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Serodiagnostic potential of immuno-PCR using mycobacterial antigen 85B, ESAT-6 and cord factor in pulmonary and extrapulmonary tuberculosis patients

Promod Mehta

Maharshi Dayanand University, India

Rapid and accurate diagnosis of tuberculosis (TB) is essential to control the disease. A novel immuno-polymerase chain reaction (immuno-PCR; I-PCR) assay was developed for the detection of mycobacterial antigen 85B (Ag85B, Rv1886c) and its antibodies in pulmonary TB (PTB) and extrapulmonary TB (EPTB) patients and the results were compared with an analogous enzyme-linked immunosorbent assay (ELISA). The amino modified reporter DNA was covalently attached with the antibody through a heterobifunctional cross linking agent succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate (SMCC). The detection limit of Ag85B by indirect sandwich I-PCR was found to be 1 fg/mL, which were 106-fold lower than ELISA. The sensitivities of 85% and 77% with I-PCR and 77.6% and 62.5% with ELISA were observed in smear positive and smear negative PTB cases, respectively with high specificity (86-90%). On the other hand, sensitivities of 84% and 63.7% with I-PCR and 68% and 47.5% with ELISA were observed in confirmed and clinically suspected EPTB cases, respectively with high specificity (92-96%). An indirect I-PCR was also developed for the detection of circulating anti-Ag85B, anti-ESAT-6 (early secretory antigenic target-6, Rv3875) and anti-cord factor (trehalose-6,6' dimycolate) antibodies from the sera samples of PTB and EPTB patients. The detection of cocktail of anti-Ag85B, anti-ESAT-6 and anti-cord factor antibodies was found to be superior to the detection of individual antibodies. Based on the detection of cocktail of antibodies, sensitivities of 89.5% and 77.5% with I-PCR and 70.8% and 65% with ELISA were observed in smear positive and smear negative PTB cases, respectively with high specificity; whereas a sensitivity of 77.5% with I-PCR and 65% with ELISA was observed in EPTB cases. The detection of mycobacterial Ag85B and cocktail of antibodies by I-PCR in the study is likely to improve the utility of existing algorithms for TB diagnosis.

pkmehta3@hotmail.com

Molecular detection and characterization of *Fusarium sporotrichioides* based on ITS2 rDNA polymorphism

Afaf I Shehata, Ekram A M Al-Sanae, Ali H Bahkali, Mohammed Abdo Yahya and Amal A Al Hazzani

King Saud University, KSA

The genus *Fusarium* contains a number of soil borne species with worldwide distribution. The presented PCR assays are highly selective and sensitive in detecting the *Fusarium* genus. In order to identify the 18 *Fusarium* isolates obtained at the molecular level, PCR analysis using primer specific for the conserved ITS DNA region of *Fusarium* genus was conducted. The data indicated that, all of the 18 isolates showed a clear band corresponding to the expected molecular size of the ITS region (431 bp). These results confirmed that all the tested samples belong to the genus *Fusarium*. Also, when all eighteen isolates of *Fusarium* species were analyzed by PCR for fumonisin producing ability using *FUM1* gene based primers, the expected DNA fragment of 183 bp was amplified only in *Fusarium verticillioides* (3 isolates), *Fusarium avenaceum* (3 isolates), *Fusarium semitectum* (1 isolate) and *Fusarium culmorum* (2 isolates) showed a positive result with *FUM1* gene set of primers. No bands were seen in other isolates of *Fusarium* spp., and the standard (*Fusarium graminearum*). In case of zearalenone, the *PKS4* gene of *F. graminearum* has been reported to be essential in the production of zearalenone. The result indicated that the expected DNA fragment of 280 bp was amplified only in *Fusarium verticillioides* (3 isolates), *Fusarium avenaceum* (3 isolates) and *Fusarium culmorum* (2 isolates) and *Fusarium graminearum*. Microsatellite-primed PCR resembles the well-known RAPD technique but is advantageous because of the ability to generate more complex banding patterns and a high degree of reproducibility. The discriminating powers of the three MP-primers [(CTG)₅, (M13) and (T3B)] used in this study were nearly the same. Cluster analyses were performed on the genomic fingerprints generated by each of the primers tested. Three dendrograms were generated with the UPGMA method. The patterns resulting from the T3B and (CTG)₅ test were more distinct and T3B was the most successful primer because it always led to high polymorphic banding patterns that were suitable for interspecies comparisons. Our results indicated that there was no association between clustering in the MP-PCR dendrogram and the geographic origin and morphological identification of the tested isolates.

afafsh@ksu.edu.sa