Characterization of mycobacterial isolates from clinical samples by using PCR-RFLP assay targeting hsp65 gene region

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ABSTRACT

Mycobacterial have long been recognized as important human and animal pathogens. Mycobacterial diseases such as tuberculosis and leprosy continue to be an important public health problem all over the world. Not only M.tuberculosis and M. leprae are important one, but mycobacteria other than tuberculosis (MOTT) are also pathogens. The immunosuppressed individual infection by HIV infection have become the most significant risk factor for disseminated NTM diseases and of these 95% are due to Mycobacterial avium complex. Thus the rapid and specific diagnosis of tuberculosis is one of most pressing needs in effort to temporary treat and control the disease. Classical identification result for mycobacteria based on culture and biochemical test may take several weeks and test fail sometimes to produce precise identification. PCR- restriction analysis (PRA) is a PCR-RFLP based, very efficient and promising approach for species identification as it gives result within one day. Several targets for PRA analysis of mycobacteria's are available which includes hsp 65kDa. The 65kDa proteins contains epitopes that are unique as well as epitopes that are common to various species of mycobacteria's.PRA is based on amplification of 439bp fragment of hsp65 gene present in all mycobacteria offers an easy rapid an inexpensive procedure to identify several mycobacterial species in single experiment. Thus the current work emphasis on quick, advanced and robust methodology for diagnosis of tuberculosis and other mycobacterial diseases.

Keywords: NTM, hsp, epitopes, PCR

I. INTRODUCTION

Mycobacterium, the genus of family of mycobacteriaceae includes 130 species till now(Euzbey 2008, Katoch 2007). Mycobacteria have long been recognized as important human and animal pathogens. Tuberculosis and leprosy are public health problems all over the world. It is believed that about one third of the world's population is infected with M. tuberculosis (Musser 1995). Tuberculosis has been called as White plague and caption of all the men of death(Ananthnarayan and Pannicker 2001).

Not only M. tuberculosis and M.leprae are important ones, but mycobacterium other than tuberculosis(MOTT) are also pathogens. The range of infection caused by MOTT or opportunistic mycobacteria is quite broad including skin infection(eg .M. marinum), cervical lymphadenitis(eg .M. avium), joint infection(eg. M. avium and M.intracellulare), bacteria in AIDS(eg M.avium) and nosocomnial infection (eg M. fortuitum and M. chelonae).

Various anti-tuberculosis drugs that are effective against infection caused by M.tuberculosis are available. These include Rifampicin, isoniazid,

pyrazinamid, ethambutol, streptomycin etc. In recent years the treatment of tuberculosis is threatened by increasing the number of patient with multi drug resistant tuberculosis especially to rifampicin and isoniazid(Paramsivan et al 1993,94)The rapid and specific diagnosis of mycobacteria is one of the pressing needs in effort to temporary treat and control the disease.

Clinical identification of bacteria based on cultural and biochemical test may take several weeks. Additional techniques such as thin layer chromatography (Marks and Szulga 1965) gas liquid chromatography(Levy Frebault et al 1983) are powerful tools but limited by the need for standardized growth conditions. Several gene probe and gene amplification techniques for detection and identification of pathogenic bacteria from culture as well as directly from the lesion have been reported(Katoch and Sharma 1997).

PCR-restriction analysis(PRA) is a PCR-RFLP based very efficient and promising approach for species identification as it gives result within one day. Several targets for PRA analysis of mycobacteria are available including hsp 65kDa(Telenti et al 1993), rRNA (Dobner et al 1996) 16S-23S rRNA spacer region(Katoch et al 2007).The hsp65 (439bp)gene coding for 65kDa heat shock protein by PCR and restriction enzyme analysis(RFLP)(Bunello et al 2001).The 65kDa protein contains epitopes that are unique as well as common to various species of mycobacteria. (Telenti et al 1993). PRA based on the amplification of 439bp fragment of the hsp65gene present in all mycobacteria offers an easy, rapid and inexpensive procedure to identify several Mycobacterial species in single experiment.(Devallosis er al 1997)

II. METHODS AND MATERIAL

Agarose ,Asparagine, Chloroform, Disodium phosphate EDTA, Ethidium bromide, Hydrochloric acid, Mac Conkey agar, Sodium dodecyl sulphate, Tris base, Lysozyme, Proteinase K Clinical sputum samples were collected and decontamination done by using 4%NaOH. Samples were cultured on LJ medium and incubated for 8 weeks at 37°C(Vestal 1977).Biochemical tests such as Catalase test, Nitrate reduction test, Tween 80 hydrolyis test was done to ensure Mycobacterium tuberculosis infection.DNA isolation(van Embden et al 1993) was done followed by amplification of DNA (Telenti et al 1993). Restriction of amplified DNA was done using HaeIII enzyme.

III. RESULTS AND DISCUSSION

In this study PCR RFLP analysis(PRA)of hsp 65 gene was applied to characterize 17 mycobacterial isolates from sputum samples of pulmonary cases.
The reference strains used were H37Rv(M. tuberculosis), N-2(M. fortuitum), J-7(M. avium), J-28(M. vaccae).

IV. CONCLUSION

The study aims to investigate the suitability of PRA technique targeting hsp 65 gene for the differentiation and identification of rapidly and slowly growing clinical mycobacteria. PCR-RFLP is a universal system of identifying mycobacteria at species and sub species level.Result analysis can be obtained in 1-2 days as compared with 2-3 weeks for biochemical tests.

V. REFERENCES

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