Rifampin에 대한 내성 마이코박테리아에서 *rpoB*의 다양한 변이

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Diverse Mutations of *rpoB* in Rifampin-Resistant *Mycobacteria*

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요 약

rifampin 내성 유전자 부위인 *rif*^{r}를 포함하는 RNA polymerase beta subunit gene (*rpoB*) (351 bp)의 DNA 서열을 분석을 이용하여 rifampin 내성 마이코박테리아의 *rpoB* DNA 변이를 조사하였다. 본 연구를 위해 국립마산병원과 결핵원으로 부터 기존의 재래 배양방법으로 동정한 rifampin에 대한 내성 마이코박테리아를 수집하였다. 본 연구실에서는 수집된 rifampin 내성 마이코박테리아에서 *rpoB* 유전자의 DNA 서열 분석을 수행하였다. 본 연구의 분석 결과로 rifampin 내성 마이코박테리아에서 *rif*^{r}를 포함한 *rpoB*의 보고되지 않은 다양한 변이들이 조사되었다.

ABSTRACT

We analyzed RNA polymerase beta subunit gene (*rpoB*) mutation of rifampin-resistant *Mycobacteria* through analysis of nucleotide sequence of *rpoB* DNA (351 bp) containing rifampin resistant region, *rif*. For this study, we collected rifampin-resistant *Mycobacteria* that were identified by conventional culture methods from Masan National Hospital and The Korean Institute of Tuberculosis. We performed sequencing of DNA nucleotides and analyzed *rpoB* gene of those rifampin-resistant *Mycobacteria.* From this analysis, we invcestigated diverse mutations of *rpoB* gene included rifampin-resistant gene, which were not reported, from those rifampin-resistant *Mycobacteria.*

Keyword

DNA sequencing, *Mycobacteria*, rifampin, *rpoB*

Ⅰ. INTRODUCTION

The *Mycobacterium* consists of more than 72 species, which include obligate parasites responsible for serious human and animal diseases, opportunistic pathogens, and saprophytic species found in nature [1]. Among *Mycobacterium, Mycobacterium tuberculosis* is the most common and important pathogen, and causes tuberculosis in 8 million new patients and 3 million deaths a year worldwide [1].

The emergence of *M. tuberculosis* and antibiotic-resistant *Mycobacteria* have increased world widely. Recently rifampin-resistant *M. tuberculosis* are associated with mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase in *M. tuberculosis*. Many cases of *rpoB* mutations have been reported from rifampin-resistant *M. tuberculosis* isolates. More than 90% of rifampin-resistant *M. tuberculosis* isolates were found within the rifampin resistant coding gene region [2].

In this study, we isolated rifampin-resistant *M. tuberculosis* by conventional culture method from clinical isolates and analyze DNA sequences of *rpoB* (351 bp) including rifampin resistant region.

II. MATERIALS AND METHODS

A. Bacteria strains and DNA preparations

Clinical isolates used in this study were provided by Masan National Hospital and The Korean Institute of Tuberculosis. Mycobacterial DNAs were prepared by the bead beater-phenol extraction method [3].

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*), was designated MF (5'-CGACCACTTCGGCAACCG-3') and MR (5'-TCGATCGGGCACATCCGG-3') [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 loadingdye; the volume was then adjusted with distilled water to 20μl. There action 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℃.

D. Nucleotide sequencing

The nucleotide sequences of the purified PCR products were directly determined as previously described [4].

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 BigDye 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 crosscheck. Determined sequences were compared with those of reference strains in GenBank to compare sequence similarities.

III. RESULTS AND CONCLUSIONS

We performed to amplify *rpoB* (351 bp) including rifampin resistant region from rifampin-resistant *M. tuberculosis isolates* by PCR (Fig. 1). Then we analyzed and compared the DNA sequences of them with those of reference strains in GenBank.

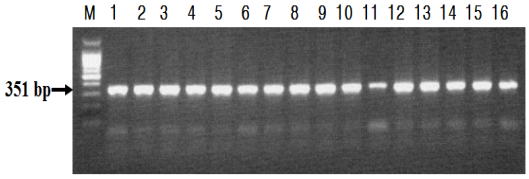

FIG. 1. Amplication products obtained by MFMR PCR from some samples of clinical culture isolates. DNA fragments of 351 bp were shown. M: marker DNA (100 bp ladder DNA), lane 1 to 16, samples of clinical *M. tuberculosis*.

In this study, forty four mutations containing 31 single change and 13 multiple change were found (Table 1). Among them, there were some mutants which never been reported recently.

TABLE 1. Comparision mutations of rifampin-resistant M. tuberculosuis

No. of Codon	Amino acid (codon)			Frequency (n=44)
Single change				
513	Gln(CAA)	→	Pro(CCA)	3
516	Asp(GAC)	→	Val(GTC)	4
		→	Tyr(TAC)	6
526	His(CAC)	→	Tyr(TAC)	4
531	Ser(TCG)	→	Leu(TTG)	14
Multiple Change				
490-531	Gln(CAG) Ser(TCG)	→	Arg(CGG) Leu(TTG)	6
526-529	His(CAC) Arg(CGA)	→	Arg(CGC) Gln(CAA)	7

ACKNOWLEDGMENTS

This study 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).

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