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Discrimination between susceptible and non-susceptible *Biomphalaria alexandrina* snails – intermediate hosts of *Schistosoma mansoni* in Western Saudi Arabia – using random amplified polymorphic DNA analysis

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ABSTRACT. Eight samples from infected and susceptible and from non-susceptible snails (*Biomphalaria alexandrina*) to *Schistosoma mansoni* infection were used in this study. Snails samples were studied at the genotypic level by RAPD analysis with two arbitrary primers. The genetic distances between samples were measured by the percentage of unshared bands. The studied samples separated into two groups according to their DNA fingerprinting. Thus this study has shown that the RAPD markers method has proved useful in discrimination between susceptible and non-susceptible snails of *Biomphalaria alexandrina*.

KEY WORDS : Schistosomiasis, *Biomphalaria alexandrina*, susceptibility, genetic diversity, RAPD-PCR, primers.

INTRODUCTION

Schistosomiasis, is a disease caused by infection with *Schistosoma mansoni* and *S. haematobium*. *Biomphalaria alexandrina* and *Bulinus truncatus* are, respectively, intermediate hosts of these parasites. *S. mansoni* and *S. haematobium* are of great economic as well as medical importance. Some 200 million people are probably infected and 500-600 million more exposed to infection (WEBBE, 1981). From laboratory observation, it has been noticed that some snails of the genus *Biomphalaria* exposed to infection with miracidia of *S mansoni*, develop infection and produce cercariae, while the rest remain uninfected. Sometimes non-susceptible snails outbreed the susceptible ones and replace them (SHOZAWA et al., 1989; MKOJI et al., 1990). These observations lead to the inquiry: do non-susceptible strains of freshwater snails occur in Western Saudi Arabia? If so, it would be beneficial to take advantage of this fact to develop methods of control by introducing a resistant strain into water containing a susceptible strain, in order to have a chance of displacing them.

Susceptibility of snails to schistosome infection depends on the metabolic status of the snail itself. One of the metabolic activities depends upon the production of reactive oxygen species by hemocytes from the snail (BENDER et al., 2005). Phagocytes play a crucial rule in host defence against pathogens, and their arsenal includes the ability to initiate a respiratory burst (HAMPTON et al., 1998). The generation of reactive oxygen species is apparently essential for efficient killing of bacteria and fungi (ROOS & WINTERBOURN, 2002; REEVES et al., 2003).

The possibility that snail susceptibility or resistance to *S. mansoni* is a consequence of differences in the respiratory burst has been addressed in previous studies with schistosome-resistant and susceptible strains of *Biomphalaria glabrata* (CONNERS & YOSHINO, 1990). HAHN et al. (2000) and DIKKEBOOM et al. (1988a; b) did not detect strain differences.

The identification of molluscs is normally based on morphological characters of the shell, renal, and reproductive systems (PARAENSE, 1975). However, the identification of some species may be complicated by the similarity among these characters (PARAENSE, 1988). Recently, molecular tools based on polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) of the ribosomal RNA intergenic spacer regions (ITS) have been used to overcome this problem (VIDIGAL et al., 1998; CALDEIRA et al., 1998; 2000). The detection *S. mansoni* infection in snails of the genus *Biomphalaria* is usually through cercariae shedding induced by artificial light exposure, or by squeezing snails between two glass slides. However, these methods cannot detect the parasite in dead snails or in the pre-patent period. In the latter, infection diagnosis is only possible after the parasite has completed its life cycle (3 to 4 weeks after infection), when cercarial release begins. Thus, molecular methods have been used to detect *S. mansoni* infection for those two situations (HANELT et al., 1997; JANNOTTI-PASSOS et al., 1997; HAMBURGER et al., 1998). Molecular techniques for *S. mansoni* detection in snails have been used as a complementary tool when the conventional techniques are not efficient. HAMBURGER et al. (1992) diagnosed *S. mansoni* in *Biomphalaria* sp., through a DNA probe marked with 32P directed to a repeated genome region of the parasite. HANELT et al. (1997) were able to detect the presence of *S. mansoni* in *B. glabrata* snails, during the pre-patent period, and distinguished *S. mansoni* from two other trematode species by amplifying its 18S region from rDNA through "nested" PCR.

The objective of this work was to determine if susceptible and non-susceptible populations of snails coexist in Western Saudi Arabia, and to characterize such strains on a molecular basis by comparing the DNA patterns of the population of snails. Thereafter, it would be beneficial to produce the non-susceptible snails in large numbers and introduce them into the field. Natural selection would fur-

ther act to increase the proportion of alleles for insusceptibility and eventually provide some measure of biological control of schistosomiasis in natural populations.

MATERIALS AND METHODS

Snail collection

One hundred snails were collected from different localities and habitats (irrigation canals and drains, current stream, ponds, dry canals etc ...) in the South-western region of Saudi Arabia, and examined individually for cercarial production. Snails were reared singly in either a 250ml or 400ml beaker with a Petri dish cover at an ambient temperature at 26ºC. Tap water, aerated for at least 24 hours, was used for humidification, and snails were fed oven-dried or fresh lettuce 2 or 3 times a week. Reproduction was by self-fertilization. Rooms in which snails were maintained and experiments conducted were kept at a temperature of about 26ºC. Eggs laid by adult snails of the first generation were reared. Each snail progeny to be isolated from rearing was numbered from "1" on, maturity being based on onset of egg laying. Each snail's progeny were maintained under standard laboratory conditions as described above to give the second and third generations of snails which were subjected to the same biological studies.

DNA extraction

Total DNA was extracted from the combined foot and eggs of selected snails. Briefly, the snail's foot and eggs were mechanically disrupted in 50mM Tris–HCl, pH 8.0 (100mM NaCl, 50mM EDTA, 0.5% SDS) and incubated overnight at 37°C with 50mg/ml proteinase K. as described by VIDIGAL et al. (1994). Following phenol/chloroform extraction and ethanol precipitation, DNA was resuspended in 10mM Tris–HCl, pH 8.0, 1mM EDTA, and DNA concentrations were estimated by comparison with known standards on 2% ethidium bromide stained agarose gels.

DNA amplification

The protocol used was that previously applied to the study of schistosome and *Trypanosome cruzi* (DIAS NETO et al., 1993; STEINDEL et al., 1993).

RAPD data analysis

Computer analyses of RAPD patterns were performed as described by HALMSCHLAGER et al. (1994). The analysis of data was based on the NEI & LI (1979) Coefficient. A dendrogram was constructed by the unweighted paired group method of arithmetic average (UPGMA) based on Jaccard's similarity coefficient by using Phoretix ID software (version 5.2).

RESULTS AND DISCUSSION

All snails were subjected to miracidia to determine their susceptibility or resistance to infection. Eight snails of *Biomphalaria alexandrina* were then selected for DNA analysis: 4 infected with *Schistosoma mansoni* and 4 free from *Schistosoma mansoni* cercariae. The primers (5'-ACCTACCGTACTATGACG-3') and (5'-GTTCCAGCC-3') were used in this study, and generated a considerable number of amplification products for comparison. Different DNA banding patterns were present in almost every isolate. Comparison of each profile for each of the two 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. Individual amplifications of both susceptible and non-susceptible snails with the two primers are illustrated in Figs 1 & 2. There are very limited differences in the amplified bands of the individuals within each population. The only reproducible differences we have noticed are between the two types of snails (susceptible and non-susceptible). The total number of bands scored was 165, with the average number of bands scored per lane being 10.3 (Figs 1 & 2). According to DNA patterns, the primers we used revealed a very high degree of similarity among the samples of the non-susceptible group, and also, separately, among the samples of the susceptible group (Figs 1 & 2). On the other hand DNA patterns of the two primers showed big differences between the samples of non-susceptible group and those of the susceptible group (Figs 1 & 2). So, the results showed that there are intrapopulation differences in *Biomphalaria alexandrina* between susceptible and non-susceptible snails.

Using RAPD-PCR, ABDEL-HAMID et al. (1999) showed that there are intrapopulation differences within a species (*Biomphalaria tenagophila*) between susceptible and non-susceptible snails. Thus, when tissues from several individuals of the same population are pooled, one can obtain a pattern which is to some extent distinctive of the strain. Also, they reported that as there is relatively restricted genetic variation between susceptible and non-susceptible snails within the same species, it is indeed possible that the susceptibility of snails to parasitic infection is genetically controlled.

Our combined data from all samples using two different primers were analyzed to produce a dendrogram (Fig. 3). According to the dendrogram constructed from RAPD data, the samples of *Biomphalaria alexandrina* split into two RAPD groups corresponding to the infection status. Our results indicated that RAPD primers were useful for distinguishing snail populations with respect to susceptible and non-susceptible properties. ABDEL-HAMID et al. (1999) suggested that RAPDs should be highly useful for phylogenetic analysis among closely related individuals. This suggestion is in agreement with both BARRAL et al. (1991) and VIDIGAL et al. (1994) who indicated that RAPD markers are a highly resolving and helpful tool for investigation of variability. They provide a simple technology that can be used to rapidly distinguish species, strains and sexes in laboratory conditions. Also, SIMPSON et al. (1993) proved that RAPD is undoubtedly a powerful approach for analysis of genetic variation and the identification of genetic markers. So, RAPD is of particular value in the study of genetic variation of snails and would allow the design of specific primers for genome analysis. RAPD is finding increasing use because of its technical

simplicity. NEWTON (1962) considered that susceptibility or resistance of a snail to infection is an hereditary character. RICHARDS (1970), RICHARDS & MERRIT (1972) and NABIH & EL-ANSARY (1990) confirmed this contention by extensive studies on the genetics of *B. glabrata* and *B. alexandrina* snails. They suggested that the specificity relationship between the parasite and its intermediate host snail is genetic. Moreover, RICHARDS (1984) showed that resistance to infection in adult snails is governed by a single gene according to mendelian genetics. This suggestion has some support from the findings of KNIGHT et al. (1991) and LARSON et al. (1996) who described the occurrence of genetic polymorphisms between resistant and susceptible snails through the use of restriction fragment length polymorphism (RFLPs) in the rRNA gene as well as RAPD assays. The differences in the DNA patterns were expected, since it was previously reported by KNIGHT et al. (2000) that there were different genetic strains of a single species of snails from the same locality and they varied in their degrees of resistance, and even among susceptible stocks certain individuals were more resistant than others. Recently, ABDEL-HAMID et al. (2006) studied genetic variations between strains susceptible and resistant to *Schistosoma* infection within *B. alexandrina* snails using random amplified polymorphic DNA analysis technique. Their results showed that 39.8% of the examined field snails were resistant, while 60.2% of these snails showed high infection rates. ABDEL-HAMID et al. (1999) reported that the availability of isogenic snail lines has made it possible to use molecular tools to determine the degree of genetic variability between them.

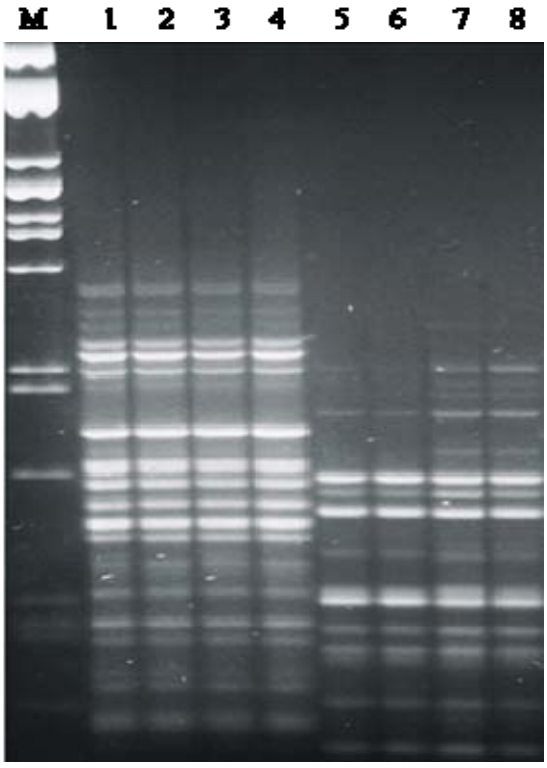

Fig. 1. – RAPD fragments generated by the primer (5'-ACCTACCGTACTATGACG-3') for eight samples of *Biomphalaria alexandrina*. Lanes: 1-4, non-susceptible samples and Lanes: 5-8, susceptible samples.

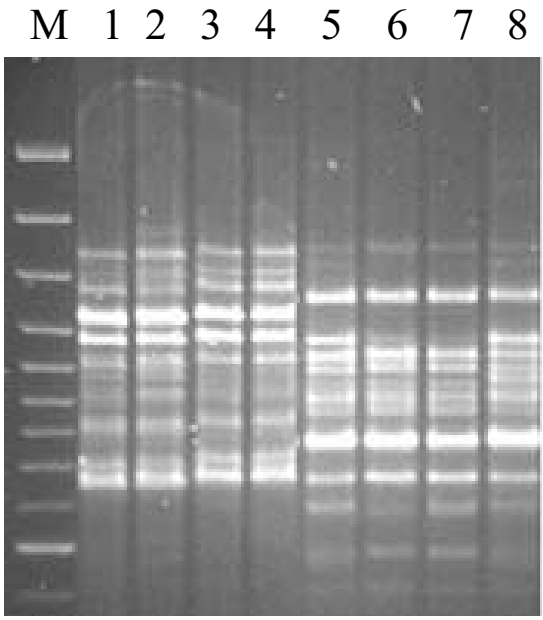

Fig. 2. – RAPD fragments generated by the primer (5'-GTTGCCAGCC-3') for eight samples of *Biomphalaria alexandrina*. Lanes: 1-4, non-susceptible samples and Lanes: 5-8, susceptible samples.

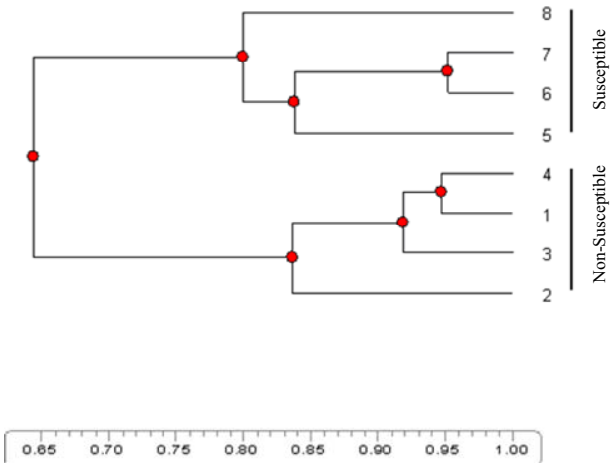

Fig. 3. – The dendrogram showing the relationships of different *Biomphalaria alexandrina* samples. Genetic distances were obtained by random amplified polymorphic DNA analysis using two different primers.

By comparing genomic DNA of susceptible and non-susceptible snails using two primers, we found a component that the susceptible ones produced that was lacking in the non-susceptible ones, that would make them non susceptible. In subsequent studies, it might be possible to insert (or delete) this component in susceptible snails by treatment with specific mutagens (ABDEL-HAMID et al., 1999).

So, we suggest that such genetically altered varieties in snails of *Biomphalaria alexandrina* could then be

released into areas endemic for schistosomes, following treatment of these areas to remove most or all of the genetically unaltered susceptible snails. This concept will lead to genetic control of schistosomiasis.

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