Title: MOLECULAR DIAGNOSTIC MODEL OF CANDIDA SPECIES

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Abstract

Candida species are human opportunistic pathogens, especially when the host immune system is compromised. Aggressive manifestations, eventually, lead the patient to death. A fast and specific diagnosis is essential to the application of appropriate treatment, lower the risks and contribute for a better prognostic. Current diagnostic tests are expensive and costing-time, and frequently result in an incorrect identification at species level. The search for a fast and reliable identification methodology at species level led us to explore the PCR-RFLP based method. The amplification of ribosomal cistron expanding around 3000 bp and subsequent restriction with Dde I endonuclease has been reported as an efficient, fast and low cost alternative, showing a reliability equivalent to the sequencing. So far our group reported 33 species-specific patterns of the Candida gender, including all species of clinical interest. However, the dynamic of analysis of the restriction profiles is the bottle neck of the process. The aim of this study is explore the possibilities of a gel-scanning based system of analysis. At this stage the reference standards strains are being analysed in double-blind tests to confirm those previous patterns determined for each reference strains and also avoid problems such as contamination and misidentification. So far four standards were confirmed such Candida albicans, Candida pararugosa, Candida thermophila and Candida mesorugosa. The others 29 standards are in the phase of genomic DNA preparation, PCR and RFLP. Future prospects will be in the direction of creating image banks of species-specific restriction patterns and train image analysis programs for recognition of these standards. The last step of our identification process is turn the tool available on the internet to the worldwide access.

Keywords: PCR-RFLP, Candida spp, diagnosis, Molecular methods.

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