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Trypanosoma cruzi ISOENZYME PATTERN AS AN EPIDEMIOLOGICAL TOOL

SY-7

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Recently enormous interest has been shown in the fields of population and evolutionary genetics and systematic with the results obtained, using several techniques provided by the biochemical and molecular biology arsenal. These techniques allowed studies on the genetic variations and the similarities as well as differences among the organisms at the level of their enzymes, other proteins and DNA. The technique more largely used has been the enzyme electrophoresis that shows differences in the primary structure of enzymes. The enzyme electrophoresis allows that many samples and several characters be compared quick and easily, once each enzyme band on a gel represent one character and a same gel can be loaded with several samples. The different molecular forms (electrophoretic bands) of a same enzyme are called ISOENZYMES. The enzyme variations can be directly related to genetic variations and the electrophoretic patterns considered phenotypic expression. Two organisms will be closer as more identical characters they share. Therefore using enzyme electrophoresis one can characterize and classify organisms at a molecular level.

As much enzyme characters one can use more reliable is the organism identification. So far about 20 enzymes have been selected and used by different authors for *T. cruzi* identification. According to the purpose one can use different set size of enzyme, a small set for the purpose of testing genetic hypothesis or epidemiological features and a larger enzyme set for the purpose of ascertaining the extent of genetic variability and the taxonomic relationship among the variants.

Isoenzyme pattern was first used for *T. cruzi* characterization by TOYÉ (1974) who found variation among the isolates. Since then several authors have been using isoenzyme patterns in *T. cruzi* for different approaches. MILES et al. (1977, 78 and 81) studying *T. cruzi* samples from north and northeast Brasil found three principal zymodemes (a group of strains sharing the same isoenzyme pattern for a set of enzymes): Z1, Z2 and Z3. Z1 strains were related with sylvatic whereas Z2 strains were related with domestic transmission cycle. *T. cruzi* Z3 was isolated mainly from armadillos and from *Panstrongylus geniculatus*, a triatomine commonly found in armadillo burrows. All three zymodemes were found infecting man and cause acute disease but in the chronic phase Z2 was the one almost exclusively found. This predominance of *T. cruzi* Z2 in patients at the chronic phase has also been reported by several authors: ROMANHA at Bambuí-MG(1982), SCHLEMPER JR. at Virgem da Lapa and Iguatama-MG (1982) and LUQUETTI et al.- Goiás (1986).

A circumstantial relationship between clinical form of Chagas' disease and *T. cruzi* zymodeme was found by MILES et al. (1981). They observed the presence and predominance of *T. cruzi* Z2 in patients from east and central Brasil. In this region some chagasic patients are known to develop megaesophagus and megacolon. On the other hand they observed the absence of *T. cruzi* Z2 in Venezuela, where the patients do not develop either megaesophagus or megacolon. Suggesting therefore that the "megas" should be due to the presence of *T. cruzi* Z2 infecting man. Nevertheless BRENIÈRE et al. (1985) studying Bolivian chagasic patients found a weak or non-existent relationship between clinical forms of Chagas' disease and *T. cruzi* isoenzyme strains. The *T. cruzi* they found infecting Bolivian patients were isoenzymatically similar to MILES Z1 and Z2 found in Brasil (TIBAYRENC & MILES, 1983). Therefore the distinct transmission cycle described at São Felipe, Bahia (MILES et al., 1977) was not observed in Bolivia. On the other hand the transmission cycle versus zymodeme situation observed in Chile (APT et al., 1987) looks similar to Brasil, with sylvatic and domestic cycles transmitted by *T. cruzi* Z1 and Z2 respectively. What is more interesting is that in some Bolivian region like Chiwisivi 64 out of 66 *T. cruzi* domestic strains were from zymodeme Z1 (TIBAYRENC & DESJEUX, 1983), found in Brasil only in the sylvatic strains. Another evidence of non-relationship between clinical forms of Chagas' disease and *T. cruzi* zymodeme was found by ROMANHA (1982) in Bambuí, Minas Gerais. Although finding three *T. cruzi* zymodemes among chronic chagasic patients none was responsible for a specific clinical form. Further APT et al. (1987) studying in Chile 107 chronic chagasic patients found no correlation between *T. cruzi* zymodeme and the prevalence of chagasic cardiopathy.

In the majority of the papers the isoenzyme bands generated on gel are only visually compared for the different *T. cruzi* strains being therefore a qualitative procedure. One can turn this qualitative into quantitative procedure by using one of the similarity indices or NEI's genetic distance equations. The equations take into account the genetic expression or frequence of different alleles for the same locus given at the end a numerical index of similarity or genetic distance between two strains. By paired comparison the strain versus similarity index or the mean genetic distance can be plotted into a graphic called dendrogram, that gives a whole relationship among the strains studied.

A controversial aspect in the interpretation of the electrophoretic results is that concerned to speciation. What would be the genetic level of divergence between two related organisms sufficient to classify them as new species? There is not a specific criterion on the genetic variation for the definition of a new species. The consensus is that the speciation do not depend only from the variations on the different loci but also from the regulatory mechanisms change. Therefore a large number of loci change is apparently not necessary to give origin a new species (GOTTLIEB, 1971). *T. cruzi* presents a very peculiar

situation at the level of genetic divergence using NEI's genetic distance equation (MILES, 1983; TIBAYRENC & MILES, 1983; GALVAN et al., 1983; TIBAYRENC et al., 1984a, 1984b; MILES et al., 1984) and very high divergence values have been found between the zymodemes. According to the number of loci analysed and the enzymes used genetic distance values up to 2.62 for Z1 and Z3, 2.16 for Z1 and Z2 and 1.63 for Z2 and Z3 were found. Furthermore these values are considered likely to be an underestimation of the real figures. When the genetic distances are compared with those of well known organisms the level of divergence between *T. cruzi* zymodemes are above those found for species in other organisms. These data raise the question about the taxonomic status of *T. cruzi* zymodemes. They also lead immediately to the hypothesis of *T. cruzi* being a complex species or a single polytypic species (TIBAYRENC & MILES, 1983).

The presence of *T. cruzi* isoenzyme "heterozigous" patterns (TAIT, 1980; ROMANHA, 1982; CHAPMAN et al., 1984a; MILES et al., 1984; TIBAYRENC et al., 1984a, 1984b), added to molecular weight determination of some enzymes (JEREMIAH et al., 1982; CHAPMAN et al., 1984b) and DNA content determination (LANAR et al., 1981; LEMESRE & TIBAYRENC, 1983) suggest diploidy in *T. cruzi* in spite of the lack of gene exchange observed (TIBAYRENC et al., 1981a; 1981b and 1984a, 1984b) and the linkage disequilibrium (QIFA ZHANG, TIBAYRENC & AYALA, 1988).

Although some *T. cruzi* strains present hybrid or heterozigous isoenzyme patterns, characteristic of genetic recombination, the Hardy-Weinberg principle has not been fullfiled. The Hardy-Weinberg principle determines the genic and genotypic frequences of a diploid population in equilibrium. A population in equilibrium occurs when there is random mating, absence of mutation, selection and migratory flux. The deviation from the Hardy-Weinberg equilibrium by *T. cruzi* strains suggest that something is acting probably interfering on the random mating. MILES (1983) suggested that the deviation is due to a founder effect, i.e., *T. cruzi* strains are selected as they are propagated from house to house. Based on the presence of hybrid isoenzyme patterns and the Hardy-Weinberg principle deviation one should suggest that *T. cruzi* might have displayed sexuality in the past, a biological characterist lost in its evolution.

Genetic considerations using 524 *T. cruzi* strains from various hosts, representing a broad geographical range, has led TIBAYRENC & AYALA (1988) to propose a clonal structure for *T. cruzi*. They showed it is not possible to cluster the parasites into a few strictly delimited groups (zymodemes). It is suggested that long clonal evolution may explain the present biological and medical variability of *T. cruzi* due to the large range of isoenzyme patterns (genotypes) presented, in a nonhierarchical structure.

Isoenzyme patterns as well as k-DNA restriction patterns have also contributed for the detection of *T. cruzi* strains mixed infection, in patients, vectors and experimentally infected mice (ROMANHA, 1979; TIBAYRENC et al., 1987 and DEANE et al., 1984).

Further ROMANHA et al., in this meeting, showed that the current parasitological procedures do not select *T. cruzi* population from chronic chagasic patients as evaluated by isoenzyme patterns.

Although at present no one can be conclusive on a possible relationship between isoenzyme marker and any *T. cruzi* biological, epidemiological or clinical aspect, inquestionably several insights have been brought about by isoenzymes to a better knowledge of the parasite. May be the focus of the questions has been approach wrongly. Instead of considering a bulk of characters (zymodeme) one should consider individual characters (isoenzyme) and try to correlate them to any aspect.

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