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Identification of Species and Strains of *Leishmania* by Using Different PCR-Based Methods

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Two different PCR-based methods were used to detect DNA polymorphisms in species and strains of the genus *Leishmania*. By amplifying genomic DNA with single non-specific primers distinctive and reproducible sets of amplification products were obtained for all *Leishmania* parasites tested. This PCR fingerprinting technique employed non-random primers which anneal to mini- and microsatellite sequences as well as primers derived from intergenic spacers for tRNA genes. The number and size of amplified DNA fragments were found to be characteristic for a given taxon. With all primers used the PCR profiles also displayed variability among isolates of a single species. By comparing PCR patterns of unidentified *Leishmania* isolates with those obtained from reference strains it was possible to identify these isolates at the species level [1].

The ribosomal internal transcribed spacer (ITS) is lying between the small subunit RNA gene and the large subunit RNA gene and occurs in nearly 200 copies in the genome of *Leishmania*. When amplified with *Leishmania* specific primers consistent size polymorphisms of the ITS regions were identified among several *Leishmania* species. By digesting the amplified ITS with different restriction enzymes varying fragment patterns were obtained for all taxa tested which could be applied to resolve main *Leishmania* complexes. The nucleotide sequences of the ITS region were almost species-specific and varied considerably between different *Leishmania* species. Only minor variations were found among strains of the same species.

A considerable degree of heterogeneity was observed among *L. tropica* and *L. aethiopica* strains. Clinical isolates of these two *Leishmania* species could be differentiated by their PCR fingerprints as well as by the RFLP patterns of the amplified ITS region. Some strains of *L. tropica* and *L. aethiopica* possessed more than one type of the ITS region [2, 3].

The information of the PCR fingerprints as well of the ITS sequences was used to construct phylogenetic trees to measure the genetic relatedness within the genus *Leishmania*.

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