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DEVELOPMENT OF A DIAGNOSTIC DNA PROBE TO DETECT *SETARIA DIGITATA*: THE CAUSATIVE PARASITE OF CEREBROSPINAL NEMATODIASIS IN GOATS, SHEEP AND HORSES

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SUMMARY

Two repetitive sequences (IpSdM and IpSdS) have been cloned and sequenced from the genome of *Setaria digitata*. When IpSdM (214 bp) and IpSdS (201 bp) were aligned, a high degree of homology (85%) was observed, indicating that they belong to the same family of repeats. IpSdM represents a complete repeating element while IpSdS consists of two partial repeating elements arranged in tandem. The elements are present in about 10 000 copies comprising 2.8% of the *S. digitata* genome. As a diagnostic probe IpSdM detects as little as 100 pg DNA of both *S. digitata* and *S. labiato-papillosa*. It can also detect a single microfilaria and a L_3 larva making it a valuable tool to monitor cattle and mosquito vector populations in the prevention of cerebrospinal nematodiasis.

KEYWORDS: *Setaria digitata*; repetitive sequences; tandem repeats; DNA probes; cerebrospinal nematodiasis.

INTRODUCTION

The diseases caused by filarial nematodes in human and livestock animals constitute a major health problem in tropical countries. The debilitating effects of these infections severely affect manpower resources in developing countries, most of which have agricultural based economies. For control and/or eradication programmes on filariasis to be effective, it is necessary to gather detailed and accurate data from epidemiological surveys. This would invariably entail the sensitive and specific detection, identification and quantification of the different stages of the parasite in both the vector and the host. Currently the method used is microscopy.

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PREPARATION AND CONFORMATION OF α-L-ARABINOFURANOSYL-PYRIDINIUM SALTS, AND HYDROLYSIS OF THE 4-BROMOISOQUINOLINIUM COMPOUND

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ABSTRACT

Tri-*O*-benzoyl-α-L-arabinofuranosylpyridinium salts can be made in acceptable yields and high stereochemical purity by the reaction of 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide and the pyridine in the presence of tetrabutylammonium bromide. Analysis of the ^{1}H-n.m.r. signals of the sugar reveals that the benzoylated compounds adopt largely the E_2 conformation whereas the debenzoylated compounds are largely in the $^oT_1$ conformation. The α-L-arabinofuranosyl-4-bromoisoquinolinium ion hydrolyses by both pH-independent and base-catalysed pathways, complicated by the reversible formation of an inert pseudo-base in alkali. The comparatively low rate of the pH-independent reaction is discussed in terms of the acid-lability of furanosides.

INTRODUCTION

Glycopyranosylpyridinium salts have proved very informative about enzymic[1–4] and non-enzymic[5,6] glycoside hydrolysis, by virtue of the absence of any possibility of acidic assistance to the departure of the aglycone, and of the conformational preferences dictated by the reverse anomeric effect of the pyridinium moiety. The preparation of glycofuranosylpyridinium salts and investigation of their hydrolytic behaviour therefore seemed likely to be fruitful. Departure of nicotinamide from the ribofuranosyl ring of NAD^+ is important biologically in the mono-[7] and poly-ADP-ribosylation of proteins[8], and simple NAD^+-glycohydrolases are also known and have been studied mechanistically[9,10]. The C–N cleavage of NAD^+ has been subject to some mechanistic investigations[11,12]; strangely, it is accelerated by anionic buffers, especially phosphate, and, in principle, is complicated by base stacking and the lability of the pyrophosphodiester group. We therefore selected α-L-arabinofuranosylpyridinium salts for investigation, since their spontaneous hydrolysis would not present these problems, yet in all probability[1–4] they would be substrates for α-L-arabinofuranosidases, which are widely distributed.

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Differentiation of *Setaria digitata* and *Setaria labiatopapillosa* using Molecular Markers

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SUMMARY

5S rRNA intergenic regions of *Setaria digitata* and *Setaria labiatopapillosa* were PCR amplified with primers designed from the 5S rRNA gene of *Brugia malayi*. The ladder-like banding patterns obtained for the amplifications were distinctly different for the two species. Four amplified products were cloned into the pBS vector and completely sequenced. DNA clones from two individual samples of *S. digitata*, Sd4 and Sd6, showed 97% sequence homology to each other.

All sequenced clones showed the presence of the spliced leader (SL) RNA gene with a 22 nucleotide spliced leader sequence. The phylogenetic tree constructed using these data and the 5S rRNA intergenic regions of several other filarial nematodes showed the *Setaria* species sharing a branch with *Dirofilaria*. RAPD-PCR analyses identified 107 bands of which 86 were polymorphic (80%). A dendrogram constructed for *S. digitata* and *S. labiatopapillosa* separated the two species into two distinct clusters. The polymorphic loci identified by the RAPD-PCR analyses can be studied further to develop species-specific probes/PCR primers for the identification of each species.

KEYWORDS: *Setaria digitata*; *Setaria labiatopapillosa*; 5S rRNA; spliced leader; RAPD-PCR.

INTRODUCTION

Setaria species are parasitic nematodes, found in the peritoneal cavity of ungulates such as cattle and buffalo and are non-pathogenic in these natural hosts. The infective larvae can be transmitted accidentally to aberrant hosts such as sheep, goats, and horses through mosquito vectors. In these aberrant hosts, the larvae may migrate along the central nervous system and cause mechanical damage and inflammatory reactions, leading to cerebrospinal nematodiasis (CSN) (Innes *et al.*, 1952).

About 43 species of *Setaria* have been identified (Wijesundera, 2001). *Setaria digitata* and *Setaria labiatopapillosa* are two species predominantly found in Sri Lanka, the former being the more common. In the past, there has been some confusion in the allocation of species of *Setaria*. While Innes *et al.* (1952) suggested that both *S. digitata* and *S. labiatopapillosa* are identical and synonymous, Yeh-Liang-Sheng (1959), on the basis of observation of distinct morphological features, suggested that the two species should be retained. This view was supported by Shoho and Uni (1977), who studied the morphological characteristics of several *Setaria* species, including *S. digitata* and *S. labiatopapillosa*, using scanning electron microscopy.

Our study focused on the differentiation of the above two species at a molecular level through analyses of the 5S rRNA intergenic region and by the use of random amplified polymorphic DNA-PCR (RAPD-PCR technique). Variation between species measured at a DNA level, as opposed to morphological findings, offers the advantages that it can be quantified and that it is not subject to environmental effects (Kazan *et al.*, 1993).

MATERIALS AND METHODS

All chemicals used were of molecular biological grade and unless otherwise specified were pur-

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A SIMPLE AND RAPID NON-RADIOACTIVE OLIGONUCLEOTIDE BASED HYBRIDIZATION ASSAY FOR THE DETECTION OF *WUCHERERIA BANCROFTI*

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Abstract. Five biotin labeled oligonucleotides was designed based on a previously cloned and characterized repetitive DNA sequence specific for *Wuchereria bancrofti*. The oligonucleotide mix (containing five probes) when used in a hybridization assay, detected as little as 100 pg of purified *W. bancrofti*, microfilarial DNA, a single infective stage larva and a single microfilaria in 50µl blood sample. A simple protocol was followed for the hybridization assay. Blood samples lysed with sterile distilled water and digested with proteinase K in the presence of a detergent were directly applied on to nylon membranes for dot blot assays. The DNA extract of mosquitos carrying infective stage larvae was eluted through sephadex G-50 minicolumns prior to blotting. The assay was also able to detect DNA extracted from microfilariae infected samples stored over five days at room temperature (28°C). This simple and rapid oligonucleotide hybridization protocol with the highly sensitive chemiluminescent-based detection has significant potential for the development of a field kit to detect *W. bancrofti* infection.

INTRODUCTION

Over 128 million people in 96 endemic countries have been estimated to be infected with lymphatic filariasis (Michael and Bundy, 1997). *Wuchereria bancrofti* alone accounts for over 90% of the human lymphatic filariasis in the world (WHO, 1992). At present, *W. bancrofti* is the only form of lymphatic filarial infection that is prevalent in Sri Lanka (Dissanaike, 1991). In 1991, around 3.5 million persons were at risk of infection while the extent of the infection has spread during a 30 year period (Dissanaike, 1991). The number of persons at risk has now increased to nine million (AFC, 1996). The percentage of new cases of filarial infection increased to 23.7 in 1996 compared with 18.3 in 1995. Furthermore the infection rate of the vector *Culex quinquefasciatus* mosquito increased from 0.64% in 1990 to 0.72% in 1996 (Ministry of Health, Sri Lanka, 1996). Therefore, the control of filariasis is important especially in the developing third world countries as it causes serious economic and social consequences by affecting many young working adults.

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The detection of the filarial parasite in the host and the vector is essential for the effective treatment of filariasis and evaluation of control programs. The accurate and specific diagnosis of filariasis in the field depends on the availability of simple, sensitive and rapid diagnostic procedures. Of the different methods available, light microscopy has traditionally been the most commonly used method for the detection of filarial parasites, especially in the field. Furthermore, the collection, dissection, and microscopic examination of vector mosquitos are the techniques currently used in epidemiological surveys to detect and characterize filarial parasites. However, these technics are not always reliable because mosquitos may carry both human and other animal filariae that cannot be distinguished either biochemically or morphologically (Sim *et al.* 1986 a,b). Although these methods are comparatively cheap they are time-consuming and labor-intensive. The use of recombinant DNA based detection strategies offer the promise of greater sensitivity, specificity and speed in demanding field situations (Rajan, 1990). Therefore, considerable effort has been expended in recent years, for the development of alternative methods using DNA probes.

DNA probes, both radioactive and non-radioactive as well as PCR based methods have been developed for the detection of lymphatic filarial parasites (Williams *et al.* 1988, 1996; Zhong *et al.*

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A sensitive polymerase chain reaction based assay for the detection of *Setaria digitata*: The causative organism of cerebrospinal nematodiasis in goats, sheep and horses

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Abstract

A sensitive PCR assay for the detection of *Setaria digitata* has been developed. Two oligonucleotide primers (17 nt) were designed from a previously cloned and characterized tandemly arranged repetitive sequence of *Setaria digitata*. Using these primers, it was possible to amplify small quantities (100 fg) of *S. digitata* genomic DNA. A simple procedure, using proteinase K and non-ionic detergent NP 40, was followed to process the host blood samples and mosquitoes harbouring L_3 larvae. The sensitivity of the polymerase chain reaction based assay surpasses the microscopic detection and the previously reported oligonucleotide based chemiluminescent detection of microfilariae in infected host blood samples and L_3 larvae in mosquitoes.

Keywords: *Setaria digitata*; Cerebrospinal nematodiasis; Diagnosis; Nematoda; PCR

1. Introduction

Setaria digitata is a filarial nematode found in the peritoneal cavity of cattle, buffalo and other ungulates. In these natural hosts, the parasite is considered to be non-pathogenic. However, the transmission of infective larvae (L_3) to abnormal hosts such as goats, sheep or horses could lead to a serious and often fatal disease called cerebrospinal

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Optimization of Repetitive Element Based PCR to Identify Polymorphism in *Magnaporthe grisea*

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ABSTRACT

One of the most serious rice diseases present in Sri Lanka is rice blast which is caused by ascomycete fungus *Magnaporthe grisea*. Polymorphic differences have been observed in *M. grisea* according the geographical region where they grow. The objective of the current research was to optimize the PCR conditions to determine the polymorphisms in *M. grisea*. *M. grisea* isolates were obtained from Matara, Kaluthara, Kurunagala and Colombo districts. Liquid nitrogen method was found as the best method over CTAB method to extract DNA from *M. grisea*. DNA samples were genotyped by using repetitive element based polymerase chain reaction (rep-PCR) with two outwardly directed primer sequences from *Pot 2* (EMBL accession Z33638). PCR reaction were optimized resulting eight to twelve bands for one isolate which lies between 100 bp to 1500 bp. This study can be extended to find the polymorphisms of Rice Blast pathogen all over the country.

KEYWORDS: *M. grisea*, Rice Blast, rep-PCR, *Pot 2*.

INTRODUCTION

Rice (*Oriza sativa*) is a one of main staple food in Asia that supplies approximately 23% of per capita energy for six billion people worldwide (Urak *et al.*, 2007). There are many serious plant diseases of rice. Rice blast is the one of the most important diseases which is caused by ascomycete fungus *Magnaporthe grisea* (Urak *et al.*, 2007). *M. grisea* can infect most parts of the plant, but infections of the leaf, node or the panicle are the most damaging phases of the disease (Ou, 1980). When *M. grisea* infects rice and produces neck rot or panicle blast, it will either kill the host plant or prevent seed germination (Ou, 1980).

Rice blast is prevalent in most of the rice growing areas in Sri Lanka. The use of disease resistant cultivars has become difficult because of the phenotypical variability of the pathogen and favourable environmental conditions during crop season (Ou, 1980). This variability of *M. grisea* varies according to the geographical region (Levy *et al.*, 1993).

DNA fingerprinting has been widely used for studying the population structure of the *M. grisea* (Suzuki *et al.*, 2006). The techniques used include Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD) and repetitive element based Polymerase Chain Reaction (rep-PCR) (George *et al.*, 1998).

Rep-PCR is a new method to determine the polymorphism in *M. grisea*. *Pot 2* elements are present in the *M. grisea* genome. *Pot 2* is a class II transposable element. This *Pot 2* element has 1857bp with a 43bp terminal inverted repeats (TIR) and 16bp direct repeats within the TIRs (Kachroo *et al., 1994*). *Pot 2* present at a large copy number of approximately 100 per haploid genome and represent one of the major repetitive DNAs by rice pathogen (Kachroo *et al.*, 1994).

Pot 2, Pot 2-TIR and MGR primers use to amplify the *Pot 2* elements in *M. grisea* by using rep-PCR. *Pot 2*-TIR and MGR are single primers. Rep PCR is based on the observation that two outwardly directed primers, complementary to the interspersed *Pot 2* element, amplify differently sized DNA fragments comprising of unique sequences lying between these elements. According to the George *et al.*, (1998) the results generated by rep-PCR of *Pot 2* displayed polymorphisms specific to the *M. grisea* strains. Due to the large number of *Pot 2* elements present in the *M. grisea* genome, a sufficient number of intervening sequences within amplifiable distance was produced with the rep-PCR technique, there by generating adequate polymorphism for the detection of genetic diversity (George *et al.*, 1998).

This study is a part of ongoing research to determine the polymorphism of *M. grisea* isolates which were gathered from agro ecological zones in Sri Lanka. Present study is mainly focused on the DNA extraction from