Abstract

The advent of HIV epidemic in recent years together with the modern medical practices have resulted in an increase in the size of the population of immunocomprosed individuals. Consequently, the infections caused by pathogenic fungi have increased rapidly and become major public health problem. Rapid and accurate identification of the pathogenic fungi to species level is critical for timely and proper treatment. Molecular typing is important in elucidating the transmission route. Both rapid diagnosis and molecular typing studies are important for consolidating control strategy.

In our previous research work, we have successfully developed rapid molecular diagnosis methods such as PCR, PCR-EIA and real-time PCR for rapid species identification of 6 *Candida spp.* and *Cryptococcus neoformans*. As a continuation of our previous effort, we have further developed a PCR-EIA method to differentiate 7 clinically frequently encountered *Candida spp.* based on their resistance characteristics to fluconazole. 3 set of primers were designed: one set is for fluconazole- susceptible species including *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida tropicalis*, one set is for *Candida glabrata* which developed resistance to fluconazole quite easily, one set is for *Candida krusei* which is innately resitance to fluconazole. Specificty of this methods were 100%. Sensitivity was less than 10fg/100µl of the fungal DNA. The identification results matched results of the phenotypical identification method employing germ tube, Viteck and API20C methods. We have also applied these molecular diagnosis methods on blood culture specimen. The method developed will be beneficial not only for clinical diagnosis but also for therapy regimen.

Reguarding molecular typing, we have developed 6 molecular typing methods, including 3 PFGE-based typing methods (PFGE-karyotyping, PFGE of *Sfi*I- and *Bss*HII), rep-PCR, MLST and AFLP methods and applied these methods to investigate the genetic profiles of *Candida albicans* clinical isolates. All these methods were able to identify clonal related isolates from the same patients. PFGE-*Bss*HII and MLST exhibited the highest discriminatory power, followed by PFGE-*Sfi*I, then by rep-PCR, then by AFLP, while PFGE-karyotyping demonstrated the lowest discriminatory power. High discriminatory power can also be achieved with a combination of typing methods with different typing mechanisms, such as rep-PCR supplementary to PFGE-based typing methods. The results also showed that the DNA type of each isolate was patient-specific and not associated with source of isolation, geographical origin or antifungal resistance. We have also applied both

PFGE-karyotyping and PFGE-*Sfi* I analysis methods to study the molecular epidemiology of long-term colonization of *C. albicans* strains from HIV-infected patients. Clonality analysis demonstrated that isolates from same patient sampled over a rather long span of time, despite of different susceptibility to fluconazole and reguardless of colonizing/infecting stage were genetically closely related. These methods will be compared and the most discriminatory typing method will be standardized and serve as the tool for future outbreak investigation and the basis for comparison basis of other typing methods. The data obtained in this study will also contribute to our attempt to establish a central genetic database of fungal pathogens and provide plateform for comparison of domestic as well as international fungal genotypes.

The publication output of this research is quite fruitful: two papers will be published in SCI journals, one paper has been submitted to SCI journal, and one article has been published in Taiwanese peer-reviewed journal, many manuscripts are in preparation and will be submitted to SCI journals.

Keywords：pathogenic fungi； rapid molecular diagnosis； species identification； molecular typing