BQ-69 TYPING OF TRYPANOSOMA CRUZI USING NUCLEAR DNA PROBES

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Different strains, isolates and clones of Trypanosoma cruzi, the ethiological agent of Chagas' disease have distinct biological and biochemical characteristics, such as morphology, virulence, tissue tropism, antigenic composition, clinical forms and pathology. It has been proposed that populations of this protozoan parasite may be genetically heterogeneous (DVORAK,J.Cell Bioch.24,1984). Some techniques have been developed to distinguish parasite strains. Miles (Parasit.Today,2,1986) has used isozyme data (zymodemes) to classify populations of T.cruzi and Morel and cols.(P.N.A.S.77,1980) characterized them by schizodeme analysis. Genetic relatedness among strains of various parasites was analysed through cloned DNA probes.

Our aim is to develop probes to be used as genetic markers to type initially different well characterized T.cruzi laboratory strains (CL,Col, Dm28, F and Y). A library of T.cruzi Y DNA was prepared in pBR322 and 30 clones were selected. We have tested some of these clones by hybridizing them to Southern blots of T.cruzi Y DNA digested with RsaI and EcoRI. Six clones that showed a band pattern with potential for discriminating among strains were used to differentiate the above mentioned laboratory strains.

Clones $pMYP_1 20$ and pMYP-7 gave rise to one single common band to all strains. Clone $pMYPR_2 42$ differentiated the Y strain from the others. Clone $pMYPR_1 22$ originated the same band pattern in strains CL/Y and Dm28/F, and a different pattern in strain Col (Colombiana). Clones $pMYPR_2 2a$ and pMYP16-20 are identical. They give rise to a complex band pattern that differentiates all strains.

We are presently testing the other clones and will start using them to type fiel isolates.

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BQ-70 DNA PROBES FOR THE IDENTIFICATION OF TRYPANOSOMES OF DOMESTICATED ANIMALS.

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Trypanosoma evansi is the causative agent of Surra, a serious disease of domesticated mammals. In bovines the infection is usually innocuous, the animal functioning as a parasite reservoir in nature.

Genomic DNA of T. evansi was extracted and totally digested with several restriction endonucleases. A large band with 23.3 kb range displayed no cleavage sites for most of restriction enzymes tested.

It was shown, by Southern hybridization, with total T. evansi DNA, that this band probably contained a repetitive DNA, which on further characterization revealed a repeat of 200 bp.

The total DNA probe was also utilized for species-specific assays, through dot-spot hybridization against other trypanosomatids. Results showed high specificity for T. evansi (when stringence conditions were used to select only repetitive DNA).

Cloning the repetitive DNA of T. evansi for further studies and better characterization are under way. It is hoped that the repetitive DNA probes can be used in field studies for diagnostic and epidemiological purposes.

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