Genetic Mutations of *rpoB* of *Mycobacteria* Resistance to Rifampin

Tae-Dong Kweon*,Young-Hee Sa*,and Seong-Karp Hong**

*Yonsei University College of Medicine

**Mokwon University

E-mail : karp@mokwon.ac.kr

Rifampin 내성 마이코박테리아의 *rpoB* 유전자 변이

권태동* · 사영희* · 홍성갑**

*연세대학교의과대학교

**목원대학교

요 약

Rifampin 내성 마이코박테리아의 RNA polymerase beta subunit gene (*rpoB*)의 변이를 of rifampin-resistant *Mycobacteria* was analyzed using nucleotide sequence of containing rifampin 내성 유전자부위인 *rif*"을 포함한 *rpoB* DNA (351 bp)의 염기서열 분석을 이용하여 조사하였다. 본 연구를 위해 마산국립병원과 국립결핵원으로부터 전통적 배양방법으로 rifampin 내성 마이코박테리아를 수집하여 그것들의 *rpoB* 유전자를 염기서열 분석을 수행하였고 최근까지 보고된 것과는 다른 변이들을 확인할 수 있었다.

ABSTRACT

RNA polymerase beta subunit gene (*rpoB*) mutation of rifampin-resistant *Mycobacteria* was analyzed using nucleotide sequence of *rpoB* DNA (351 bp) containing rifampin resistant region, *rif*. For this purpose, we collected rifampin-resistant *Mycobacteria* that were identified by conventional culture method from Masan National Hospital and The Korean Institute of Tuberculosis and performed analysis of nucleotide sequence of *rpoB* of them. We found various mutations of *rpoB* linked rifampin resistant gene from rifampin-resistant *Mycobacteria*. From this study, we identified mutations of different codons from codons that have been reported recently.

Keyword

Mycobacteria, nucleotide sequence, rifampin, *rpoB*

Ⅰ. INTRODUCTION

The genus *Mycobacterium* has more pathogenic species including obligate parasites responsible for serious human and animal diseases, opportunistic pathogens, and saprophytic species found in nature [1]. *Mycobacterium tuberculosis* is the most common and important pathogen, and causes tuberculosis in 8 million new patients and 3 million deaths a year worldwide. In addition, the discrimination of *M. tuberculosis* and nontuberculous mycobacteria (NTM) during the early stages of infection is necessitated by the increasing rate of NTM infections. Moreover, a novel advent of antibiotic-resistant *M. tuberculosis* has been most dangerous phase as a human and animal pathogen. The gene of *rpoB* is a kind of house-keeping gene and encodes the β-subunit of RNA polymerase. It is also related to rifampin resistance in mycobacteria. Recently, partial rpoB

sequences suitable for the mycobacteria identification were reported [2].

To analysis of mutation of rifampin-resistant *Mycobacteria,* we perform to identify by conventional culture method from Masan National Hospital and The Korean Institute of Tuberculosis and analyze nucleotide sequence of *rpoB.*

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 Masan National Hospital. Mycobacterial DNAs were prepared by the bead beater-phenol extraction method.

B. Amplication of DNA

A set of primers, which was previously used to amplify *rpoB* DNA (351 bp) encompassing the *rif r* (region associated with rifampin resistance in *Mycobacteria*) [5], was designated MF (5'-CGACCACTTCGGCAACCG-3') and MR (5'-TCGATCGGGCACATCCGG-3'). Template DNA (approximately 50 ng) and 20 pmol of each primer (MF and MR) were added to a PCR mixture tube (Accu-Power PCR PreMix; Bioneer, Daejeon, Korea) containing 1 U of Taq DNA polymerase, 250 μM dNTP, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 1.5 mM $MgCl_2$, and gel loading dye; the volume was then adjusted with distilled water to 20μl. The reaction mixture was subjected to 30 cycles of amplification (5 min at 94℃, 1 min at 95℃, 30 s at 68℃, 1 min 20 s at 72℃), and this was followed by a 10 min extension at 72℃.

C. DNA Nucleotide sequencing

The nucleotide sequences of the purified PCR products were directly determined as previously described [2].

For the sequencing reaction, 60 ng of PCR amplified DNAs, which were purified using a QIAEX II gel extraction kit (QIAGEN, Hilden, Germany), 5 pmol of either the forward or the reverse primer, and 4 μl of Big Dye Terminator v2.0 100 RR mix (Perkin-Elmer Applied Biosystems) were mixed, and the contents were adjusted to a final volume of 10 μl with distilled water. The reaction was run for 30 cycles of 10 s at 96 °C, 5 s at 60 °C, and 4 min at 60 °C. Both strands were sequenced as a cross check. Determined sequences were compared with those of reference strains in GenBank to compare sequence similarities.

III. RESULTS AND CONCLUSIONS

We performed analysis of genetic mutations of rifampin-resistant Mycobacteria using DNA amplication of *rpoB* (3.7 kb) by PCR (Fig. 1) and nucleotide sequencing method. From the specimens that were collected and analyzed, we found 17 rifampin-resistant *M. tuberculosis* strains.

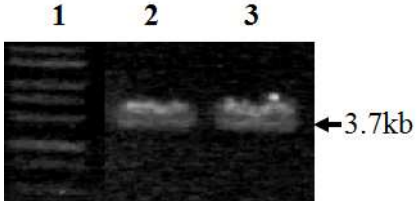

Fig 1. DNA amplication of *rpoB* (3.7 kb) of *M. tuberculosis* by PCR method for analysis of DNA nucleotide sequence (1; DNA marker size, 2 and 3; amplified *rpoB* gene).

Among them, there were five mutations in four sites within *rpoB* gene of 17 rifampin-resistant *M. tuberculosis* strains containing mutants that have never been reported recently (Table 1).

TABLE 1. Comparision of mutation in *rpoB* region of rifampin-resistant *Mycobacteria tuberculosis*

site of *rpoB* codon	Mutation (DNA)	Mutation (amino acid)	Frequency (*n*=17)
510	CAG→CAC	Q→H	4
516	GAC→TAC	D→Y	3
526	CAC→TAC	H→Y	3
	CAC→CGC	H→R	3
531	CAC→TAC	H→Y	4

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0014780) and (2011-0024912).

REFERENCES

[1] S. K. Hong, B. J. Kim, Y. J. Yun, K. H. Lee, E. C. Kim, E. M. Park, Y. G. Park, G. H. Bai, and Y. H. Kook, "Identification of *Mycobactreium tuberculosis* by PCR-linked reverse hybridization using specific *rpoB* oligonuceotide probes," *J. Microbiol. Methods.* vol. 59, pp. 71-79, 2004.

[2] B. J. Kim, S. H. Lee, M. A. Lyu, S. J. Kim, G. H. Bae, S. J. Kim, G. T. Chae, E. C. Kim, C. Y. Cha, and Y. H. Kook, "Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*)," *J. Clin. Microbiol.*, vol. 37, pp. 1714-1720, 1999.