Differentiation of four *Mycobacterium* Species using DNA-DNA Hybridization Method using Specific Probes

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ABSTRACT

DNA-DNA hybridization method with four oligonucleotide-specific probes was used simultaneously for differentiation and identification of four *Mycobacterium* species (*Mycobacterium tuberculosis*, *M. avium, M. intracellulare, and M. kansasii*). This DNA-DNA hybridization method with 4 oligonucleotide-specific probes, which targets in the *rpoB* region of 4 Mycobacteria species, respectively, was tested on 322 clinical isolates. Using DNA-DNA hybridization method, we detected *M. tuberculosis* (282 strains), *M. avim* (7 strains), *M. intracellulare* (9 strains), and *M. kansasii* (3 strain) from 322 clinical isolates. This result was compared with conventional biochemical test and *rpoB* DNA sequence analysis of this clinical isolates. We confirmed identification of *Mycobacterium tuberculosis*, *M. avium, M. intracellulare, and M. kansasii* with high sensitivity (100 %) and specificity (100 %). This DNA-DNA hybridization method could be performed within 4 hours at least. Therefore, we suggest that DNA- DNA hybridization method using 4 *rpoB* DNA probes of *Mycobacteria* could be used for accurate, rapid, convenient detection and identification of *Mycobacterium tuberculosis*, *M. avium, M. intracellulare, and M. kansasii* in clinical samples.

Keyword

DNA-DNA Hybridization, *Mycobacterium*, *rpoB,*

Ⅰ. INTRODUCTION

Mycobacteria infections including *M. avium* and *M. intracellulare,* severe clinical problem in AIDS patients, *M. kansasii* as well as *Mycobateria tuberculosis* causing tuberculosis are very dangerous and important pathogens clinically. Therefore, it is a very critical point to differentiate and identify them for therapy of patients.

In this study, we tested DNA-DNA hybridization using four each specific oligonucleotide probes for the detection and identification of four mycobacteria from 322 clinical isolates.

II. MATERIALS AND METHODS

A. Bacteria strains and DNA preparations

Clinical isolates used in this study were provided by the Korean Institute of Tuberculosis and Department of Clinical Pathology, Seoul Nsational University Hospital. Mycobacterial DNAs were prepared by the bead beater-phenol extraction method.

B. Amplication of rpoB DNA

A set of primers, which was previously used to amplify rpoB DNA encompassing the *rif r* (region associated with rifampin resistance in *Mycobacteria*) [1], was labeled with biotin and designated BioMF (5'biotin-CGACCACTTCGGCAACCG3') and BioMR (5'biotin-TCGATCGGGCACATCCGG3'). The reaction mixture was subjected to 30 cycles of amplification (5 min at 94℃, 1 min at 95℃, 30 s at 68℃, 1min 20s at 72℃), and this was followed by a 10 min extension at 72℃.

C. DNA hybridization using a microtiter well plate

DNA hybridization was performed as previously described [2], with minor modification (Fig. 1). Briefly, four oligonucleotide-specific probes was designed from the known *rpoB* sequences of *Mycobateria tuberculosis, M. avium, M.*

intracellulare, and *M. kansasii* [1].

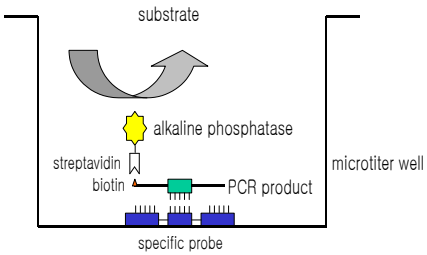

Fig. 1. DNA-DNA hybridization method in microtiter well plate.

D. Nucleotide sequencing

The nucleotide sequences of the purified PCR products were directly determined as previously described [1]. Determined sequences were compared with those of reference strains in GenBank to compare sequence similarities.

III. RESULTS AND CONCLUSIONS

DNA-DNA hybridization method was performed for differentiation and identification of four *Mycobacteria.* The cut off value for DNA-DNA hybridization test using these 4 probes were set at 0.2. Some strains other than members of the *Mycobacteria* did not react with 4 probes coated in this micro-titer well plate (Fig. 2).

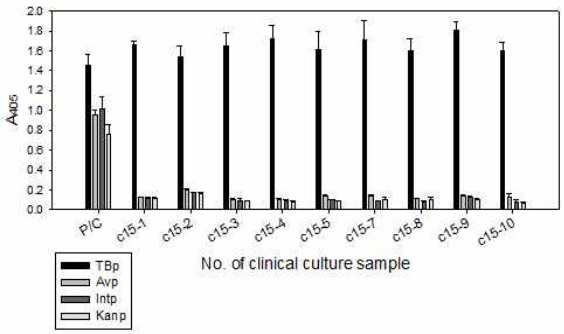

Fig. 2. A example of DNA-DNA hybridization test result using four probes from clinical culture samples.

This DNA-DNA hybridization test was also performed with 322 clinical culture samples. Four *Mycobacteria* were differentiated and identified (Fig. 2). Clinical samples were identified as *M. tuberculosis* (282 strains), *M. avim* (7 strains), *M. intracellulare* (9 strains), *M. kansasii* (3 strain) and non-*Mycobacteria* strains (21 strains) among 322 strains (Table 1).

TABLE 1. Comparision of the results obtained by DNA-DNA hybridization method and other methods for identification of clinical culture samples

	Methods		
Identification	DNA-DNA hybridization	Sequencing of *rpoB*	Biochemical test
M. tuberculosis	282	282	282
M. avium	7	7	7
M. intracellulare	9	9	9
M. kansasii	3	3	3

Sensitivity and specificity of this DNA-DNA hybridization method performed with 322 culture samples was 100%, respectively. Twenty one samples were identified as non-*Mycobacteria* by analysis of sequencing of *rpoB* and biochemical test.

There have been developed so many methods and used to detect and identify *Mycobacteria.* These method involved conventional biochemical test, PCR-based methods [3], and liquid culture-based Bactec mycobacterial detection systems [4]. However, up to date, a proper and precise method for *Mycobacteria* has been insufficient.

From this study, we confirmed that this DNA-DNA hybridization method could be useful method for differentiation and identification of four *Mycobacteria* (*M. tuberculosis*, *M. avim*, *M. intracellulare* , and *M. kansasii*).

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