Calmodulin Intergenic Spacer: A Useful Marker for the Identification and Characterization of *Leishmania* Species

A. Miranda[1], J.E. Calzada[1], A. Saldaña[1], F. Samudio[1,2], J. Castillo[1], S. Puga[1], H. Paz[1], J. Pascale[1] , A. Brandao[2]

[1]Instituto Conmemorativo Gorgas para Estudios de la Salud- Panamá

[2]Instituto Oswaldo Cruz- Brasil

INTRODUCTION

Calmodulin is a protein that modulates the interaction of calcium ion with several other proteins in various cellular activities and is a key gene in the metabolism of the cell. (1). DNA coding region of calmodulin is very conserved but mutations of calmodulin intergenic spacer has been recently demonstrated to be specific for the major lineages of *T. cruzi,* a related protozoa (2). We have analyzed the calmodulin intergenic spacer in *Leishmania* spp., and proved that it is a useful marker for the specific identification of *Leishmania* species.

METHODS

DNA:

1.Reference strains

Identification	Strain	International Reference
2306	*L.(V.) panamenisis*	(MHOM/PA/98/WR2306)
566	*L.(V.) braziliensis*	(MHOM/BR/1975/M2903)
2355	*L.(V.) braziliensis*	(MHOM/PA/02/WR2355)
565	*L.(V.) guyanensis*	(MHOM/BR/1975/M4147)
2771	*L.(V.) peruviana*	(MHOM/PE/05/WR2771)
1023	*L.(V.) lainsoni*	(MHOM/BR/1981/M6426)
561	*L.(L.) mexicana*	(MHOM/BZ/1982/BEL21)
575	*L.(L.) amazonensis*	(IFLA/BR/1967/PH8)
579	*L.(L.) chagasi*	(MHOM/BR/1974/PP75)

2. Ten isolates from Panamanian patients

PCR and Sequencing: Primers 3utrcal and 5utrcal were designed to amplify the calmodulin intergenic spacer (2).
PCR products were cloned and transformed for sequencing.

RESULTS

- All *Leishmania* species amplified a product of an approximate size 1.2 Kb, which corresponds to one intergenic spacer (Fig.2).
- Depending on the *Leishmania* species, calmodulin gen has two or three copies in the genome, separated by the calmodulin intergenic spacer containing both Untranslated Regions (3' UTR and 5'UTR) and an intergenic segment (Fig.3).
- Sequences of the amplified region revealed that the reference strains present several mutations which provide a clear distinction between them (Fig 4 and 5).
- Panamanian isolates from patients showed homology with the *L. panamensis* reference sequence. Interestingly, an intraspecific mutation consisting of cytosine repetitions (8 to 11) was observed in these samples. The implications of this mutation will be further analyzed.

DISCUSION

Accurate identification of *Leishmania* species is important for monitoring clinical outcome, adequately targeting treatment, and evaluation of epidemiological surveillance (3). In Panama, the main etiologic agent is *L. panamensis,* although sporadic cases by *L. amazonensis* have been reported (4). Panama provides a biologic corridor for the entrance of pathogens and reservoirs of infections from Central and South America. In this sense, the etiologic agent of visceral leishmaniasis (*L. chagasi*) has never been detected in the country, but has been reported in neighboring countries. Furthermore, its main natural vector (*Lutzomya longipalpis*) and potential reservoirs are present in Panama (5). Our results indicate that calmodulin intergenic spacer is a useful molecular marker to genotype *Leishmania* strains to the species level. The major genetic variations were observed in the UTR´s particularly in the 3' UTR, which could affect the differential expression of calmodulin and consequently influence *Leishmania* phenotype.

Figure 1. Leishmaniasis lesions in Panamanian patients

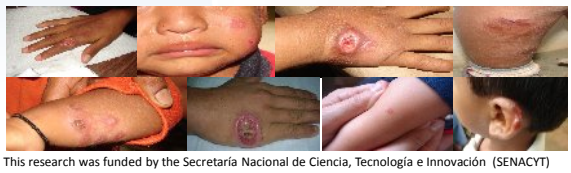

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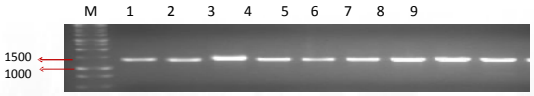
Figure 2. PCR of the *Leishmania* calmodulin intergenic spacer



1: *L. amazonensis*; 2: *L. mexicana*; 3: *L. chagasi*; 4: *L. braziliensis* (566); 5: *L. guyanensis*; 6: *L. panamenisis* ; 7: *L. peruviana*; 8: *L. braziliensis* (2355); 9:*L. lainsoni*; M: 1Kb

Figure 3. Genomic organization of the calmodulin gene in *Leishmania*

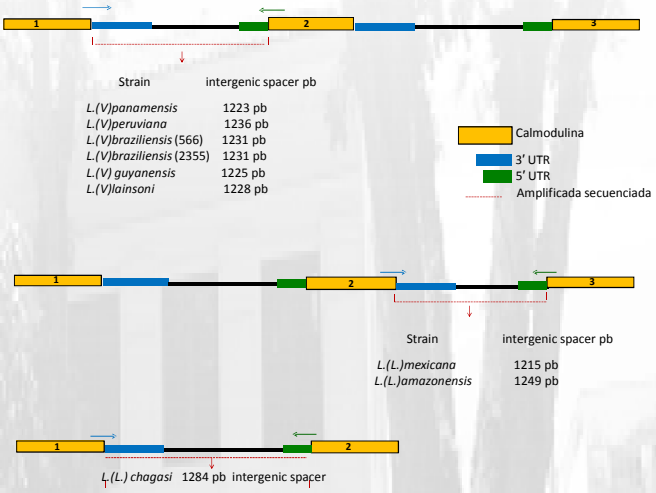


Figure 4. Specific mutations in *Leishmania Viannia* strains

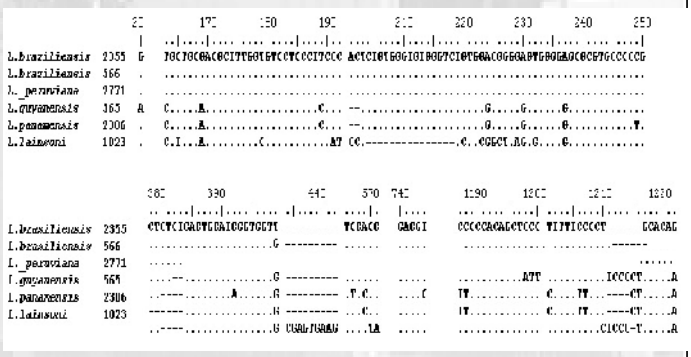
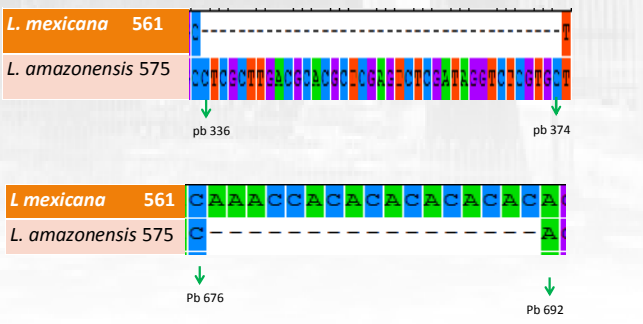


Figure 5. Mutations observed in *(L.) mexicana* y *L. (L.) amazonensis*



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