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MOLECULAR IDENTIFICATION TOOLS FOR BIVALVES: A REVIEW

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The Bivalvia class constitute an important molluscan class that exert its effect on human society an important food component and sentinel organism indicating environmental contamination. Accurate species identification is a prerequisite for management of aquaculture, diversity studies and for human consumption. Presence of different species of bivalves on the global markets for human consumption has given rise to several commercial frauds based on species substitution. Reliable and rapid techniques are required to identify seafood species in different products and thus prevent wilful or unintentional fraudulence. This article reviews the molecular tools used by different researchers in the last two decades. By reviewing virtually all genetic markers used during the last two decades of bivalve molecular research, it is obvious that the best tools for bivalve molecular study are mitochondrial COI and nuclear ITS2.

Keywords: Molecular markers; bivalves; DNA barcoding; ribosomal RNA; ITS.

1. INTRODUCTION

"There are more than 20,000 species in class Bivalvia with a wide distribution both in freshwater and marine environment. They impart its importance especially in aquaculture as a source of food, it has a strong economic impact upon human society. Bivalves are commercially exploited and utilised as food throughout the world. Besides, it is of particular interest as an indicator of the sentinel organism reflecting the level of environmental contamination in a manner amenable to both short term and long-term monitoring" [1]. "Drastic ecological changes in the habitat affect the distribution and abundance of clams in estuaries. Commonly taxonomic ambiguities exist due to morphologic variability. Accurate species

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identification is very important in determining similar species for aquaculture management, biodiversity-studies and population dynamics" [2]. Despite the fact that advanced researches have been initiated towards problems related to production, quality and maintenance of better stocks, scant attempts have been made towards the study of the distribution, systematic position and affinities of bivalve species. Presence of different species of bivalves on the global markets for human consumption has given rise to several commercial frauds based on species substitution. Reliable and rapid techniques are required to identify seafood species in different products and thus prevent wilful or unintentional fraudulent.

"This review examined the molecular methods and tools commonly used by specialized researchers. Molecular tools are methods based on DNA sequencing that can provide information about the evolutional modifications of species through its structure. There were two major categories of molecular markers used for studying bivalves: mitochondrial (16S rDNA, 12S rDNA, COI) and nuclear (ITS1, ITS2, 18S rDNA)" [3]. Mitochondrial DNA is a small circular molecule involve in respiration processes. Different molecular techniques that researchers have used to identify bivalves *viz*. allozyme electrophoresis, DNA barcoding using mitochondrial DNA COI, ribosomal RNA and histone genes, 12sRNA, 16s mitochondrial RNA, 18s RNA, small ribosomal subunit RNA, PCR-RFLP of ITS and 5s rDNA gene. This paper presents a literature review concerning the versatility of molecular approaches in bivalve identification and its management.

2. ALLOZYME ELECTROPHORESIS

"Non-morphological criteria for mollusc species identification were carried out through allozyme electrophoresis" [4,5]. "Old molecular tool Protein electrophoresis has been replaced by DNA sequencing and enzymes are still used as useful molecular markers. Major disadvantages of the technique include only nucleotide substitutions that modifies the electrophoretic mobility of the molecules are easily tracked. Since the same band of the isoenzyme representing two different alleles bearing identical mobility and cause risk in data interpretation, these techniques cannot distinguish evolutionary relations and null alleles" [6].

With the help of PCR, numerous DNA markers allowing genetic comparisons at population level have been proposed. DNA markers are categorised in to two: PCR based (RAPD, AFLP) and non-PCR based (RFLP). All methods use gel electrophoresis and represent a valuable source of information on bivalve biology, ecology and conservation. According to [7] "the high sensibility for detecting a wide array of polymorphisms; the possibility of automatization; low costs; the detection methods that can be based on fluorescence instead than radioactivity; the opportunity of analysing a large number of samples are the major advantages". "While the disadvantages of RAPD as dominant markers are the high sensibility of this technique (although its high reproducibility in a given laboratory, but results have been inconsistent when repeated in another laboratory)" [8].

3. DNA BARCODING

"DNA barcoding is an important taxonomic tool that identifies organisms quickly and accurately by comparing the similarities and differences in DNA sequences with a series of sequences of reference taxa" [9]. Even though [10] proposed "Cytochrome c oxidase I (COI or Cox I) has been proposed as a universal biological barcode, its suitability has only been tested in a few groups" [11-13]. "Mitochondrial genes are preferred to nuclear genes since they occur in numerous copies in each cell, thus it is more useful on older archival material" [10]. "However, COI has not been proven to be the optimal gene for molecular identification" [14,15]. "The genes 12S, 16S and cytochrome b (CytB) have not been suggested as barcoding genes for marine fauna, but are commonly used in reconstructions of molluscan phylogeny" [16-18]. In molluscs, these genes may be useful alternatives to COI effect of barcoding, or may provide useful complementary information useful in species descriptions.

"Mitochondrial DNA (mtDNA) has been extensively studied in several groups of animals, mainly for taxonomic and phylogenetic purposes. Simple maternal inheritance, absence of recombination, and high substitution rates make mtDNA suitable for molecular studies. Use of mtDNA to estimate evolutionary relationships in bivalves requires great care because few bivalve molluscs exhibit gender-associated mtDNA heteroplasmy" [19]. Moreover, the number of loci available for mtDNA analysis is limited.

"One of the most frequently used regions of the mitochondrial (mt) DNA is cytochrome c oxidase subunit 1 (CO1). It is the most used marker in molecular studies and barcoding" [20-22] "COI has phylogenetical signal stronger than any other mitochondrial markers, as it can discriminate not only between strongly related species but also among phylogroups belonging of the same species. CO1 shows distinct divergence and it provides valuable information in species identification to complete

taxonomic data, systemic position and phylogeny" [23].

"Mitochondrial DNA fragments (mtDNA), simple genome structure,can be used as genetic markers for phylogenetic research in animals. The standardized short sequence of mtDNA can be used to identify an organism to the level of a species called the DNA code" [24,25,10]. "COI gene, part of the mitochondrial genome, is widely used as a DNA barcode marker. It has a universal primer that can determine phylogenetic signal ranges that are greater than other mitochondrial genes" [26,10]. "It can be used successfully in analysing phylogenetics at the species level and at higher taxonomic levels" [27] and "for taxonomy studies of various groups of animals such as fish" [28-30], birds [31], and insects [10].

"12s and16s mitochondrial RNA markers have been shown to be useful for analyzing relationships among bivalve species" [29,30]. "The invertebrate mtDNA 16S rRNA gene has been useful for resolving species through family level relationships between bivalves" [32-38].

4. RIBOSOMAL RNA AND HISTONE GENES

"One of the nuclear genes (H3) and the large subunit of the mitochondrial ribosomal RNA gene, 16S genes are fairly well-established markers for molecular systematics of shellfish and are typically used for high-level (family, order, class) analyses" [39,40]. "Histone and rRNA genes in invertebrates, they are usually organized in tandem arrays clustered in one or more chromosomal positions, although other organizations have also been described" [41]. The evolutionary dynamics of both histone gene and rDNA clusters has been analysed in only a few groups of invertebrates. As histone gene clusters were extremely conserved in number and location in all these groups, 45S and 5S rDNAs showed high degrees of variation. In comparison with 45S and 5S rDNA clusters, notable differences in number and location of the histone H3 gene clusters were found, especially between mussels and clams.

5. PCR-RFLP OF ITS

"The RFLP assay is quite robust and rapid for distinguishing larvae by performing only one detection test. This test will be useful to monitor the possible introduction and dispersion of wide spreading bivalves, either by studding of ship hulls, presence in ballast water or due to the aquaculture" [42]. "The PCR-RFLP method is an assay for rapid identification of both adults and larvae. It is also a low-cost method that yields satisfactory results without the cost of sequencing the samples" [43].

6. 5S RDNA GENE

"Ribosomal DNA internal transcribed spacer (ITS) sequence variation has generally proven to be a powerful tool for studying phylogenetics and for species identification" [44,45]. "It has been used in a wide range of invertebrates including molluscs" [46,47]. "Several researchers showed that the ITS sequences show more divergence than their flanking regions and are easily amplified. Hence, they can be used to distinguish between related species and to infer phylogenetic relationships from population to families and even higher taxonomic levels" [48,49]. "The difficulty of using these sequences stems from the occurrence of multiple copies of each genome, which opens the possibility of intra-individual and intra-specific variation. Many researchers have shown that methods such as PCR amplification alone or PCR amplification followed by restriction analysis or sequencing were used to differentiate between related bivalve molluscs" [50-56]. "Molecular phylogenetic studies based on ribosomal gene analysis have been a valuable tool for investigating evolutionary relationships between the bivalve families" [57-62]. "Internal transcribed spacers (ITSs) are sequences located in the eukaryotic ribosomal DNA (rDNA) between the 18S and 5.8S rDNA genes (ITS1), and between the 5.8S and 28S rDNA genes (ITS2). Many researchers used either the entire internal transcribed spacer region or one of its spacers to resolve phylogenetic relationship of closely related taxa" [63-67].

ITS1 of nuclear ribosomal DNA has been used as molecular marker to study phylogenetic relationships among bivalve species and population [68-72]. It has also been used to identify bivalve species [73,74]. "The full degree of ITS2 sequence variation differs substantially between taxa and has been utilised as an excellent marker for species distinction, since it is a relatively fast evolving sequence" [75-80]. It can also be used as a molecular marker [81-87]. "The complete sequence of ITS was used in phylogenetic analysis of mussels and clams" [88-89].

7. CONCLUSION

With the advent of new tools, simple approach to the identification and management of bivalve population in the early days has transformed to molecular studies. In earlier days, researchers depended on single molecular markers for inferring phylogenies, phylogeographies, genetic structure, etc, while today there are great numbers of molecules to answer the

question. Considering this fact, a common methodology probably is not have been well defined due to numerous directions and approaches those researchers used to identify and classify the species, or populations. In order to get a clear picture about bivalve species relationships, it is mandatory to have a common molecular tool. In phylogenetics, COI (cytochrome oxidase subunit I) was proved to be a good marker with a strong signal appropriate for inferring relationships. Although this marker has been used in earlier molecular studies, now its utility is being questioned [90]. As mitochondrial DNA is a circular molecule made up of genes that act together in the process of evolution, focusing on that single gene can lead to an incomplete assessment of the whole genetic variability. COI can be used as the standard marker when inferring the phylogeography of bivalves. It is a sufficient signal in assessing the temporospatial distribution of genes. If different researchers use different markers, the obtained results will be different leading to ambiguities. ITS2 is a marker that has enough discrimination power to distinguish different population structures. Phylogenetic studies of Bivalvia can be better performed through a combination of genes such as COI and 16S rDNA or 18S rDNA rather than using a single target gene. It is clear that a common methodology should be the best solution for bivalve population studies. By reviewing virtually all genetic markers used during the last two decades of bivalve molecular research, it is obvious that the best tools for bivalve molecular study are mitochondrial COI and nuclear ITS2.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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