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Original Research Article

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) BASED MOLECULAR CHARACTERIZATION OF EVOLUTIONARY ADVANCEMENT AMONGST HIERARCHAL FIVE VERTEBRATE SPECIES

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ABSTRACT

Random Amplified Polymorphic DNA is molecular technique with unique advantage of utility in genetically isolated species without prior genomic information. The prime goal of the present study was to identify molecular characterization and understands the evolutionary advancement among five different vertebrate species. On the basis of the analysis of RAPD profiles amplified by six arbitrary primers that gave the best results in producing species-specific bands (OPG-04, OPG-12, OPG-13, OPG-16, OPG-17 and OPG19), the polymorphic bands were unique in 31.4%, 23.2%, 19.2%,11.3% and 10.8% of rats, avain, gecko, toads and murrels respectively. Based on results, dendrogram constructed for phylogenetic relationship shows wistar rats were most unique and distinct.

KEY WORDS: Random Amplified Polymorphic DNA, wistar rat, Gecko, Murrel, Toads, Dendrogram, Phylogeny.

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INTRODUCTION

Random Amplified Polymorphic DNA is molecular technique now available, which allows evolutionary biologists to determine the genetic characters of a different variety of closely related animals. The discovery of the RAPD had a major impact on the research of eukaryotic genomes and contributed to the development of various DNA markers. RAPDs are particularly useful to study the genetic structure of populations because they reveal polymorphisms in non-coding regions of the genome (Caccon et al, 1997). Different primers produce RAPDs that have been used extensively as molecular markers. RAPDs also have the advantage that no prior knowledge of the genome is necessary for this application [1-2].

The RAPD markers method has been reported

to be an efficient tool to differentiate geographically and genetically isolated population, and has been used to verify the existence of population of species that have arisen either through genetic selection under different environmental conditions or as a result of genetic drift. The prime goal of the present study was to identify molecular characterization and understands the evolutionary advancement among five different hierarchal vertebrate species.

MATERIALS AND METHODS

This study was conducted at Nizam's Institute of Pharmacy, Mediciti Institute of Medical Sciences, (Hyderabad, India) and Genetics laboratory. This study was approved by the Nizam's Institute of Pharmacy, (Reg.No. 1330/ ac/10/ CPCSEA dated 30 June 2014).

DNA Isolation: Genomic DNA was isolated from tissue samples by pure link DNA kit life technologies (USA).

Primers: The 20 ten-nucleotide random primers of arbitrary sequence were tested for their potential use in the differentiation of five i nvestigated species. A set of 20 primers was purchased from Eurofins, Inc.

Experimental strategy: In an initial analysis, all the primers were screened for their usefulness in amplifying species or breed-specific DNA fragments. Pooled DNA samples were used as templates. On the basis of the criteria of band pattern quality, reproducibility and the presence of species or breed-specific fragments, only some of the primers were chosen for further analysis. These primers were then applied to study 36 individuals representing five investigated species.

RAPD (Random Amplified Polymorphic DNA): RAPD reactions were performed in a volume of 25 ìl containing: 80 ng of genomic DNA, 2 ìM of arbitrary primer, 0.20 mM dNTPs, 1.33 mM MgCl2, 1 unit of Taq polymerase and 1 × reaction buffer (10 mM Tris-HCl pH 8.8, 1.5 mM MgCl2, 50 mM KCl, 0.1% Triton X-100) provided by the enzyme supplier. Negative controls (lacking DNA) were set up for each reaction mastermix to check for DNA contamination. Reaction mixtures were overlaid with 30 ìl of autoclaved mineral oil in order to avoid evaporation. Amplifications were carried out in a biorad thermocycler and the cycling conditions. RAPD-PCR products were size-fractionated in a 1.8% agarose gel with the addition of ethidium bromide. The results of electrophoresis were documented with the use of the biorad computer system (Figure 6.1 to 6.6). Molecular weights of amplified DNA fragments were estimated by means of Bio-Capt (version 99) and Bio-1D programs.

Statistical Analysis: RAPD profiles of 36 individuals were characterized as matrices of zeros and ones by scoring bands on agarose gel as their presence ("1") or absence ("0"). Only the bands that met the criteria of clarity and reproducibility were scored. Matrices of zeros and ones characterizing RAPD profiles of the four investigated species were also made, by combining the profiles for individuals. Character matrices for species were then subjected to RAPD distance programs. A triangular matrix of pair wise distances was calculated using the Dice algorithm based upon the formula by NEI and LI (1979). Then, the Unrooted dendrogram based on the Neighbor-Joining method of SAITOU and NEI (1987) was constructed by means of NJTREE 2.0 and TDRAW 1.14 programs (Jin and Ferguson, University of Texas Health Science Center,Houston) available in the RAPD distance package.

RESULTS

Access results make it possible to conclude that RAPD analysis can be a powerful tool for developing molecular markers useful in distinguishing between species and for studying their phylogenetic relation. A number of species-specific bands were added within RAPD profiles composed by these primers: 31.4% of all the polymorphic bands were exclusive to the Rat, 23.2% were unique to the Avian, and 19.2% were diagnostic for the Gecko and 11.3% for the Toads and 10.8% for Murrel (Fig.6.7). By performing PCR we can strength achieved the previous RAPD genetic variation results further we can come to the conclusion genetic variation in the 5 species.

Phylogenetic analysis: On the basis of the analysis of RAPD profiles amplified by six arbitrary primers that gave the best results in

producing species-specific bands (OPG-04, OPG-12, OPG-13, OPG-16, OPG-17 and OPG19). A Neighbor-Joining tree display phylogenetic affinity of the five investigated species was constructed. The dendrogram in weed out and no out group is specified, however, the phylogenetic branch of the Wistar Rat seems to be the most distinct.

Fig. 1: RAPD profiles of individual animals representing five species (amplified by primer OPG-4). Lane 1, 2 Murrel, lane 3, 4 Toads, lane 5, 6 Gecko, lane 7,8 Avian, lane 9,10,11 Wistar rat, lane 12 is 1 kb ladder.

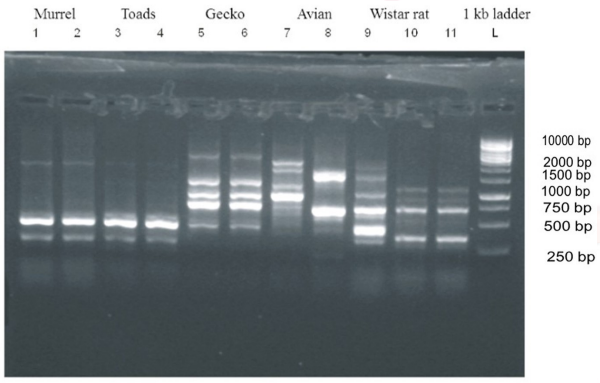


Fig. 2: RAPD profiles of individual animals representing five species (amplified by primer OPG-12). Lane 1, 2 Murrel, lane 3, 4 Toads, lane 5, 6 Gecko, lane 7, 8 Avian, lane 9, 10, 11 Wistar rat, lane 12 is 1 kb ladder.

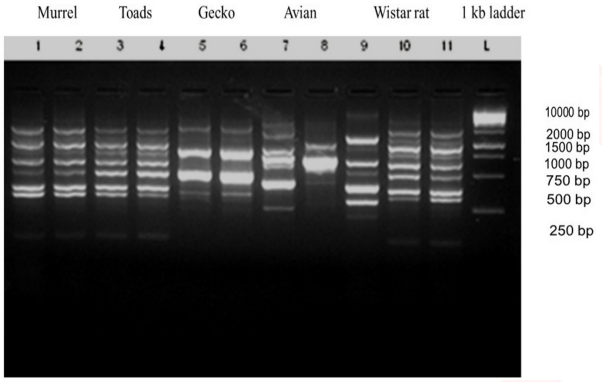


Fig. 3: RAPD profiles of individual animals representing five species (amplified by primer OPG-13). Lane 1 is 1kb ladder , lane 2 ,3,4 Murrel ,lane 5,6,7 Toads ,lane 8,9,10 Gecko ,lane 11,12 Avian, lane 13,14 Wistar rat, lane 15 is 50bp ladder.

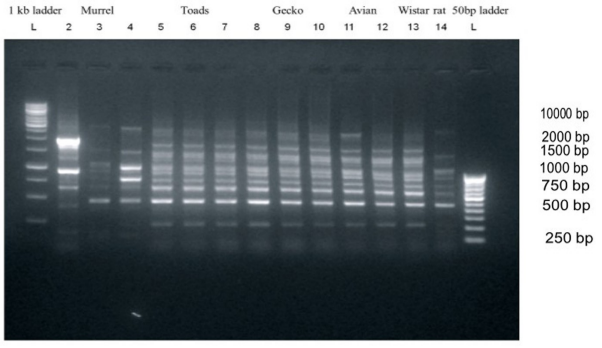


Fig. 4: RAPD profiles of individual animals representing five species (amplified by primer OPG-16). Lane 1 is 1kb ladder, lane 2, 3, 4 Murrel, lane 5,6,7 Toads, lane 8,9,10 Gecko, lane 11, 12 Avian, lane 13, 14 Wistar rat, lane 15 is 50bp ladder.

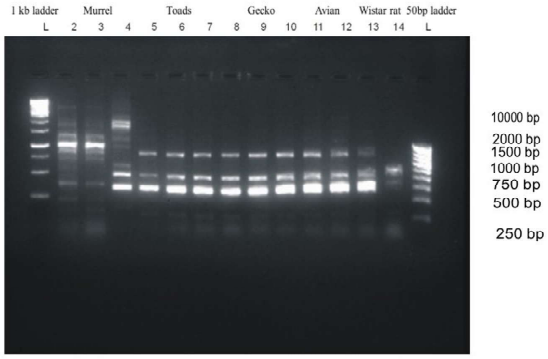


Fig. 5: RAPD profiles of individual animals representing five species (amplified by primer OPG-17). Lane 1 is 1kb ladder, lane 2, 3, 4 Murrel, lane 5,6,7 Toads, lane 8, 9, 10 Gecko, lane 11, 12 Avian, lane 13,14 Wistar rat, lane 15 is 50bp ladder.

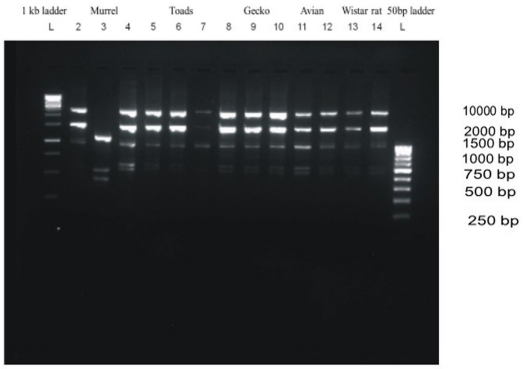


Fig. 6: RAPD profiles of individual animals representing five species (amplified by primer OPG-19). Lane 1, 2, Murrel, lane 3, 4, Toads, lane 5, 6, Gecko, lane 7, 8, Avian Lane 9, Wistar rat 10, 11, lane 12 is 1 kb ladder.

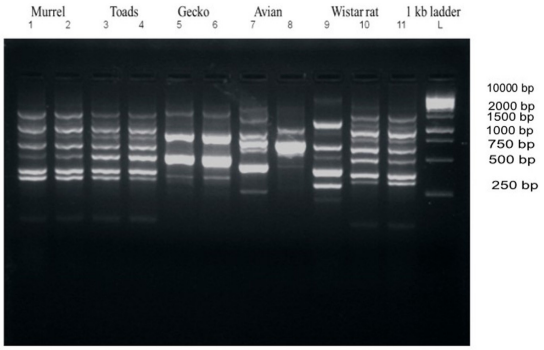
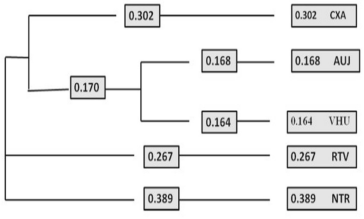


Fig. 7: Dendrogram showing hypothetical phylogenetic relations of the Avian (CXA), Toads (AUJ), Murrel (VHU) and Gecko (RTV), and Wistar rats (NTR). Numbers inside branches and brackets indicate patristic distances to individual species.



DISCUSSION

The RAPD technique has been used to study genetic variation in several species, so far RAPD marker and studies were reportedly used to distinguish the genetic variability in various species which are as follows mosquito species and populations, black aspergilli, Gliricidia, Medfly, Cocoa, fish species, bacterial species and parasitic protozoans [3-11].

The most recent phylogenetic studies on cichlid fishes were done to learn their phylogenetic relationships and provide information to the classical hypothesis using RAPD markers. In another study reported in species, subspecies and strains of tilipa in order to segregate the commercial important species among them using RAPD markers [12]. High levels genetic variability was discovered using RAPD markers same broodstock of sea bass (Dicentrarchus labrax) and several genetic markers were discovered in tropical fish species representing 7 families [13]. Study conducted in 2 laboratory strains of zebrafish has generated 721 strain-specific utilizing the RAPD markers [14-15].The RAPD analysis was done in sexually reproducing and clonal organisms using markers and it is learned that these markers are more apt for clonal organisms and it was used to determine clonal identity as they have asexually breeding property [16-17]. Species and Clone-specific markers were identified in hydroids and in fungal mycelia [18].

Even though the usefulness and value of RAPD markers is not quite clear in taxonomic and phylogenetic studies, usage of these markers in diagnostic purposes. RAPD markers unique to individuals from one species within a genus will be species-specific (inter-specific) [19]. Generation of genus-specific markers is possible only if the gene fragment is turned out to be a unique polymorphism in the individuals belonging to a certain genus. Inter-specific gene flow and hybrid identification can be determined by employing the species specific markers. Similarly, identification of hybrid populations is possible by choosing population-specific markers [20]. Species-specific RAPD markers were used to find out the inter-specific gene flow between 2 iris species, Iris hexagona and I. vulva [21]. Similarly, identification of F1 hybrids from different inbred lines of maize was done using the AP-PCR [19].

Determination and detection of paternity and kinship relationships among large progenies of dragonfly was done among the individuals belonging to a given species using RAPD polymorphism [20]. In a study, consisting of anax parthenope males and ovipositing females. The males were believed to guard a female in order to ensure a consequent mating partner rather than immediate fertilization. The RAPD analysis has helped in identification of a father among a population consisting of several unrelated males, the guarding male, the guarded female and offspring clutches and as a specific RAPD marker was identified in the guarding male and in the offspring which was rare in the whole population. The diagnostic RAPD markers have helped in parentage analyses (present in only 1 of the putative parents) in the off springs. A high frequency of non-parental RAPD bands has been reported in primate pedigrees [22]. However, lower frequencies of non-parental RAPD bands were found in beetles (Nicrophorus tomentosus) and strawberries (Fragaria vesca) [23]. In a study conducted in Nectomys squamipes, a semiaquatic rodent species distributed along watercourses using RAPD analysis in order to assess genetic distance and the genetic structure of populations [24].

In another study, the molecular characterization of Dicentrarchus labrax embryonic cells (DLEC) was done using Random amplified polymorphic deoxyribonucleic acid analysis by PCR (RAPD-PCR) to detect DNA alterations in environmental genotoxic studies [25].Similarly, in another study on Zebra fish was done, where in RADP primers were identified in laboratory strains of zebra fish which have shown extensive genetic polymorphism in developmental stages [26]. RAPD analysis in Atlantic Coast striped bass which exhibit exceptionally low levels of genetic variation, and it is found that gene flow is enough to hinder the fixation of alternate genetic markers, but it is not enough in stopping the development of significant divergence in frequencies of RAPD markers [27].

Coming to the present studies on RAPD among various vertebrate species, no attempt has been

made previously elsewhere. The use of randomly amplified polymorphic DNA (RAPD) protocol to examine genetic variation and to generate DNA fingerprints of vertebrates paved the way for more future studies to differentiate between five species existed in this study. The randomly amplified polymorphic DNA (RAPD) protocol was reliable, simple to set up, fast and large areas of genomic DNA screened as the study proved that consequently this study agrees with earlier studies used the same protocol [28]. As well as it needs only minute amount of DNA, no prior information about DNA sequence required as that required in the study of mitochondrial and nuclear genes amplification and no hazardous radioactive chemicals used. These advantages make it more preferable than other techniques. The RAPD-PCR amplification with a single ten-nucleotide primer to produce a DNA fingerprint of DNA fragments was affecting essentially with Primer, DNA template and reaction conditions. The number of bands on the agarose gel depends on the number of appropriately oriented and target sites present in DNA in that species or individual.

The results of RAPD profiles showed strongly differentiated fingerprints of the five species, so discrimination among the tested species was easy. The RAPD technique is less laborious compared with other fingerprinting techniques, producing results with low statistical error. But, this method needs accurate work and multiple ten-nucleotide primers should be used to generate a number of molecular markers to establish fingerprints. Therefore DNA fingerprint similarity is being used to infer the level of genetic variation within and between natural populations. RAPD variation differed among primers and species since the amplified bands per primer were ranged from 0 and 1 and also the bands scored in the five species ranged from 0.164 to 0.389, in neighboring joining tree. The fact that RAPD-PCR technique surveys numerous loci in the genome makes the method particularly attractive for analysis of genetic distance and phylogeny reconstruction, thus effectively by using RAPD genetic variation among five species is evaluated.

CONCLUSION

RAPD analysis is a very easier, efficacious method for deciphering phylogenetic relationships between various animal species. RAPD study of five hierarchal vertebrate animals of lower vertebrates show definite phylogenetic pattern with uniqueness of polymorphic DNA showing ascending order amongst vertebrate species. The uniqueness of DNA content was maximally unique in the highest vertebrate (rat) in this study.

Conflicts of Interests: None

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