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Molecular Identification of Some *Schistosoma mansoni* Isolates in Saudi Arabia

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Abstract: In this paper, nine geographical isolates of *Schistosoma mansoni* (three from Egypt, three from Saudi Arabia and 3 from Puerto Rico) were studied at the genotype level by RAPD analysis with two arbitrary primers. The genetic distance was measured by the percentage of unshared bands. The RAPD results showed that the Egyptian strains were closely related to Saudi strains but Puerto Rico strains clustered in different group. These results demonstrated the usefulness of RAPD for displaying the differences of inter- and intra-species of *Schistosoma*. It also suggested that genetic diversity among different geographical strains of *Schistosoma mansoni* from different localities had occurred.

Key words: *Schistosoma mansoni* · RAPD · Egypt · genetic variation

INTRODUCTION

Schistosomiasis is caused by infection with blood flukes of the genus *Schistosoma*, of which three species (*S. mansoni*, *S. haematobium* and *S. japonicum*) are the main causative agent of the disease in man [1]. This chronic helminthic infection affects more than 200 million people throughout the world and 600 million live in endemic areas where they are at risk of infection [2]. This wide distribution of the disease makes the study of genomic variability extremely important.

There are well known two distinct geographical strains of *Schistosoma mansoni*, the Puerto Rican and the Egyptian strains [3-5]. However, a new geographical strain namely, Saudi Arabian strain has been reported based on differences with the Egyptian strain shown by scanning electron microscopy [6, 7].

Early studies of population structure in schistosomes used isozymes to look for genetic differences between populations of *S. mansoni*: those that are transmitted through rodents and populations that infected humans [8, 9]. A substantial amount of phenotypic variation has been observed in schistosome species, it has been more difficult to quantify the degree of genetic differentiation among these same populations [10]. More rigorous studies of population structure and genetic subdivision are needed to clarify our understanding of schistosome epidemiology [11]. Ideally, such studies would employ genetic markers that are highly polymorphic and inherited in a Mendelian codominant fashion. The data available from isoenzyme analysis of laboratory strains of schistosomes from Africa, Asia, the Caribbean and Brazil [12] indicate that there is relatively restricted diversity in these species and strains with no distinct genetic constitutions.

Several studies have shown that the RAPD technique can be applied successfully to helminths [13-19] for estimating differentiation among parasite populations [20]. Recently, there have been important advances in the application of recombinant DNA techniques. A number of DNA-based methods have been developed to investigate genetic diversity, to differentiate strains and species and to analyze phylogenies of schistosomes. Dias-Neto *et al*. [13] have demonstrated that RAPD (random amplified polymorphic DNA markers) allows the identification of strains of *S. mansoni* and species of *Schitosoma*. They reported that, random amplified polymorphic DNA (RAPD) showed very discrete variability in different strains of *S. mansoni* adult worms, however, all of them were derived from laboratory maintained populations [18]. Pillay *et al*. [21] have also demonstrated intraspecific DNA polymorphisms among the isolates of *S. mansoni.*

The aim of this investigation was to confirm the differences between the closely similar isolates of

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Schitosoma mansoni isolated from different localities (Saudi Arabia, Egypt and Puerto Rico) in which the morphological and physiological characters were not enough for obvious separation.

MATERIALS AND METHODS

Parasite isolates: Nine isolates of repeated twice *S. mansoni* were obtained from different places: SMS1,2,3 (from Saudi Arabia); SME1,2,3 (from Egypt) and SMP1,2,3 (from Puerto Rico).

Isolation of genomic DNA: DNA was extracted from individual adult worms as follows: frozen worms were individually homogenized with a pestle in 100 µL of 10 mm Tris-HCl pH8; 1mm EDTA, 10 mm NaCl and 70 mm sucrose (extraction buffer). The pestle was rinsed in an Eppendorf tube with 10% sodium dodecyl sulphate and 12 µL of proteinase (10 mg mL^{-1}). The homogenate was incubated at 57°C for 2 h, then extracted once with an equal volume of phenol and once with chloroform. The solution was adjusted to 03 m with sodium acetate and precipitated at 20°C overnight with 2 volumes of absolute ethanol. DNA was pelleted the following day by centrifugation (15000 g, 20 min, 4°C), then rinsed with ethanol 70%, dried and finally resuspended in 100 µL of TE (10) mm Tris-HCl, pH8 and 1 mm EDTA). This extraction protocol yielded sufficient DNA for approximately 12 RAPD reactions worm [22].

RAPD - PCR amplification: Two arbitrary primers A7 and A8 (5'-GAAACAAATG-3' and 5'-GTGACGTAGG-3', respectively) were used as described [23]. The random amplification procedure was performed essentially as described [24] PCR reaction mixture was subjected to electrophoresis in a 1% agar gel and photographed.

Data analysis: Computer analysis of RAPD patterns was performed as given by Halmschlager *et al.* [25]. Basically, the formation obtained from agarose gel electrophoresis was digitalized by hand to a two - discrete - character - matrix (0 and 1 for absence and presence of RAPD bands). Dendrogram was calculated by using the Jukes - Cantor option in the DNADIST program and application of the FITCH program to the computed distance matrix (PHYLIP package [26]). For running DNADIST, the two discrete characters of 0 and 1 had to be converted to Guanine and Thymine in the RAPD data matrix. Complete alignment of data was performed with CLUSTALX software and then the cluster analysis will be ready by using Treecon programme [27].

RESULTS AND DISCUSSION

Nine isolates of *S. mansoni* were used in this study. The two primers (5'-GAAACAAATG-3' and 5'-GTGACGTAGG-3') used in this study, generated a considerable number of amplification products for comparison. A different DNA banding pattern was present in almost every isolates. Comparison of each profile for each of the primers was based on the presence (1) versus absence (0) of RAPD amplimers that migrated to the same position in the gel. Bands of the same size obtained by the same primer were scored as identical, but only bands repeatable in at least two experiments with the same primer at different times were evaluated. All two primers revealed high similarities between each type of isolates (Figs. 1 & 2). Anou *et al*. [28] reported that isolates of *Schistosoma japonicum* showed identical RAPD patterns by using seven primers.

The combined data from all isolates of *S. mansoni* by using two different primers were analyzed to produce a dendrogram (Fig. 3). According to dendrogram constructed from RAPD data, the isolates of *Schistosoma mansoni* isolates split into two RAPD groups (RAPD I and RAPD II). RAPD I cluster divided into two subgroups RAPD I A and RAPD I B. RAPD I A included *S. mansoni* isolates from Saudi Arabia (SMS) and RAPD I B included *S. mansoni* isolates from Egypt (SME). This result indicates that those two groups of isolates related and slightly far from the Puerto Rico isolates which clustered in RAPD II. Dendrogram revealed a correlation between clusters and geographical origin of isolates. Those results revealed a clear and significant genetic differentiation among the three local populations of adult schistosomes. These came in agreement with findings of Sire *et al.* [22]. Differences in snail infectivity [29, 30] and drug susceptibility [31-33] were also reported. The study of ribosomal DNA (rDNA) has shown that schistosomes exhibit both interspecific and intraspecific differences. It has been suggested that the variation in the major tandemly repeated copies of the gene can be used for species identification [34-37], whereas low copy numbers variants that exhibit intraspecific polymorphism can be used for strain identification [34, 35, 38].

This study has shown that there is considerable genotypic and phenotypic variability among *S. mansoni* isolates obtained from different geographic regions. When applied to the study of Schistosomes, the RAPD markers method has proved useful in analyzing the different problems associated with their genetic diversity. Its main additional advantages over the other techniques

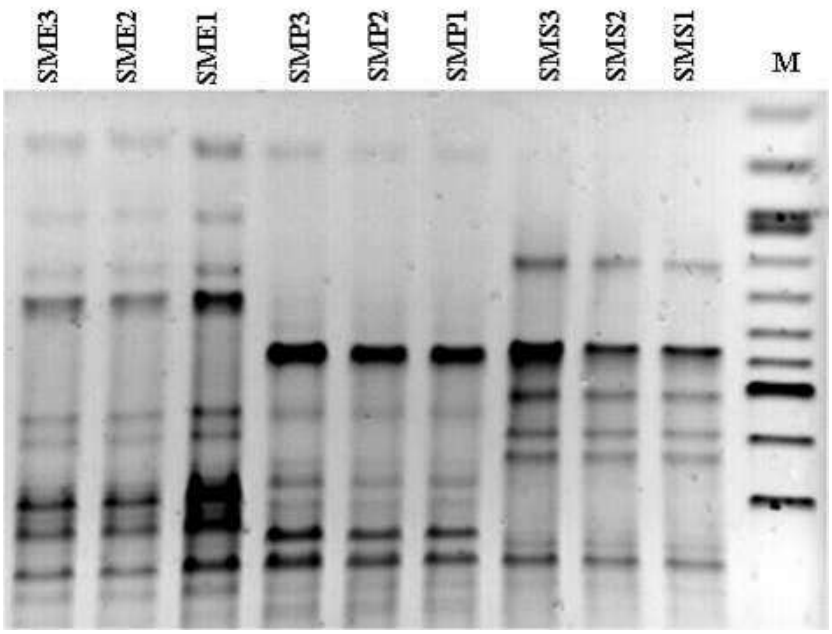

Fig. 1: RAPD fragments generated by the primer A7 (5'-GAAACAAATG-3') for nine isolates of *Schistosoma mansoni* from Saudi Arabia (SMS), Egypt (SME) and from Puerto Rico (SMP)

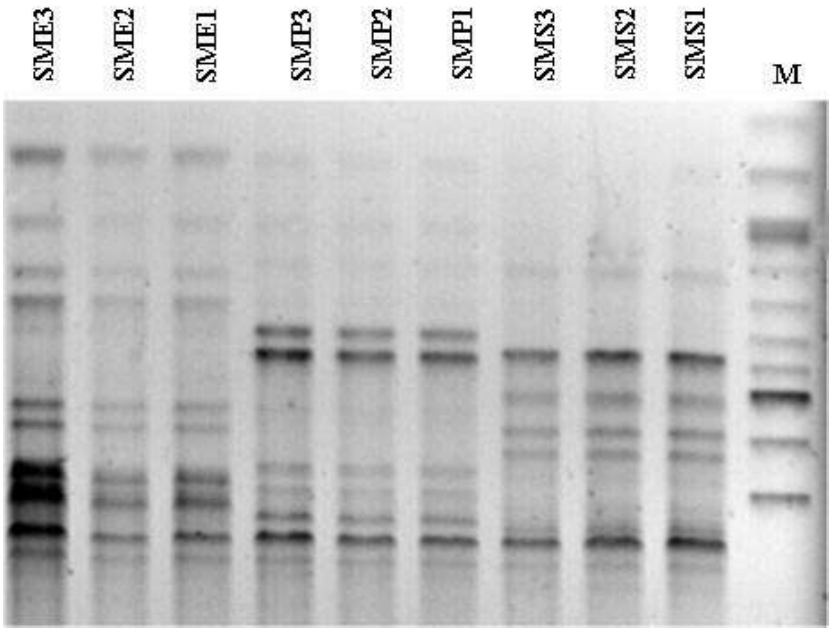

Fig. 2: RAPD fragments generated by the primer A8 (5'-GTGACGT AGG-3') for nine isolates of *Schistosoma mansoni* from Saudi Arabia (SMS), Egypt (SME) and from Puerto Rico (SMP)

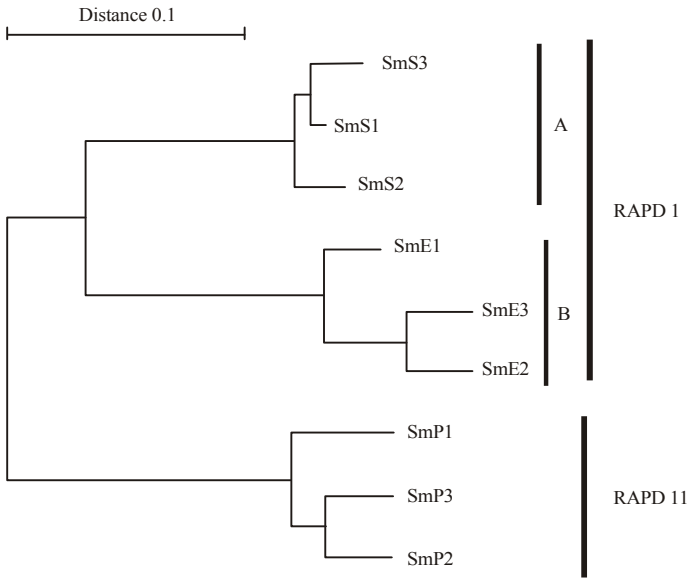

Fig. 3: The dendrogram showing the relationships of different *schistosoma* isolates based in two different primers results

are speed, reproducibility, technical simpleness, high resolution and considerable reduction of the amount of DNA for routine analysis. Using this technique, markers which can be used to discriminate between species or strains were revealed quickly and without the need for sequence information or radiolebelling.

In conclusion, RAPD markers are highly resolving and helpful tool for investigation of genetic variation within the genus *Schistosoma* and the species *S. mansoni*. They provide a single technology that can be used to rapidly distinguish species and strains. Compared with other techniques of biochemistry and molecular biology, we can understand this endemic disease better.

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