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EVALUATION OF PTYCHOBOTHRIDEAN CESTODES USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS FROM FRESH WATER FISHES IN GODAVARI BASIN (M.S.) INDIA

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ABSTRACT

Ptychobothridean genera like *Senga* and *Circumoncobothrium* are the common parasites of fresh water fishes. The genotypic study of these parasites was taken by RAPD. The RAPD profile of these two parasites were not similar to each other as depicted by the band pattern in picture. These results suggest the presence of inter-specific polymorphism among cestode parasites of two different genera for RAPD analysis. The present study demonstrated that genetic differentiation of cestode parasites could be accomplished on the basis of genomic variation with polymorphic band pattern using RAPD. All the detected bands (PCR product) were polymorphic and band size ranged from 500-5000 bp in length. The RAPD of profiles using GBO-31, GBO-32, GBO-33, GBO-34, GBO-35 and GBO-36. Primers were able to characterize inter-specific polymorphism among the two genus (*Senga* and *Circumoncobothrium*). Genetic analysis suggests that *Senga* and *Circumoncobothrium* show genetic diversity with respect to RAPD patterns using all the six primers used for the present study. The genetic distance between the analyzed genuses ranged from 0.14 to 0.80. The differentiation of the two parasites on the basis of genetic markers could greatly facilitate study on the biology of these parasites.

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KEY WORDS : Cestode, Godavari basin, Ptychobothridae, RAPD.

Introduction

The molecular markers are a useful tool for assessing genetic similarity, variation and resolving quantitative traits identities. Among the molecular markers random amplified polymorphic DNA (RAPD) is increasing its speedy process and simplicity.

The RAPD (Random amplified polymorphic DNA) polymerase chain reaction (PCR) is a technique recently developed[16,19] that employs random oligodeoxy nucleotide primers in a PCR to generate polymorphic markers. The polymorphism detected provide a simple method of gene mapping[9,12,16,17] and species or strain differentiation[1-4,11,13-18].

It has been argued that DNA finger printing is so essential to behavioural ecology and population biology that it would be highly unfortunate to have its application limited to a few specialized laboratories rather than to the broad community working in these fields.

Recently, considerable progress has been achieved in studies on the phylogeny of tapeworms (Eucestoda) and new hypotheses based on morphological life cycle, ultra structure and molecular data have been proposed[6-8,10].

Principle of RAPD analyses

The PCR based RAPD technique is an attractive complement to conventional DNA

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TABLE - 01 : Melecular size mapping of RAPD profile of *Senga* and *Circum onchobothrium.*

Lane No.	Description	No of bands detected (Quantity One™)
1 and 14	1 kbg scale marker	07
2	*Senga* RAPD with GBO-31	08
3	*Circumoncobothrium* RAPD with GBO-31	09
4	*Senga* RAPD with GBO-32	13
5	*Circumoncobothrium* RAPD with GBO-32	11
6	*Senga* RAPD with GBO-33	01
7	*Circumoncobothrium* RAPD with GBO-33	04
8	*Senga* RAPD with GBO-34	02
9	*Circumoncobothrium* RAPD with GBO-34	08
10	*Senga* RAPD with GBO-35	02
11	*Circumoncobothrium* RAPD with GBO-35	08
12	*Senga* RAPD with GBO-36	11
13	*Circumoncobothrium* RAPD with GBO-36	10

fingerprinting. RAPD analysis is conceptually simple. Nanogram amounts of total genomic DNA are subjected to PCR using short synthetic oligonucliotides of Random sequences. The amplification protocol differes from the standerd PCR condition[5] in that only single random oligonucleotide primer is employed and no. primer knowledge of the genome subjected to analysis is required. The technique essentially scans a genome for these small inverted repeats amplifies intervening DNA segments of variable length.

Material and Methods

For the genotypic study of cestode parasites, fresh water fishes were dissected. Parasites were collected from its intestine and kept in absolute alcohol. These fresh materials were used for further processes.

Parasite worms samples were separated into different sterile eppendrof tubes for DNA isolation. The tissue was chopped using sterile surgical blade and then subjected to high salt tissue DNA isolation protocol. Briefly, the parasitic tissue macerate was resuspended in 0.9% saline and pelleted for further processing of genomic DNA isolation. TNES buffer 50Mm Tris-Hcl (PH 7.4), 100mM Nacl, 2mM etylenediaminetetracetic acid 1% Nonidet P-40, lysoenzyme and proteinase K (10µg/ml) were used for nucleic acid isolation. This method employed Nacl and SDS lysis followed by phenol: chloroform: iso-amyl alcohol, 58:48:2), and then with $CHCl_3$: isoamyl alcohol (24:1). DNA was then precipitated with 2% Sodium acetate and absolute ethanol. Dried DNA was dissolved in nucleases free water. Quality assessment of genomic DNA was performed by 1% agarose gel electrophoresis as well as DNA was quantified using Qubit TM fluorometer (Invitrogen USA) for measurement of DNA concent critical in all downstream application that contains PCR interventions.

Six RAPD primers used in this analysis were GBO-31, GBO-32, GBO-33, GBO-34, GBO-35 and GBO-36. Every RAPD reaction mixture comprised of 25 ng of genomic DNA, 20 pM of the appropriate RAPD primer; 200 pM of dATP, dCTP, dGTP,and 1.0 U of Taq DNA polymerase were all provided in the gene Ombio PCR research kit (geneOmbio technologies Pune). Reaction products were analysed by electrophoresis through 1.5% (Wt/vol) agarose (promega corporation, Madison, WI 53711 USA) gel slabs (10cm by 16cm

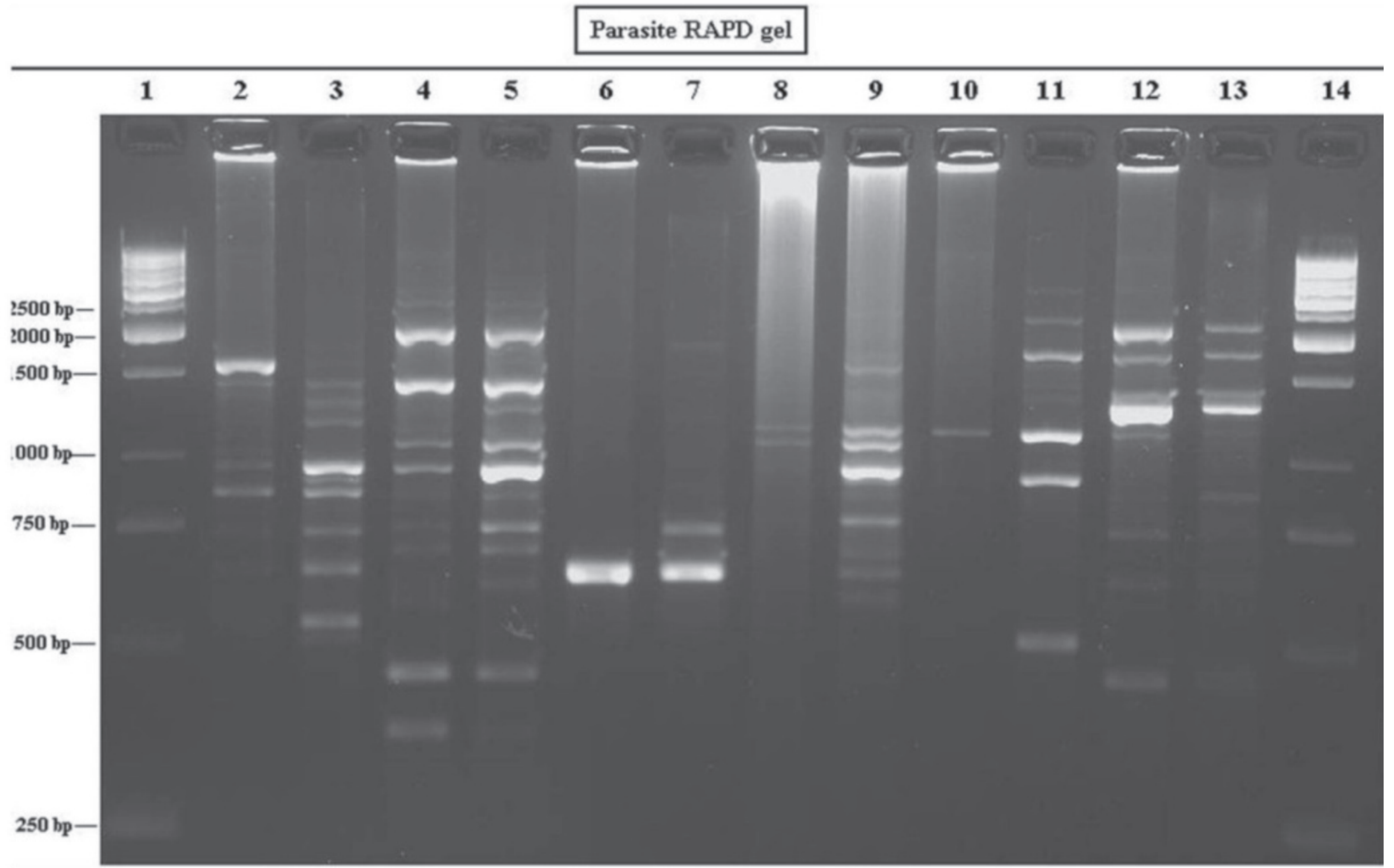

Fig. 1 : RAPD profile of *Senga* and *Circumoncobothrium* using six different RAPD primer.

TABLE -2 : RAPD DNA Genetic analysis of *Senga (*NX) and *Circumancobothrium* (NY) by using primers and estimation of common amplicons (Nxy), similarily coefficients (SC) and genetic distance (GD) analysis profile

S No	Name of primer	Nx. (No. of amplicons in parasite *Senga*)	Ny. (No.of amplicons parasite *Circumonco-bothrium*)	Nyx (No. of amplicons common)	Sc=2Nxy (Nxy+Ny) (Simmilarity Coefficient)	GD=1-SC (Genetic Distance)
1	GBO-31	8	9	1	0.71	0.29
2	GBO-32	13	11	2	0.83	0.17
3	GBO-33	1	4	3	0.40	0.60
4	GBO-34	2	8	4	0.40	0.60
5	GBO-35	2	8	5	0.20	0.80
6	GBO-36	11	10	6	0.86	0.14

by 6mm) with 0.5×Tris-borate EDTA as the resolving buffer. A Gel were stained with ethidium bromide, placed over a source of UV light, and then photographed. The molecular sizes of DNA fragmentes were determined using Gene ruler 1kb lader (Fermentas international Inc, Canda) DNA size standard.

Genetic Analysis:-

For estimation of similarity-coefficient (SC), the formula proposed was used to generate computer algorithm and transformed into GD (Genetic Distance) Analysis profile. The SC was converted into GD using the equation GD = 1- SC. Total number of allels amplified was calculated using the formula. NX + NY= NXY, Where Nx, Ny and Nxy were total amplicons detected in parent strain, mutant strain and those common in the two samples respectively.

Results

The RAPD analysis was performed with different samples of cestode parasites using six different RAPD primers. Images in the below section show the profiles generated with primers GBO-31, GBO-32, GBO-33, GBO-34, GBO-35, and GBO-36 against DNA from two samples. For given

TABLE-3 : Scoring of polymorphic bands (using Quantity One^{TM} BIORAD Software)

S No	Name of primer	No. of polymorphic bands
1	GBO-31	5
2	GBO-32	4
3	GBO-33	3
4	GBO-34	6
5	GBO-35	8
6	GBO-36	3

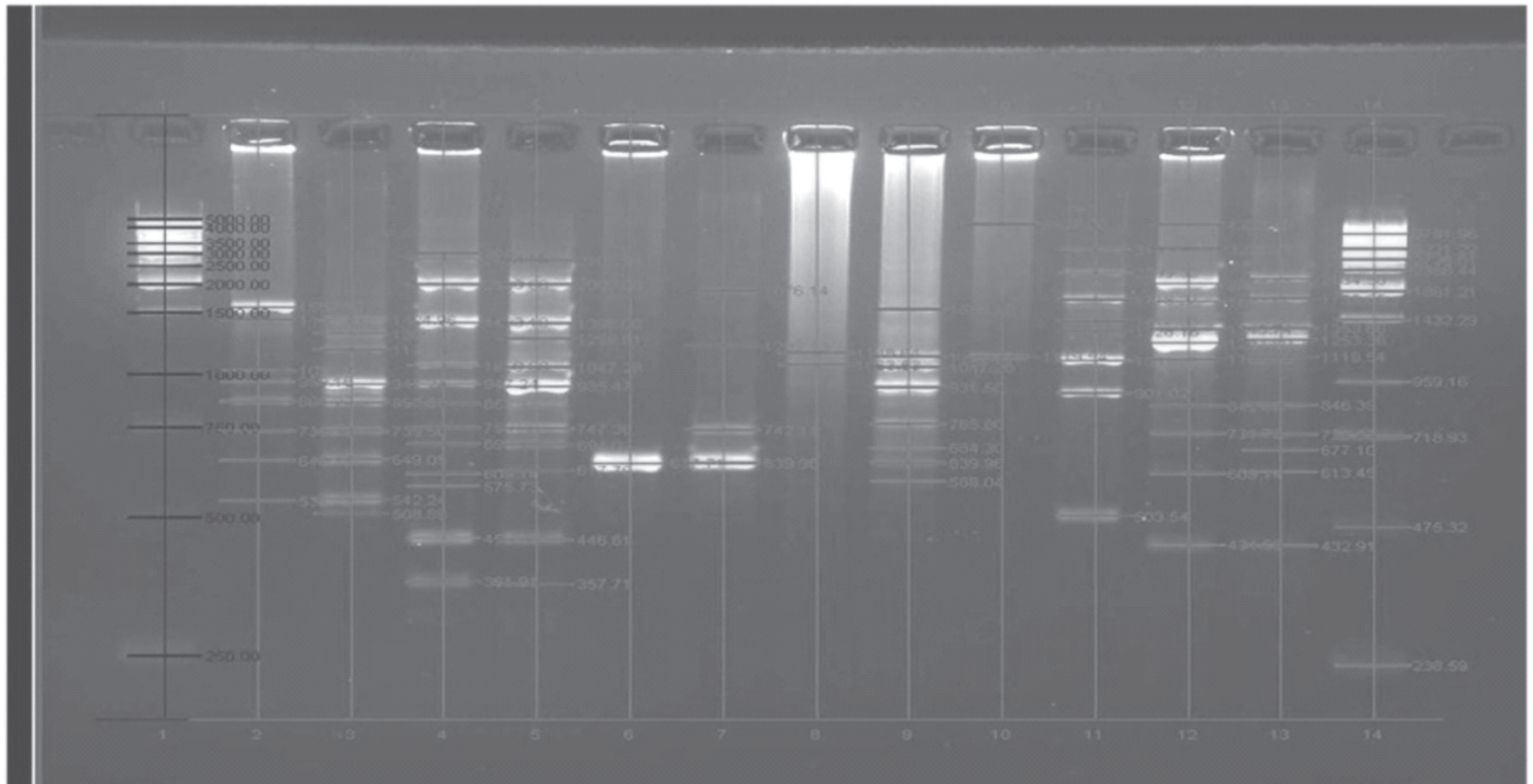

Fig. 2 : Molecular size mapping of (RAPD) profile of Senga and Circumoncobothrium with ten different RAPD primers using Quantity One^{TM}(Bio Rad) software.

primers, the profiles of DNA fragments found for both the samples were not similar. With each of the primers, DNA fragment length polymorphisms were observed although several amplicons were of identical size and hence were considered to be the same allel. The reproducibility of the RAPD profiles was confirmed by generating identical.DNA fragments from re- extracted DNA, as well as ultra diluted (1:100) RAPD product solution generated from previous reactions. For each primer, the array of DNA fragments consisted of bands of high as well as low intensities when stained with ethidium bromide and detected under ultra violet light (260 nanometer). The higher intensity bands were possibly due to the amplification of repetitive DNA sequence, the influence of neighbouring sequence on hybridization to the target sequence, or fewer mismatches to the target sequence. In contrast, the low intensity bands may possibly have PCR amplification caused due to higher degree of mismatch between the primer and the target sequence.

Conclusion

From above observation it can be concluded that both parasites *i.e. Senga* and *Circumoncobothrium* show genetic diversity with respect to RAPD patterns using all the six primers used for the present study. Hence it can be concluded that the major polymorphic bands obtained by RAPD analysis are useful for differentiation of the cestode parasite genus.

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