Journal of Bacteriology Research Vol. 2(1), pp. 005-008, February, 2010
Available online at http://www.academicjournals.org/jbr
ISSN 2006- 9871 © 2010 Academic Journals

Full Length Research Paper

Utility of IS*1245*-IS*1311* based PCR typing system for *Mycobacterium avium* isolates obtained from clinical and environmental sources

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Accepted 14 December, 2009

In the present study, twenty four *Mycobacterium avium* isolates obtained from clinical (sputum) and environmental (water and soil) samples were analyzed by a PCR typing method. Gel electrophoresis patterns showed that a single PCR reaction generated 21 profiles. Based on the relatedness of the PCR band patterns six isolates could be grouped in three clusters. Results have the implication that the PCR based system could be used as a simple and rapid method for molecular typing of *M. avium* isolates with high discriminatory index.

Key words: IS*1245*, IS*1311*, *Mycobacterium avium*, PCR typing.

INTRODUCTION

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental organisms. The important species of NTM associated with human disease are: *Mycobacterium avium*, *M. intracellulare*, *M. kansasii*, *M. marinum*, *M. ulcerans*, *M. scrofulaceum*, *M. smegmatis* etc. Water and soil have been reported as common reservoirs of NTM. These mycobacteria are naturally resistant to most of the anti-tuberculosis drugs but may be sensitive to some aminoglycosides, fluoroquinolones or new macrolides. Among the NTM, members of *M. avium* complex (which included two species *M. avium* and *M. intracellulare*) are present in all natural sources and can be isolated from water, soil, air, plants, house dust, food samples, and even from animals such as chickens and pigs (Inderlied et al., 1993). These organisms, particularly *M. avium* isolates, are the most important among the NTM that have assumed clinical prominence in the post-AIDS era (Katoch, 2004).Till date, no human-to-human transmission of *M. avium* has been established, and environmental sources of *M. avium* infections have been described (Inderlied et al., 1993; Katoch, 2004).

Isolation and identification of *M. avium* relies on methods used to detect bacilli as well as blood cultures. Strain typing methods are often employed for epidemiological investigations of bacterial infections. Various methods described for typing of *M. avium* strains include serotyping (Thoen et al., 1975), phage typing (Crawford and Bates, 1985), multilocus enzyme electrophoresis (Yarkus et al., 1992), pulsed-field gel electrophoresis (Pestel-Caron et al., 1999), restriction fragment length polymorphism and hybridization with specific probes (Guerrero et al., 1995), random amplified polymorphic DNA (RAPD) (Matsiota-Bernard et al., 1997) and PCR based typing (Picardeau and Vincent, 1996). Among the described methods for *M. avium* typing, pulsed-field gel electrophoresis is considered as the 'Gold Standard' (Yoder et al., 1999); however, the procedure is technically cumbersome and hence would not be useful for a laboratory with limited resources.

A PCR based typing method has been described by Picardeau and Vincent (1996) that targets two insertion elements present in the *M. avium* genome, namely IS*1245* and IS*1311*. This system is based on amplifica-

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tion of the DNA between closely spaced copies of these repeat elements. Thus, agarose gel electrophoresis of the PCR products gives a band pattern corresponding to the DNA fingerprint of the isolate. This method was proposed as a rapid technique for investigating small numbers of *M. avium* isolates and the results were comparable to PFGE (Pestel-Caron et al., 1999). In the present study we have investigated the utility of this PCR based system for molecular typing of *M. avium* isolates obtained from clinical and environmental sources.

MATERIALS AND METHODS

M. avium isolates

A total of 24 *M. avium* isolates and one reference *M. avium* strain (TMC-724) were taken from the collection of Mycobacterial Repository Centre of our Institute. Among the *M. avium* isolates included in this study eight were obtained from sputum samples (HIV status not known) collected in Delhi (n = 4), Chennai (n = 2), Jaipur (n = 1), and Lucknow (n = 1). Sixteen isolates were obtained from environmental sources (Parashar et al. (2004)). Environmental isolates included those obtained from water (n = 12) and soil (n = 4) samples collected in Agra. Isolates were maintained on LJ medium and freshly subcultured before being used for further investigations.

DNA isolation

Colonies were scraped from the LJ slant and suspended in 400 µl TE (10 mM Tris, 1 mM EDTA pH 8.0). Cells were killed by boiling in a water bath at 95°C for 20 min. DNA was purified as per a previously described procedure (van Embden et al., 1993). DNA was dissolved in TE and kept at -20°C until use. For parallel sets of PCR reactions, DNA was prepared from the isolates exactly as described by Picardeau and Vincent (1996). Briefly, colonies were taken in 50 µl of TE containing 1% Triton X-100 and boiled in a water bath for 30 min. Cells were pelleted and the supernatant was used as such in PCR without further purification.

IS*1245*-IS*1311* based PCR typing

Molecular typing based on PCR targeting IS*1245* and IS*1311* was done as described earlier (Picardeau and Vincent, 1996). Breifly, amplification reactions were performed with 50 µl volume containing 1X Taq polymerase buffer, 1.5 mM $MgCl_2$, 200 µM (each) dNTPs, 1 µM (each) primers (PA, 5´-CAGAGCCTCAGGCGA-3´, and PB, 5´-CAGAGCCTCACGCGGA-3´) and 2U of *Taq* polymerase. Two microlitre of template DNA was added to the reaction mix. The 35 PCR amplification cycles carried out in a GeneAmp® 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) were denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min with a final extension at 72°C for 10 min. Reactions were kept at 4°C until use PCR products were electrophoresed on 1.5% agarose gel (Bangalore Genei), stained with ethidium bromide and scanned by Gel Documentation System (Bio-Rad Laboratories, USA). The gel electrophoresis band patterns were analyzed and band sizes were calculated using the accompanying analysis software Quantity One. DNA of reference *M. avium* strain (TMC-724) was used with each set of PCR reactions and its band patterns were used as control to compare different agarose gels.

RESULTS AND DISCUSSION

In the present study, band patterns of *M. avium* isolates observed after PCR typing were polymorphic (Figure 1). Band patterns of *M. avium* isolates were analyzed based on the number of PCR bands and their size range (Table 1). This analysis shows that there were a minimum of two bands to a maximum of nine bands. The range of numbers of DNA bands in clinical and environmental isolates was 3 - 9 and 2 - 8, respectively. In contrast to another study from India, reporting zero numbers of bands in eight clinical *M. avium* isolates (out of 65 isolates analyzed) (Kumar et al., 2006), none of the isolates in our study had zero bands. Molecular sizes of the DNA bands were in the range of 100 bp to 2174 bp indicating that results of PCR typing system can easily be analyzed after agarose gel electrophoresis. Both intense and faint bands were observed after agarose gel electrophoresis, although band intensity was not considered as a discriminatory factor (Picardeau and Vincent, 1996). Among twenty four isolates analyzed in this study, a total of twenty one band patterns were observed after gel electrophoresis. Eighteen isolates had unique band patterns. Based on the relatedness of band patterns, six *M. avium* isolates obtained from water were grouped in three clusters (two isolates in each cluster) (Table 2). Isolates of clusters A and B had identical PCR band patterns specific to their clusters. Two isolates of cluster C showed four shared bands while three bands were unique to either one or another isolate suggesting that these isolates were closely related rather than identical. On the basis of these results a discriminatory index of the PCR based typing system was calculated to be 0.989 (Hunter and Gaston, 1988). The results of the present study are based on the isolates at our Mycobacterial Repository Centre and do not represent the true picture of the isolates from this region; hence, the results need to be interpreted cautiously from epidemiological point of view.

Earlier study carried out by Yoder et al. (1999) had established links between *M. avium* isolates obtained from patient and food samples using the same PCR based typing method. In our study we were not able to obtain patient isolates from the Agra region, and therefore the same comparison was not possible. Still the study is significant as it reconfirms the utility of PCR typing method for DNA fingerprinting of *M. avium* isolates obtained from both patient and environmental isolates in different settings (Yoder et al., 1999). Moreover, to the best of our knowledge, this is the first study that describes the application of this PCR typing system for *M. avium* isolates obtained from soil and water samples.

RAPD analysis of *M. avium* is another PCR based system that has been used by investigators for comparing strains (Matsiota-Bernard et al., 1997). However, major limitation of RAPD is its poor reproducibility (Matsiota-Bernard et al., 1997; Penner et al., 1993). Previously Pestel-Caron et al. (1999) have reported that the PCR

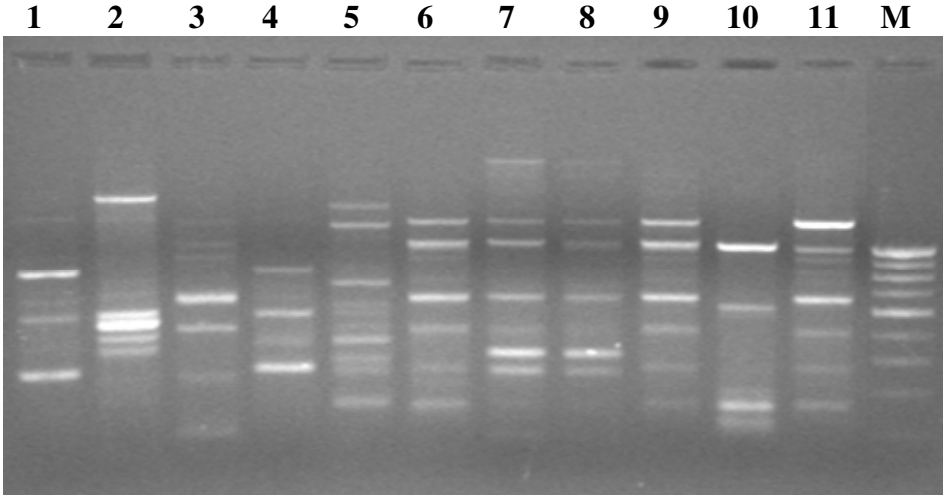

Figure 1. PCR typing patterns of *M. avium* isolates after agarose gel electrophoresis and ethidium bromide staining. Lane 1, TMC-724; lanes: 2 to 11, isolates of *M. avium*; M, 100 bp DNA ladder

Table 1. Analysis of PCR typing results.

M. avium source	No. of PCR bands	Molecular sizes (bp) of PCR bands
Sputum	3 - 9	117 - 1866
Water	3 - 7	100 - 2174
Soil	2 - 8	181 - 1278

Table 2. Environmental *M. avium* isolates showing relatedness based on PCR band patterns.

Cluster	Isolate code	Source	Molecular sizes (bp) of PCR bands						
A	ICC-1330	Water	1324	1091	786	688	525	384	272
	ICC-1622	Water	1301	1091	786	676	525	384	272
B	ICC-1339	Water	2174	1309	1099	682	513	433	368
	ICC-1345	Water	2136	1309	1080	671	513	433	368
C	ICC-1309	Water	1078	741		572	354	282	204
	ICC-1332	Water	1056	761	648		351		212

ICC Indian culture collection.

typing system can generate DNA fingerprints of *M. avium* isolates with 90% reproducibility. In view of these observations it has been thought that PCR typing system may serve as an alternative DNA fingerprinting method for the characterization of *M. avium* strains. Moreover, we performed PCR in parallel reactions using DNA samples from the same *M. avium* isolates isolated either according to the procedure of Picardeau and Vincent (1996) or the procedure of van Embden et al. (1993). The results of these parallel PCR reactions showed similar band pattern after agarose gel electrophoresis. Hence, we propose that tedious DNA purification steps need not be performed before processing for the PCR typing method, which further simplifies the whole procedure of this typing system.

Based on these results we conclude that PCR typing based on IS*1245* and IS*1311* offers a rapid and technically less demanding approach for characterization of *M. avium* strains obtained from both clinical as well as environmental sources. This approach of strain characterization may particularly be useful for resource-poor settings in developing countries that might have majority

of infections due to *M. avium*. Further validation of this method requires confirmation by testing of more clinical strains by different laboratories.

ACKNOWLEDGEMENTS

We acknowledge the financial support provided by Department of Biotechnology, Govt. of India. LEPRA, UK is gratefully acknowledged for providing reagents for the experimental work in our laboratory. GPSJ was supported by a Senior Research Fellowship grant from the Council of Scientific and Industrial Research, New Delhi, India.

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