Testing and Validating PCR-RFLP of Heat-shock Protein 70 Gene for Further Use as a Universal Tool for Leishmania Identification and for Replacing MLEE

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Abstract:

In an attempt to overcome the limitations of multilocus enzyme electrophoresis (MLEE) - gold standard tool for *Leishmania* identification - PCR-based methods have been developed and employed. Studies performed by our group (da Silva et al., 2010) and colleagues (Montalvo et al., 2010) have demonstrated that the target hsp70 differentiates many New and Old World *Leishmania* species through PCR-RFLP and DNA sequencing. These findings suggest that such approach might represent a universal and accurate tool for *Leishmania* species identification. Based on that, we aim to validate hsp70 PCR-RFLP as a substitute for MLEE for *Leishmania* typing. To construct an hsp70 PCR-RFLP panel we have used 18 reference strains, representing *Leishmania* species of the New and Old World. PCR conditions and primers were the same from a previous study (da Silva et al., 2010). The DNA was properly amplified and the 1400bp fragments observed in 1% agarose gels. Hsp70 PCR products were digested with HaeIII and MboI. Products were subjected to 12.5% acrylamide gel electrophoresis (Genephor) and silver stained. The combination of enzymes allowed differentiation of most species analyzed. Species from the *L. donovani* complex (*L. donovani* and *L. infantum*) could not be distinguished as well as those from the *L. mexicana* complex (*L. amazonensis* and *L. mexicana*). However, Montalvo et al., (2010) indicated MluI and RsaI to discriminate species from the *L. donovani* and *L. mexicana* complexes, respectively. Our results, combined with those obtained by other researchers, indicate that hsp70 PCR-RFLP could be used as a universal tool for *Leishmania* identification, replacing MLEE. However, more isolates for each species should be used to validate the hsp70 PCR-RFLP for *Leishmania* species identification. Advantages of hsp70 PCR-RFLP in comparison to MLEE are: (i) it can be applied to low cell density cultures; (ii) a standardized protocol can be developed and results can be easily compared among different laboratories; (iii) a panel of PCR-RFLP pattern can be built and disponibilized at internet; (iv) it can be used in cases where the parasite is isolated but cannot be cultivated, a common situation for *Leishmania*; (v) DNA sequencing could be employed if new RFLP profiles are observed.

Key words: Leishmania, Heat-shock protein 70, PCR-RFLP, MLEE