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STUDY OF POLYMORPHISM AMONG *L. guyanensis* ISOLATES BY RFLP OF rRNA ITS GENE

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Leishmania guyanensis Floch, 1954 and its variants seems to be restricted to the Amazon basin, although it has been reported in other regions. It is of interest that the geographic distribution of this species is limited to the north of the Amazon river, and is the most common species found in this region. The *L. guyanensis* population is very homogeneous by its enzymatic profile with few enzymatic variants being observed in this species Cupolillo et al., 1994. *Am. J. Trop. Med. Hyg., 50:* 296-311). Even though the enzymatic profile of *L. guyanensis* population has shown almost no variation among the different isolates, serodeme and kDNA analysis have shown micro-heterogeneity in this population (Grimaldi et al., 1991. *Am. J. Trop. Med. Hyg., 44:* 645-661; unpublished data).

Ribosomal RNA genes are highly conserved and have proven useful in phylogenetic studies of distantly related trypanosomatids. Typically, eukaryotic rRNA genes are found as tandem repeat units separated by a non-transcribed spacer (NTS) region. The NTS evolves much more rapidly than the regions encoding the mature rRNAs and have been widely used in comparasions of more closely related species, including *Leishmania.* Similarly, the internal transcribed sequences (ITS) show extensive variability. They are relatively small and flanked by highly conserved segments to which PCR primers can be designed (Cupolillo et al., 1995. *Mol. Biochem. Parasitol., 73:* 145-155).

In attempt to study the amount of variability found among *L. guyanensis* isolates, we performed the PCR amplification of the ITS gene of about 30 samples. The PCR product were digested using different restriction enzymes and the profiles were observed in poliacrylamide gel. Some enzymes did not show any polymorphism and others a small amount of variability. Numerical analysis of these data will better evaluate the level of polymorphism found among *L. guyanensis* isolates, which will be compared with the level observed among other species belonging to the *Viannia* subgenus.

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MULTIPLE PRIMER RAPD ANALYSIS FOR STUDYING GENETIC DIVERSITY AND MOLECULAR TAXONOMY OF *LEISHMANIA SPP.*

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In order to study the genetic diversity of the *Leishmania* genus, Random Amplification of Polymorphic DNA (RAPD) was performed on a set of 18 *Leishmania* stocks representative of the main species of the genus. We tested on these sample one hundred decamer primers in parallel with samples of others microorganisms (*T. cruzi, T. congolense, Candida albicans, M. tuberculosis*). Several lines of results have been reached:

(i) Twenty three primers gave easily scoreable multiband profiles. The combined use of a fair set of these primers will make it possible to perform both, highly discriminative strain identification and population genetic analyses.

(ii) Some of them showed a synapomorphic specificity, that is to say: they can specifically identify given phylogenetic subdivisions, either at a subspecific or at a specific level. For example, one primer showed a profile specific of *Leishmania peruviana.* Other primers revealed different profiles between *L. guyanensis, L. braziliensis, L. lainsoni and L. chagasi .*

(iii) Lastly, some primers appeared to be linked with specific virulence patterns. Some of them, for example, seemed to be able to distinguish the stocks isolated from mucocutaneous forms from the stocks isolated from cutaneous forms for *L. braziliensis.* The great interest of the RAPD method in comparison with isoenzyme analysis is to allow the purification and sequence characterisation of those fragments which are of a specific interest, for designing probes and PCR diagnoses.

(iv) The comparison with other microorganisms studied in parallel with the same technique, gave a clear and reliable idea of the real divergence between *Leishmania* taxa. For example, the genetic diversity within the *braziliensis/guyanensis* complex is comparable to that obtained for the *M. tuberculosis* species, in the same way the genetic diversity of that the *Viannia* sub-genus and *T. cruzi cruzi* are comparable. The comparison between various microorganisms show clearly that the genetic divergence level for a same taxon is very different.

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