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Research brief

Trypanosoma cruzi: Two genetic groups in Paraná state, Southern Brazil

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Abstract

This paper presents the genetic characterization of *Trypanosoma cruzi* strains isolated from chronic chagasic patients, triatomines, and sylvatic reservoirs from Paraná state, Southern Brazil, using the RAPD and SSR-PCR techniques. It has shown the presence of both phylogenetic groups of *T. cruzi* (I and II), describing for the first time the existence of *T. cruzi* II in Paraná state.

Keywords: *Trypanosoma cruzi*; RAPD; SSR-PCR; Paraná state

Trypanosoma cruzi, the etiologic agent of Chagas' disease, is widely distributed in the American continents, occurring from the South of the United States to the South of Argentina. It circulates among humans, vectors, and sylvatic and domestic reservoirs. The interaction of the parasite with natural reservoirs and triatomine bugs is known as the sylvatic transmission cycle and the interaction with humans and domestic mammals as the domestic transmission cycle. The increase in the latter is attributed to ecological, social, and economic conditions, which have allowed contact between poor rural people and the sylvatic cycle. Recently, Aufderheide et al. (2004) reported results of molecular studies showing that humans were a link within the sylvatic cycle. In Latin America, around 15 million people are infected by the parasite (WHO, World Health Organization) and in Brazil, 3.5 million (Dias, 1997). Paraná state has been considered the fourth most endemic area in Brazil, with an estimate of 166,511 infected people (Silveira and Resende, 1994). In Paraná, the prevalence of chagasic infection is 4% among rural people (Camargo et al., 1984). In the north and northwest of the state, *T. cruzi* strains were isolated from chronic chagasic patients, from sylvatic reservoirs and from triatomines captured in the peridomestic environment. Strains isolated from chronic chagasic patients showed a homogeneous biological behavior in mice (Araújo et al., 1999), a drug susceptibility gradient that varied from 0 to 100% (Toledo et al., 1997) and a well correlated genetic grouping (Gomes et al., 1998). Furthermore, *T. cruzi* strains isolated from triatomines captured in the same region displayed low virulence in mice (Cardoso et al., 2000). *T. cruzi* is a heterogeneous species consisting of several sub-populations and now is classified into two major groups, named *T. cruzi* I and *T. cruzi* II (Anonymous, 1999).

Random amplified polymorphic DNA (RAPD) and simple sequence repeat anchored polymerase chain reaction amplification (SSR-PCR) are techniques widely used in the study of the genetic variability of this parasite (Oliveira et al., 1997; Steindel et al., 1993). In this paper *T. cruzi* strains isolated from humans, triatomines, and sylvatic reservoirs from the north and northwest

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Paraná state, Brazil, were characterized using these techniques.

Table 1 shows the parasite strains, hosts, isolation methods, and the localities where they were isolated. For comparative analyses between these strains and the groups *T. cruzi* I and II, two reference stocks, Esmeraldo strain (*T. cruzi* II) and Sylvio clone (*T. cruzi* I) were used. The strain 150, a human isolate, was different from other strains in molecular, biochemical, and biological behavior (Gomes et al., 2003) and due to this fact it was also used as reference. *T. cruzi* strains were kept in LIT medium at 28 °C until they reached 1×10^9 cells/ml. The cells were washed by centrifugation in KRT (Krebs–Ringer-Tris) buffer and the cellular mass was stored at −20 °C before use. The DNA extraction and quantification was performed in accordance to Macedo et al. (1992).

Table 1
Trypanosoma cruzi strains isolated from chronic chagasic patients, sylvatic reservoirs, and triatomines from north and northwest Paraná state, Brazil

Strain	Host	Isolation method	Localities
G1	*Didelphis* sp.	Hemoculture	Sarandi
G2	*Didelphis* sp.	Hemoculture	Doutor Camargo
G3	*Didelphis* sp.	Hemoculture	Maringá
G249	*Didelphis* sp.	Hemoculture	Floresta
F3	*Triatoma sordida*	Xenoculture	Paiçandu
A21A	*T. sordida*	Inoculation in mice	Paiçandu
N914A	*T. sordida*	Xenoculture	Floresta
N120B	*Panstrongylus megistus*	Inoculation in mice	Doutor Camargo
2052	Human	Hemoculture	Miraselva
328	Human	Hemoculture	Congonhinhas
379	Human	Hemoculture	Londrina
399	Human	Hemoculture	Primeiro de Maio

RAPD amplification was carried out as described by Oliveira et al. (1997) using the primers M13F-40 "forward" (5′GTTTTCCCAGTCACGAC3′), L15996 (5′C TCCACCATTAGCACCCAAAGC3′), and λGT11-F (5′GACTCCTGGAGCCCG3′) in a thermocycler, MJ Research PTC-150, with a final volume of 10 μl. SSR-PCR amplification was carried out as described by Oliveira et al. (1997) with modifications proposed by Gomes et al. (1998), using the primer (CA)8RY (R = purine, Y = pyrimidine). The amplified products for both techniques were electrophoresed through a 4% nondenaturating polyacrylamide gel and silver stained.

The multiple band profiles of *T. cruzi* strains on polyacrylamide gel obtained by RAPD and SSR-PCR were visually scored and analyzed for polymorphism based on the presence and absence of bands. Distance matrixes between strains for RAPD data and for SSR-PCR data were obtained by the complement of Jaccard's similarity and clustering was analyzed with the algorithm UPGMA (Unweighted Pair Group Methods of Arithmetic Means), using the FreeTree program (Pavlicek et al., 1999). Bootstrap analyses were based on 10,000 resamplings.

Fig. 1A shows a representative result of the amplified band patterns with the primer M13F-40. Very similar band patterns were found in *T. cruzi* strains isolated from vectors and reservoirs, but these were very different from those in strains isolated from humans. Bands with about 670 and 900 bp were only seen in strains from vectors and reservoirs, while one band of about 950 bp was present only in strains isolated from humans. With this

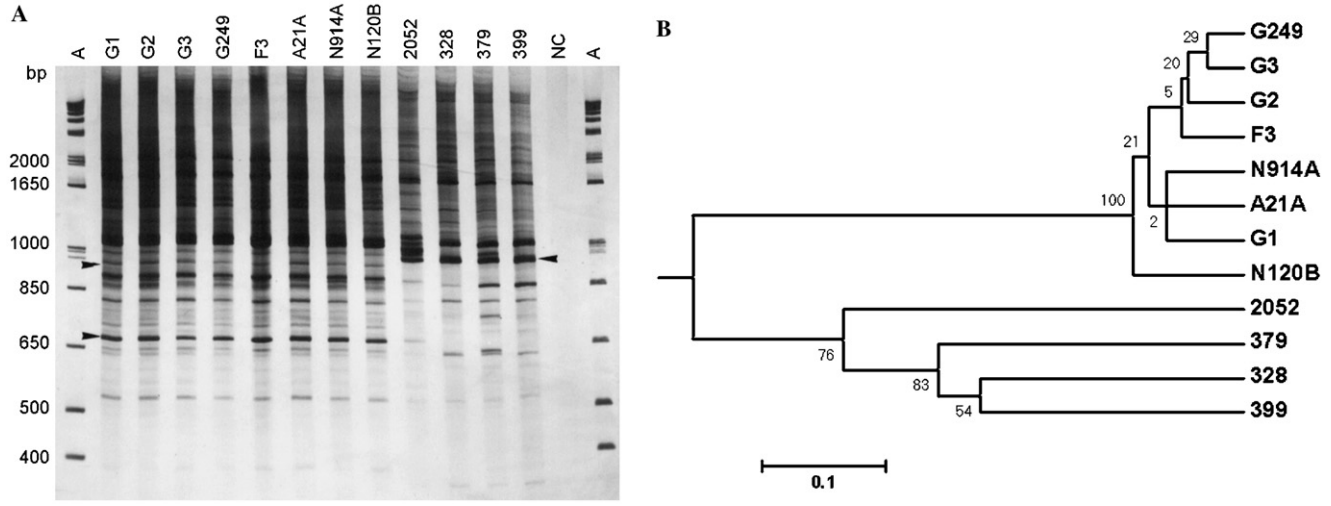

Fig. 1. (A) RAPD profiles of *T. cruzi* strains using the primer M13F-40 "forward." On the left and the right are indicated the molecular weight size markers (A) of some DNA fragments of 1 kb Plus DNA ladder scale (Gibco BRL). G1, G2, G3, and G249 represent the strains isolated from sylvatic reservoirs; F3, A21A, N914A, and N120B from triatomines; 2052, 328, 379, and 399 from humans. NC represents the negative control. (B) Phenogram of 12 *T. cruzi* strains, constructed by the UPGMA method based on the complement of Jaccard's similarity coefficient from SSR-PCR markers. The number on each node indicates bootstrap probability based on 10,000 resampling.