DETECTION OF BRUGIA MALAYI AND BRUGIA PAHANGI PARASITES BY BIOTINLABELED DNA PROBES

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ABSTRACT

Morphologically, the larvae of Brugian parasites are difficult to differentiate by conventional methods. Recently, radioactive labeled DNA probes[2,3] have been developed to distinguish the larvae of these parasites. However, these probes have a short shelf-life and are hazardous to the users. Two oligonucleotide DNA probes have been tested, one is specific for B.malayi and the other specific for B.pahangi. They were each labeled with Biotin in three different ways by using : a one-tailed 30mer biotinylated uridine residues, a two-tailed 30mer biotinylated uridine-thymidine residues. The dot blot assays were tested at various temperatures (30^0C-80^0C) using different concentrations of parasite DNAs (12.8ng-0.1ng). Our preliminary results indicated that the sensitivity and specificity of the biotinylated DNA probes, with a two-tailed 45mer biotinylated residues, were highly acceptable for field use.

INTRODUCTION

B.malayi and *B.pahangi* are found sympatrically in some regions of Asia, including Indonesia; they can be transmitted by the same vectors, like *Mansonia* mosquitoes.

Morphologically, it is difficult to distinguish the larvae of those parasites. Recently, a monoclonal antibody[1] and DNA probes[2] had been developed to differentiate the species and stages of the parasites.

McReynolds et al[2] had cloned and sequenced the repeated DNA sequences of Brugian parasites. A 322 base pairs repeated sequence was observed when DNA samples from *B.malayi* and *B.pahangi* were digested with the restriction enzyme HhaI.

A 644 base pairs dimer of the repeated sequences from *B.malayi* was inserted into the plasmid pBR322. The copy number of the repeats in *B.malayi* was estimated to be 30,000. The repeats are arranged in direct tandem arrays and comprised about 12 % of the genome. The cloned sequence (pBma 68) was subsequent used as a probe for *B.malayi*. However, this DNA probe cross hybridized with *B.pahangi* in non-stringent conditions. Subsequent to this work, Williams et al[3] had cloned and sequenced 15 copies of each repeats from *B.malayi* and *B.pahangi*. A comparison of these consensus repeats revealed 93 % homology between the two species except for one 65 base pairs region with only 72 % homology. Utilizing this region of divergence, two synthetic oligonucleotide probes were developed, each specific for *B.malayi* or *B.pahangi*.

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The disadvantages of radioisotopes for labeling DNA probe is its short shelf-life, expensive, time consuming, require a safe waste disposal system, and could be hazardous to the investigators. Therefore, the probes were tried to be labeled with Biotin similar to that described by Langer et al.[4].

Three different biotinylated DNA probes were tested to determine the optimal condition for hybridization.

MATERIALS AND METHODS

DNAs from the microfilariae of *B.malayi* and *B.pahangi* were isolated, purified, and spotted onto nitrocellulose filters with a concentration of 12.8ng; 6.4ng; 3.2ng; 1.6ng; 0.8ng; 0.4ng; 0.2ng; and 0.1ng respectively. Briefly the assay is performed as described: DNA was denatured in 0.3M NaOH for 10 minutes at room temperature. An equal volume of 4M ammonium acetate was added, and the samples were incubated on ice and then spotted onto nitrocellulose filters. The baked nitrocellulose filters were incubated in a solution containing 5X Denhardt's, 6X SSC, 1ng/ml salmon sperms DNA, 10mM EDTA, and 1% SDS, and then hybridized to the biotinylated DNA probes, which were prepared by the New England Biolabs. Afterwards the filters were washed and incubated in a subtrate solution for 2 hours. Labeling with biotin was performed in three different ways: (A) a one-tailed 30mer biotinylated uridine residues, (B) a two-tailed 30mer biotinylated uridine residues, and (C) a two-tailed 45mer, biotinylated uridine-thymidine residues. The dot blot assay was performed at various temperature using lambda DNA and pBma68 as negative and positive controls and incubated overnight. A blue colour indicated a positive result.

RESULTS AND DISCUSSION

Our preliminary results indicated that the biotinylated DNA probes with a two-tailed 45mer biotinylated uridine-thymidine residues gave the best sensitivity (12.8ng-0.1ng) compared to the two other biotinylated probes. The colour reaction on the nitrocellulose filter was easy to read, and therefore this labeling method was decided to be used in subsequent experiments. It was speculated that the spacer coupled by the unlabeled thymidine residues provided an efficient reaction between the substrate and the biotin molecules. The best specificity was obtained when the dot blot was performed at a temperature of 75^0C. At this temperature, there was no cross hybridization of the heterologous DNA probe to the parasite DNAs at all the concentrations tested. However, a dot blot assay at 75^0C was impractical for field use. To decrease the hybridization temperature to 35^0C-37^0C, we added 50 % formamide to the hybridization solution. According to Williams (personal communication) every 1% of formamide solution will decrease the hybridization temperature by 0.65^0C. Our preliminary experiments indicated that the biotinylated DNA probes are easier to use for the following reasons :

(1) The filters can be read with the naked eyes without having to autoradiograph the results.
(2) It has a long shelf-life (one year).
(3) It is not hazardous to the investigators/ technicians.
(4) It does not require a safe waste disposal system.

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QUESTIONS AND ANSWERS :

1. Question: You mentioned that the probe you used could detect also L_1 & L_2.
   But could you explain what part supposed to be responsible for the common reaction. They have to be different in their differentiation stage antigen.

   Answer : The probe can detect the DNA parasite that can be isolated from L_1, L_2, L_3 or microfilariae. These probes can't differentiate the stage antigens of the parasites (because these probes only can detect the DNA parasite).
   To differentiate the stage antigens, we can use the monoclonal antibody.