Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Parasitology 110 (2005) 108–113
Experimental Parasitology
www.elsevier.com/locate/yexpr

Trichomonas vaginalis: intrastrain polymorphisms within the ribosomal intergenic spacer do not correlate with clinical presentation

Augusto Simões-Barbosa *, Tatiana Teatini Lobo, Joaquim Xavier, Sandra Elisa Carvalho, Eduardo Leornadecz

Universidade Católica de Brasília, Programa de Pós-graduação em Ciências Genômicas e Biotecnologia, SGAN 916, Brasília, DF 70790-160, Brazil

Received 8 July 2004; received in revised form 16 December 2004; accepted 16 December 2004
Available online 24 March 2005

Abstract

Trichomoniasis presents a broad spectrum of clinical patterns ranging from asymptomatic to severe vaginitis and cervicitis. Despite its importance, very little is known about the genetic relatedness of its causative agent, *Trichomonas vaginalis*, and the clinical phenotypes. To address this question, analysis of restriction length polymorphism (RFLP) within the intergenic spacer of the ribosomal DNA (IGS) from 60 clinically defined isolates of *T. vaginalis* was performed. This is the first description of the IGS polymorphism of *T. vaginalis*. As expected, a considerable number of patients were asymptomatic (28%) while only 12% presented both leukorrhea and macular colpitis, the most evident symptoms of trichomoniasis. The IGS–RFLP with the use of eight restriction enzymes showed absence of correlation between the genetic relatedness of the isolates and symptomatology. Further studies are necessary to evaluate the importance of the IGS polymorphism to the parasite virulence and clinical phenotype.

Index Descriptors and Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; rDNA, ribosomal DNA; IGS, intergenic spacer of the ribosomal RNA gene; ITS, internal transcribed spacer of the ribosomal RNA gene; HSP, heat shock protein; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RAPD, random amplified polymorphic DNA; UPGMA, unweighted pair-group methods analysis

Keywords: Protozoa; *Trichomonas vaginalis*; Ribosomal RNA gene; Ribosomal intergenic spacer; Polymorphism; RFLP; Trichomoniasis; Clinical

1. Introduction

Trichomonads are flagellated amitochondrial anaerobic protists, representing one of the most ancient branches of eukaryotes. Living in the urogenital and alimentary tracts of a wide range of hosts, some species are pathogenic especially those restricted to bovine and human urogenital tracts. *Trichomonas vaginalis*, the etiological agent of human trichomoniasis, is recognized as the principal non-viral sexually transmitted pathogen worldwide (WHO, 1995).

Clinical investigations of trichomoniasis were previously undertaken (Fouts and Kraus, 1980; Krieger and Alderete, 1999; Rein, 1990; Wolner-Hanssen et al., 1989). Women are generally affected by the disease showing a wide variation in symptoms. The infection affects the vulva, vagina, uterine cervix, and secondarily the urinary tract. Remarkably, a copious leukorrhea is observed. Although there is variation in color, a typical leukorrhea comes out as a frothy malodorous vaginal discharge. Another aspect of clinical importance is the appearance of the vagina and cervix, termed macular colpitis or "strawberry cervix." In this case, erosion of the cervical epithelium and punctate hemorrhages on the cervical wall are observed. However, not all patients present such typical clinical appearance. In addition, patients may also experience abdominal pain,

* Corresponding author. Fax: +55 61 347 4797.
E-mail address: sbarbosa@pos.ucb.br (A. Simões-Barbosa).

0014-4894/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.exppara.2004.12.012

irritation, and discomfort. Certainly, at least part of this pathological manifestation could be a result of the genetic ability of the parasite to express virulence factors which promote cytoadherence, cell detaching, and cytolysis (Petrin et al., 1998). Therefore, the existence of virulent and non-virulent isolates of *T. vaginalis* could be genetically determined and assessed by the use of genetic markers.

In this context, few reports have investigated the genetic relatedness of *T. vaginalis* isolates to its correlation with their phenotypic or clinical appearance and some of them present conflicting results. Isolates of *T. vaginalis* from symptomatic and asymptomatic patients could be grouped based on their zymodeme pattern (Proctor et al., 1988; Vohra et al., 1991). No concordance between genetic relatedness and metronidazole resistance or geographic origin was found when subtyping isolates of *T. vaginalis* by restriction fragment length polymorphism (RFLP) within the HSP 70 gene (Stiles et al., 2000). In contrast, Snipes et al. (2000) and Vanacova et al. (1997) have found correlation of random amplified polymorphic DNA (RAPD) patterns with metronidazole susceptibility in *T. vaginalis*. Although using the RAPD technique, the work done by Hampl et al. (2001) and Vanacova et al. (1997) partially contradicted each other, concerning the correlation between the presence of *T. vaginalis* RNA virus or geographic origin with the genetic relatedness of the isolates. Interestingly, these works concur with an indicative correlation of the genetic relatedness of the isolates and virulence level, experienced in animal models (Vanacova et al., 1997; Hampl et al., 2001). The ribosomal region has also been used to investigate the molecular epidemiology of trichomoniasis. Only one mutation was observed in the internal transcribed spacer (ITS), but critically related to metronidazole resistance in *T. vaginalis* (Snipes et al., 2000).

Although the ribosomal intergenic spacer (IGS) does represent a variable region to explore intraspecific genetic variation, it has not been used in *T. vaginalis* yet. Here, we have developed primers to amplify the IGS of *T. vaginalis*. The result of IGS–RFLP pattern was crossed with the clinical appearance and symptoms of 60 *T. vaginalis* infected patients.

2. Methods

2.1. Tricomonal isolates and DNA extraction

Clinical isolates of *T. vaginalis* were obtained in a previous study of diagnosis performance with a randomized population of women attended by gynecology clinics of the public health centers in Brasilia (Regional Norte-HRAN), capital of Brazil (Lobo et al., 2003). Parasites were then cultivated for three days in TV-Pouch (Biomed Diagnosis) following two months in Diamond's modified TYM medium supplemented with bovine fetal serum (10%), and with penicillin G (2000 U/ml) and streptomycin sulfate (2 mg/ml), and amphotericin B (25 μg/ml) at 37 °C. However, for this study, we used DNA samples obtained from vaginal swabs since not all positives samples resulted in growth. The extraction was performed by the use of the GFX genomic extraction kit, according to the manufacturer's recommendations (Amersham). Qualitative and quantitative analysis of the DNA samples were performed by electrophoresis in Tris–borate–EDTA buffer and agarose gels stained with ethidium bromide, as described elsewhere (Sambrook et al., 2001).

2.2. PCR–RFLP

A pair of oligonucleotides (IGSf2 and IGSr2) were developed for the amplification of the ribosomal IGS of *T. vaginalis*. The IGS corresponds to the region between the 28S rDNA and 18S rDNA in eukaryotes (Fig. 1), and it is supposed to be very polymorphic. Primers IGSf2 and IGSr2 were designed based on the sequences that are deposited in GenBank (Accession Nos. AF202181 for 28S rDNA and U17510 for 18S rDNA) and thermodynamic parameters were used as default by Primer3 (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi). The sequence of the oligonucleotides IGSf2 and IGSr2 are, respectively: 5′-CAC GTG ATG TTG GAC CGA TA-3′; 5′-CAA TCG CAT GTA TTA GCA CCA-3′ (Fig. 1).

The procedure for PCR amplification was standardized. It included 1 min 30 s of pre-denaturation, followed by 40 cycles each consisting of 1 min of denaturation, 1 min of annealing, and 1 min 30 s of extension. A final

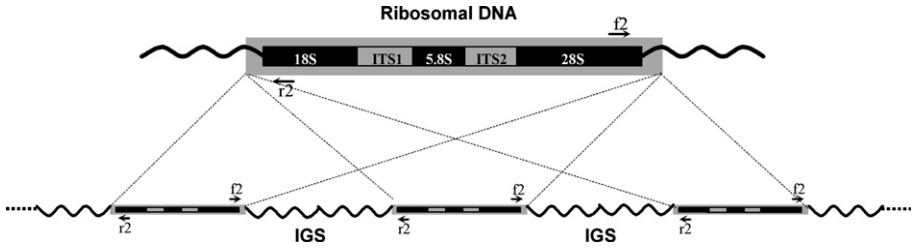

Fig. 1. A schematic representation of the eukaryotic ribosomal genes. The ribosomal genes 18S, 5.8S, and 28S are separated by the internal transcribed spacers, ITS 1 and ITS 2. Hundreds of copies of these rDNA units are dispersed in tandem repeats and interspersed by a non-transcribed IGS. Oligonucleotides IGSf2 and IGSr2, indicated by arrows, anneal at 3′ and 5′ of 28S and 18S, respectively, resulting in the amplification of the IGS region.