection of gene of interest from natural population into hatchery bred population. The use of SSR in aquaculture will aid genetic monitoring of farm stock in relation to breeding programs in order to monitor genetic variability, identify advantageous crosses, and reduce inbreeding and precision in the selection of genetically improved stock) [47]' Microsatellite markers technique will enhance effective management of wild fish stocks [78].

4. Limitations of Microsatellite

Microsatellite is unarguably of great potential in fish biotechnology; however, there are limitations in various aspects of its use. In gene mapping, the use is disadvantageous in that they exist as unknown DNA fragment [12] When used to study phylogeny, primer developed from one taxon may be ineffective on all the taxon for which the genotype entails. Homoplasy causes prejudice in the genetic analysis of natural population, hence cause a deterrent in the identification of conservation units [79].

High cost involved in producing species-specific microsatellite and labor intensity as a result of personnel time involved in the development of the primersis anothercon in the use of microsatellite [80], while the existence of null alleles that is alleles that do not amplify in PCR reactions is also a major deterrent in the use of microsatellite marker [81] and due to polymerase slippage during replication, small size differences between alleles of a given microsatellite locus (as minute as 2 bp in a locus comprised of di-nucleotide repeats) is possible.

5. Conclusion

Biotechnology has opened streams of opportunity in the advancement of genetic resources in aquaculture and if properly utilized will not only boost production but acceptability, marketability and effective management of aquatic resources

Microsatellite markers on the other hand have several advantages in fisheries and aquaculture which outweigh its concern, its high level of polymorphism makes provision for good resolution power which will enhance genetic tagging and parentage identification, its potential are vast with revolutionary ability for absolute size determination which allow for digital storage and comparisons of large number of individuals. The rate of technological advancement with microsatellite is geometrical, little effort and attention has been given to the use of microsatellites in the area of marker assisted selection for species like catfish and tilapia in other

to meet the ever increasing demand.

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