

ZYMODEMES OF *TRYPANOSOMA CRUZI* ISOLATED  
 FROM WILD MAMMALS IN BOLIVIA

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*Trypanosoma cruzi*, the etiological agent of Chagas' disease, is a very heterogeneous population of parasites circulating among man, vectors and wild and domestic reservoirs. Until now, few data have been reported about the *T. cruzi* isoenzymatic strains circulating in the Bolivian sylvatic reservoirs. In the present study we reported the isoenzyme typification of 9 *Trypanosomatidae* stocks isolated from wild Bolivian mammals.

The stocks were obtained from wild mammals caught in the Yungas and Alto Beni regions of Bolivia (North East from La Paz) by means of blood culture: 2 ml of sterile heart blood were introduced into 3 ml of LIT medium and the cultures amplified to a good concentration of parasites for the isoenzyme typification. These stocks were analyzed by isoenzyme electrophoresis carried out in cellulose acetate plates. The eleven enzyme systems assayed were as follows: glucose-6-phosphate dehydrogenase (EC 1.1.1.49)(G6P), glucose-6-phosphate isomerase (EC 5.3.1.9)(GPI), glutamate dehydrogenase (EC 1.4.1.2)(GD1), glutamate dehydrogenase (NADP+)(EC 1.4.1.4)(GD2), isocitrate dehydrogenase (NADP+)(EC 1.1.1.42)(ICD), malate dehydrogenase (EC 1.1.1.37)(MDH), malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) or malic enzyme (EC 1.1.1.40)(ME), peptidase 1 (ficin, EC 3.4.22.3, formerly EC 3.4.4.12, substrate: leucylleucylleucine)(PEP1), peptidase 2 (bromelain, EC 3.4.22.4, formerly EC 3.4.4.24, substrate: leucyl-L-alanine)(PEP2), phosphoglucomutase (EC 5.4.2.2, formerly EC 2.7.5.1)(PGM), and phosphogluconate dehydrogenase (EC 1.1.1.44)(6PGD). The stocks were classified according to their genetic distances (average number of codon differences between populations) (Nei, 1972, *Am. Nat.*, 106: 283-292). We used as reference samples: (a) the three zymodemes previously described by Miles

et al. (1980, *Trans. R. Soc. Trop. Med. Hyg.*, 74: 221-237) referred to as Z1, Z2 and Z3; (b) the Chilean Tulahuen strain.

Out of 9 stocks, 5 were identified as *Trypanosoma cruzi* (2 of them already reported, 3 of them close to two variants already reported). These 5 *T. cruzi* stocks were isolated from 4 *Didelphis marsupialis* (opossum) and 1 *Dasypus novemcinctus* (armadillo). Two interesting features should be emphasized regarding the enzyme profiles of the *T. cruzi* stocks: (a) 1 of them appeared identical to the Tulahuen strain, and also exhibited typical heterozygous pattern for the GPI, like the Tulahuen strain (Tibayrenc & Desjeux, 1983, *Trans. R. Soc. Trop. Med. Hyg.*, 77: 73-75). Until now such zymodeme, with typical heterozygous patterns, had been reported only in domestic transmission cycles (Tibayrenc & Miles, 1983, *Trans. R. Soc. Trop. Med. Hyg.*, 77: 76-83; Tibayrenc et al., 1984,

TABLE

Genetic distances among sylvatic Bolivian stocks and reference Brazilian zymodemes

Sylvatic strains*	Sylvatic strains*				Reference strains		
	Nº 20	Nº 43	NP 1	NP 2	Z1	Z2	Z3
Nº 20	—						
Nº 43	1.58	—					
NP 1	0.24	1.60	—				
NP 2	0.78	0.69	0.85	—			
References							
Z1	0.64	1.03	0.37	0.85	—		
Z2	1.34	0.84	1.36	1.26	1.65	—	
Z3	1.77	1.24	1.79	1.07	2.26	0.85	—

\* Sylvatic strains: Nº 20 and Nº 43, reference number from Tibayrenc classification (Tibayrenc et al., 1986, *Proc. Natl Acad. Sci. USA*, 83: 115-119), NP = New profiles.

ISOENZYMIC STRAINS*				
Enzymes	W. S	Tula	Z1	Z2
ME	■	■ ■	■ ■	■ ■
MDH	■	■ ■	■ ■	■ ■
PGM	■	■ ■	■	■
PEP 2	■	■	■	■
6PGD	■	■ ■ ■	■	■

\* W. S = Wild stocks unrelated to *T. cruzi*, Tula = Tula-huen *T. cruzi* reference strain, Z1 and Z2 = reference Brazilian *T. cruzi* strains.

Isoenzymic patterns of wild *Trypanosomatidae* stocks and *Trypanosoma cruzi* reference strains.

*Trans. R. Soc. Trop. Med. Hyg.*, 78: 519-525). (b) Even for this very limited sample, the distance values are spread over a continuum and show that the zymodemes recorded here are impossible to be clustered into few well-defined groups or "principal zymodemes": they exhibit a non-hierarchized structure (see Table), a result already noted for a more extensive set of stocks by Tibayrenc et al. (1986, *Proc. Natl Acad. Sci. USA*, 83: 115-117).

The 4 other stocks were not identified as *T. cruzi*. They shared the same isoenzyme profiles and were isolated from 1 *Bradypus tridactylus* (sloth) and 3 *Coendou prehensilis* (porcupine). The malic enzyme and the malate dehydrogenase enzyme systems did not reveal 2 loci as in *T. cruzi* and three other enzyme systems (PGM, 6PGD and PEP 2) presented new patterns never reported for the extensive set of stocks of *T. cruzi* studied by Tibayrenc et al. (1986, *Proc. Natl Acad. Sci. USA*, 83: 115-119) (see Fig.). The taxonomic status of these stocks is presently under analysis. Further details will be included in a more elaborated paper.

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