DIFFERENTIATION OF *CANDIDA ALBICANS* STRAINS BY MICROSATELLITE MULTIPLEX PCR GENOTYPING

P. Sampaio,[1] L. Gusmão[2], C. Alves[2], A. Amorim[2,3], and C. P ais[1*]

[1]Centro de Biologia da Universidade do Minho, Departamento de Biologia, 4710-057 Braga, Portugal
[2]Instituto de Patologia e Imunologia Molecular da Universidade do Porto, R. Roberto Frias, s/n 4200 Porto, Portugal
[3]Faculdade de Ciências, Universidade do Porto, Portugal

A search in the genome database of the pathogenic yeast *C. albicans* was conducted for sequences containing microsatellite repeats. Ten sequences, located outside and inside known coding regions, were selected and primeres were designed, in the nonvariable flanking regions, for locus-specific amplification. Based on the amplification efficiency, species specificity, and observed polymorphism, five sequences were selected for further characterization. The location of the selected *loci* was determined by PFGE followed by hybridization with specific probes. The results obtained showed that each microsatellite were assigned to a different chromossome indicating an even distribution throughout the genome. The polymorphism of these new microsatellite *loci* was investigated in order to evaluate their applicability to accurately differentiate strains. A multiplex PCR strategy was developed allowing the simultaneous screening of the five markers, followed by GenScan analysis of the products, providing a rapid and accurate methodology for genotyping large numbers of strains. A total of 122 *C. albicans* strains, including 80 independent clinical isolates and multiple isolates from the same patient were analysed using this multiplex system. Seventy-eight different genotypes were observed resulting in a discriminatory power of 0.98. When multiple isolates obtained from the same patient were studied the results showed that in different body sites, patients can harbour distinct clones but the infecting population at each body site is monoclonal. These new microsatellites proved to be a valuable tool to differentiate *C. albicans* strains and when compared to other molecular genotyping techniques, revealed to be simple, efficient and reproducible, being suitable for application in large scale epidemiological studies. Allele nomenclature based on the number of repeat sequences rather than fragment size is proposed for the characterization of each strain and contribute for the construction of a public database in light of what is already in use for other organisms.