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COMPARATIVE STUDY OF RANDOM AMPLIFIED POLYMORPHIC DNA OF *TRYPANOSOMA CRUZI* ISOLATES FROM PARAGUAY

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Abstract: The variation of Paraguayan *Trypanosoma cruzi* isolates was examined using the unweighted pair group method analysis (UPGMA) based on the resulting matrix of the Dice similarity coefficients of random amplified polymorphic DNA (RAPD) profiles. These isolates were also compared with the established strains isolated from Brazil, Chile and Colombia in South America. Of 9 Paraguayan isolates, 3 isolates were similar to the Berenice, Y and Sao Felipe strains (zymodeme type; Brazilian Z2), respectively, isolated from Brazil, and 4 strains were similar to the Tulahuen strain from Chile. There was no isolate similar to the Colombiana I strain (Z1) from Colombia.

Key words: *Trypanosoma cruzi*, Chagas' disease, Paraguay, random amplified polymorphic DNA (RAPD), unweighted pair group method analysis (UPGMA)

INTRODUCTION

Chagas' disease caused by *Trypanosoma cruzi*, is widely distributed in South and Central America and represents a considerable health problem. In Paraguay, 20-30% of the population is infected in endemic areas (Rojas de Arias *et al.*, 1984), where *Triatoma infestans* is a responsible vector for the domestic transmission of *T. cruzi* (Canese, 1978). In Paraguay, about 30% of the patients infected with *T. cruzi* have cardiac symptoms, but digestive symptoms are rare (Canese *et al.*, 1972). The multiform symptoms of Chagas' disease might be caused by genetic differences in *T. cruzi* strains (Miles *et al.*, 1981).

The classification and identification of *T. cruzi* strains has mainly depended on by zymodeme analysis (Ebert, 1982; Miles and Cibulskis, 1986; Higo *et al.*, 1997). Schizodeme analyses have been adopted for classification of *T. cruzi* strains using kinetoplast DNA restriction fragment length polymorphism (Carreno *et al.*, 1987; Mimori *et al.*, 1992) and polymerase chain reaction (PCR) methods (Avila *et al.*, 1990). Recently, random amplified polymorphic DNA (RAPD) analysis using arbitrarily-primed polymerase chain reaction (AP- PCR) have been also adopted for classification of *T. cruzi* strains because of its sentitivity and besides the point that it does not require sequence information for primer design, and recommended the RAPD analysis method as a useful tool for the study of parasite populations (Steindel *et al.*, 1993; Brisse *et al.*, 1998; Gomes *et*

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al., 1998).

In the present study, we conducted an RAPD analysis on *T. cruzi* isolates from different endemic areas of Paraguay and compared them with established strains from Brazil, Chile and Colombia using the Dice similarity coefficient.

Materials and Methods

Parasites:

Seven *T. cruzi* isolates were from Chagas' disease patients, 3 males and 4 females, and two isolates were from vector insects in endemic areas of Paraguay, South America. Three strains were isolated from patients of the acute Chagas' disease with Romana's sign or oedema; JAG strain from Ita Moroti in Department of Paraguari, MG from Ygatimi in Canindeyu and RF from Altos in Cordillera. Two were from seropositive (IgG) patients without the symptoms of the disease; DG from Paraguari in Paraguari and LO from Tobati in Cordillera. The other patients have chronic chagasic cardiopathy; CIR from Quiindy in Paraguari and ARM from Villeta in Central. Two strains were isolated from *Triatoma infestans*, 201 and 204, captured in two houses of Ypahu in Paraguari, which is a highly endemic area of Chagas' disease (Table 1). Five established *T. cruzi* strains, i.e., Colombiana I (Z1 by zymodeme classification) from Colombia, Y, Berenice (Brazilian Z2) and Sao Felipe (Brazilian Z2) from Brazil, and Tulahuen from Chile, were used for comparison with the Paraguayan strains. The *T. rangeli, Leishmania amazonensis, L. guyanensis* and *L. mexicana* were also examined for comparison.

DNA preparation:

T. cruzi epimastigotes were cultured in a Schneider's medium with 15% fetal bovine serum at 26°C and washed with physiological saline, then the parasite pellets were suspended in SE buffer (0.15 M NaCl, 0.1 M EDTA, pH 8.0), lysed with sarkosyl and digested with proteinase K at 60°C. The kinetoplast DNA-enriched materials were collected by centrifugation (16,000 rpm for 90 min at 4°C), extracted by phenol/chloroform and precipitated by ethanol (Mimori *et al.*, 1992). These DNAs were used as the templates for AP-PCR.

PCR amplification and polyacrylamide gel electrophoresis:

The protocol of AP-PCR was based on our method for *Leishmania* parasites (Mimori *et al.*, 1998). Each amplification reaction was done in a final volume of 15 μl reaction solution containing 200 μM each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 30 ng template DNA, 150 ng primer, 0.3 Units r*Taq* DNA polymerase (Takara), and was performed on a GeneAmp PCR System 2400 (Perkin Elmer). The following 6 primers were tested for the amplification of *Trypanosoma* DNA: FL-S (5'-GCCATGCAGCAGCCCTTCAATTAC-3'), M-13 (5'-GTAAAACGACGGCCAGT-3'), LS-3 (5'-AAGTGTTGATACCCACTTTGT-3') FL-AS (5'-GTGCTTCTCTTAGAGCTTATATAAGCC-3'), PDR2-S (5'-CTGGTAAGTTTAGTCTTTTTGTC-3') and PDR2-AS (5'-GTGCCAAGCTTGCATGCCTG-3'). The reaction mixture was subjected at 94°C for 5 min for the initial denaturation, and was subjected to the initial 10 cycles though the following profiles: 94°C for 30 sec for denaturation, 37°C for 30 sec for annealing and 72°C for 30 sec for extension, followed by 30 cycles where the annealing step was altered to 60°C. Finally, the mixture was subjected to 72°C for 7 min, and stored at 4°C. Each 7 μl of PCR product were analyzed by electrophoresis in 6% polyacrylamide gels

Table 1 Paraguayan *Trypanosoma cruzi* isolates used in this study

Isolate	Host (Age and sex)	Phase	Locality
RF	Man (7, M)	Acute	Altos (Cordillera)
JAG	Man (12, M)	Acute	Ita Moroti (Paraguari)
MG	Man (41, M)	Acute	Ygatimi (Canindeyu)
DG	Man (45, F)	Indeterminate	Paraguari (Paraguari)
LO	Man (15, F)	Indeterminate	Tobati (Cordillera)
ARM	Man (59, F)	Chronic cardiopathy	Villeta (Central)
CIR	Man (65, F)	Chronic cardiopathy	Quiindy (Paraguari)
	Vector		
201	*Triatoma infestans*		Ypahu (Paraguari)
204	*Triatoma infestans*		Ypahu (Paraguari)

at 100 V for 1 hr. The gels were stained by the silver nitrate staining.

Data analysis:

Phenetic trees based on the Dice similarity coefficient (Dice, 1945) between all possible pairs in an analysis group were constructed. The similarity coefficient between an isolate 1 and an isolate 2 was calculated in the following formula. The similarity coefficient $S=2a/2a+b+c$; a=the number of bands present in both isolates, b=the number of bands present in an isolate 1 and absent an isolate 2, and c=the number of bands present in an isolate 2 and absent in an isolate 1. These data were compared by means of an UPGMA. The phenon line, which was marked on the phenetic tree of UPGMA, is the average of the similarities among all possible

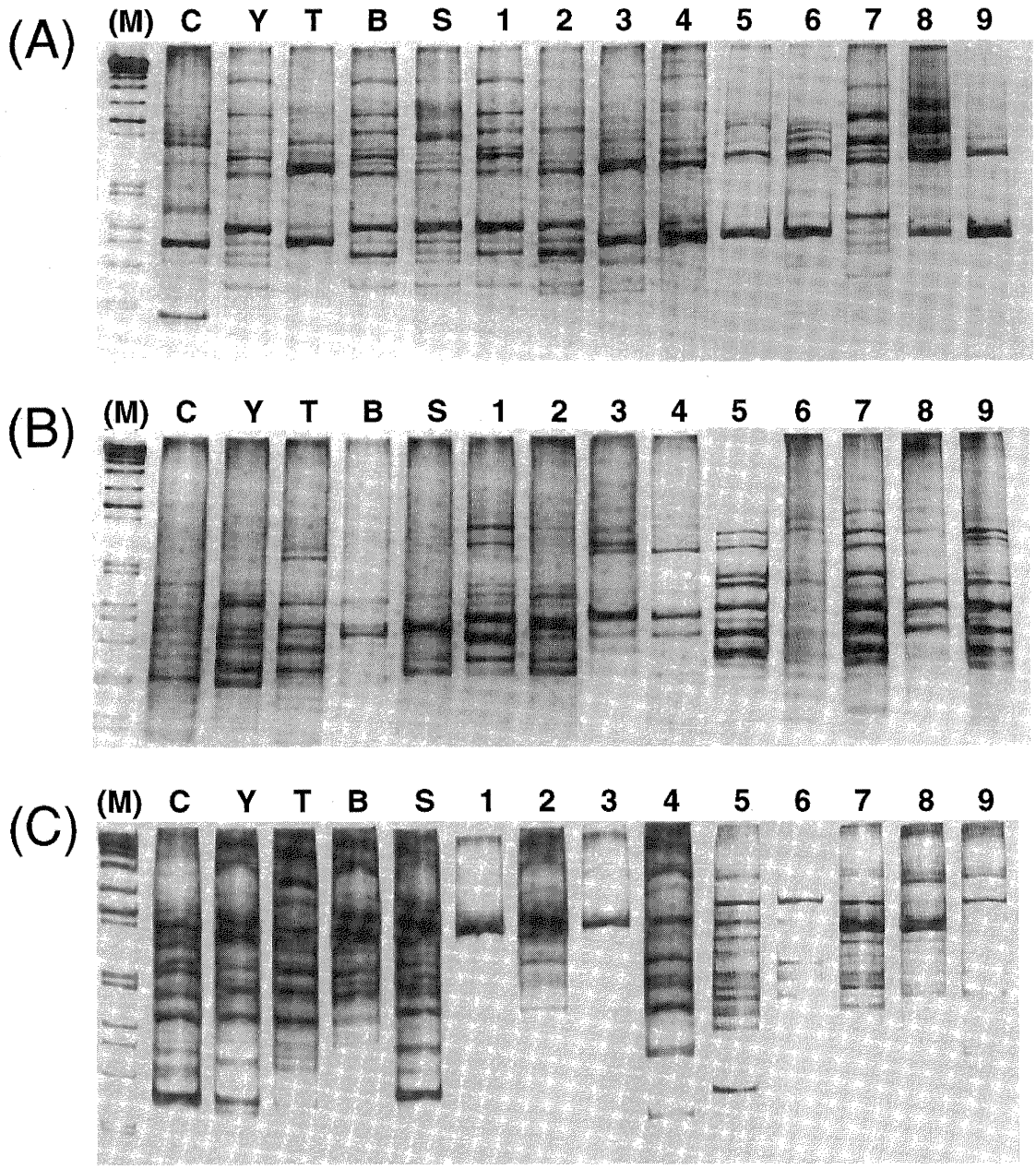

Figure 1 RAPD products of established and Paraguayan *Trypanosoma cruzi* strains using arbitrary primers FL-S(A), M-13(B) and LS-3(C) separated on 6% polyacrylamide gels stained with silver. (M); 1 kb ladder. C; Colombiana I, Y; Y strain, T; Tulahuen, B; Berenice, S; Sao Felipe, 1; CIR, 2; JAG, 3; RF, 4; MG, 5; DG, 6; LO, 7; ARM, 8; 201 and 9; 204.

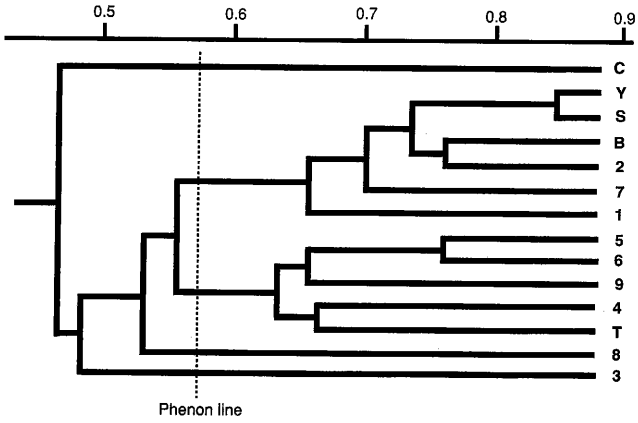

Figure 2 UPGMA dendrogram of the established and Paraguayan *T. cruzi* strains based on the Dice similarity coefficients of RAPD profiles (average data of the matrix with the primers FL-S, M-13 and LS-3). The vertical bar indicates the phenon line. C; Colombiana I, Y; Y strain, T; Tulahuen, B; Berenice, S; Sao Felipe, 1; CIR, 2; JAG, 3; RF, 4; MG, 5; DG, 6; LO, 7; ARM, 8; 201 and 9; 204.

pairs, and indicates separation among groups (Steindel *et al.*, 1993; Vidigal *et al.*, 1994).

Results and Discussion

The random amplified polymorphic DNA (RAPD) patterns of AP-PCR were compared among Paraguayan isolates and established strains of *T. cruzi, T. rangeli* and *Leishmania* strains. Six arbitrary primers were tested for the amplification of parasite DNA, and three primers (FL-S, M-13 and LS-3) were found to give the clear consistent DNA fingerprinting patterns for the strain-comparison as shown in Figure 1. The Colombiana I strain had a DNA profile very different from those of the others. The Y, Berenice and Sao Felipe strains were similar to each other. The DNA profile of the Tulahuen were slightly different from the others. There was a similar tendency of the division of Paraguayan and established strains among RAPD profiles with each primer. The DNA profiles patterns of *T. rangeli* and 3 *Leishmania* strains were completely different from those of *T. cruzi* strains (data not shown). As well as the zymodeme analysis, RAPD was successfully used for classification of *T. cruzi* variants (Steindel *et al.*, 1993; Brisse *et al.*, 1998; Gomes *et al.*, 1998). Moreover, it was indicated that RAPD analyses might make a major contribution to the study of the genetics of the snail, *Biomphalaria glabrata*, an intermediate host for Schistosomiasis (Vidigal *et al.*, 1994). Our conditions of PCR method and arbitrary primers were different from those of previous research, however, we were also able to construct the appropriate grouping of *T. cruzi*. This method is an useful technique for the classification of *T. cruzi* strains without requiring much amount of DNA.

Dice similarity coefficients between two strains were calculated according to the method described in materials and methods. The construction of the UPGMA dendrogram of Paraguayan and established strains was based on the average data of the matrix of Dice similarity coefficients in the RAPD profiles with 3 primers. These strains of *T. cruzi* were divided into 5 distinct groups as indicated by the position of the phenon line. Of 9 Paraguayan isolates, 3 isolates (CIR, JAG and ARM) were similar to the Berenice, Y and Sao Felipe strains isolated from Brazil (zymodeme type; Brazilian Z2), respectively, and 4 strains (DG, 204, LO and MG) were similar to the Tulahuen strain from Chile. There was no Paraguayan isolate similar to Colombiana I (Z1) from Colombia in this study. The classification by present RAPD profiles were also paralleled to analyses of our restriction enzyme analysis previously reported (Mimori *et al.*, 1992) and those of zymodeme analyses (Ebert, 1982; Higo *et al.*, 1997). We were able to construct the appropriate grouping of *T. cruzi*. The RAPD profiles were easy and convenient as a classification of *T. cruzi* organisms.

It is interesting that the strains in the same groups; 3 strains (CIR, JAG and ARM) and 3 strains (DG, 204 and LO) were isolated from near endemic areas, respectively. The ubiquity observed in the strains studied in the work, as well as in other investigations has been explained by the clonality criteria used mainly in studies employing zymodeme techniques (Tibayrenc *et al.*, 1984). The fixed heterozygosity observed in several loci over time and in distant geographical areas is due to the absence of meiotic segregation in *T. cruzi* populations (Tibayrenc and Ayala, 1988; Lewicka *et al.*, 1995). In this study, we found no correlation between the pathogenicity and the DNA profiles of *T. cruzi* strains. A further detailed analysis for more different strains might clarify this correlation in this country.

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