

## 결핵으로 인한 폐 실질 파괴 환자의 객담에서 분리한 *Mycobacterium lentiflavum* 1예

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### Isolation of *Mycobacterium lentiflavum* from a Patient with a Lung Destroyed by Tuberculosis

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*Mycobacterium lentiflavum* has recently been described as an emerging human pathogen without regard to the immune status of the host. We herein report on *M. lentiflavum* isolated from a respiratory specimen of a patient. Although the organism described in this case seems to be a colonizer of a lung destroyed by tuberculosis, the current methods for species identification of nontuberculous mycobacteria have to be re-evaluated so as not to underestimate these organisms. (*Korean J Lab Med* 2007;27:124-7)

**Key Words :** *Mycobacterium lentiflavum*, 16S rRNA gene, Clinical specimen

## INTRODUCTION

*Mycobacterium lentiflavum* is one of the slow-growing nontuberculous mycobacteria (NTM), whose colony is characterized by smallness, smoothness, and scotochromogen. The biochemical characteristics are similar to *Mycobacterium avium* and closely related to *Mycobacterium simiae* and *Mycobacterium genavense*[1].

*M. lentiflavum* may be isolated from clinical specimens as a simple contaminant or may even be the causative organism of disease, both in immunocompetent and immunosuppressed patients[2-4]. Haase and his colleagues reported *M. lentiflavum* as an etiological agent of cervi-

cal lymphadenitis in children[5]. The prevalence of the cases in which *M. lentiflavum* isolation may be clinically significant is approximately 10%[4].

The isolation of NTM always raises the question of its clinical significance because NTM can be found in nature. Especially in countries where tuberculosis is still an issue of public health, NTM, as the name means, is easily overlooked as a contaminant in contrast to *M. tuberculosis*. However, NTMs are no longer simple contaminants, thus the accurate identification of a NTM species presages various other necessary purposes such as diagnosis, treatment planning and patient prognostication.

Several molecular typing methods were used for species identification of NTM strains, including PCR-restriction fragment length polymorphism (PCR-RFLP) and the sequencing of some genes. In Korea, due to the simplicity and relatively cheap expenses, many laboratories prefer PCR-RFLP to sequencing, and the *rhoB* is the common gene for identification of NTM species[6, 7]. Unfor-

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tunately, the *rpoB* PCR-RFLP is insufficient in the accurate identification of some NTM species, especially for those species that are either newly defined or infrequently isolated from clinical specimens.

Here we report on a *M. lentiflavum* from a respiratory specimen, which exhibited the phenotypic property of scotochromogen, as identified by sequencing analysis of 16S rRNA, the fragment including the hypervariable region (*E. coli* nt 180-420)[8, 9] or the *hsp65* gene[10]. The clinical significance, though, is still unknown as this is the first report of *M. lentiflavum* from clinical specimens in Korea.

## CASE REPORT

During a physician's follow-up in 2002, it was discovered that a 69-yr-old man, with symptoms of hemoptysis and a previous history of tuberculosis, had developed a new radiological lesion of the chest (Fig. 1). The man had had a history of pulmonary tuberculosis and had been taking medication for one year at another hospital about 18 yr ago. He had tested positive serologically for hepatitis B virus surface antigen and negative for HIV. His chest CT showed a newly discovered nodule in the left lower field of the lung previously damaged by tuberculosis. Bron-

choscopic findings were free of malignancy, and culture of his bronchial washing specimen had no growth of mycobacteria. After the bronchoscopic examination, NTM were cultured from three successive sputums with a degree of 10 to 20 colony growth during a 14-month period.

The colonies were identified as *M. lentiflavum* by the sequencing of either 16S rRNA or *hsp65* with 100% similarity of studied fragment. However, in the case of *rpoB*, the organism could not be designated as any specific species due to the lack of reference sequences. The drug sensitivity results performed by the Korean Institute of Tuberculosis revealed resistance to isoniazid, rifampin, streptomycin, ethambutol, kanamycin, capreomycin, para-aminosulfuric acid, ofloxacin, and pyrazinamide; and susceptibility to only prothionamide and cycloserine. Thereafter, for three years, the patient displayed no changes in symptoms and in X-ray findings, and the patient received no treatment except for symptomatic therapy. None of the sputum cultures tested positive for AFB.

## DISCUSSION

The present case represents the first isolation of *M. lentiflavum* from a respiratory specimen in Korea, although its clinical significance is still unknown. It could only be



Fig. 1. (A) Simple chest PA done on May 13th, 2002: Lung destroyed due to tuberculosis with volume loss of both upper lung field. No definite change since May, 1998. (B) Routine chest CT done on April 25th, 2002: A new nodular lesion on left lower lung field (arrow) compared with October 30th, 2000.

a colonizer in the damaged lung, although, with the new radiological lesion, we could find no evidence of neoplasm or reactivation of tuberculosis. The same NTM species isolated during a 14-month interval is thought to be an true NTM pulmonary disease by the definition of the British Thoracic Society[11].

*M. lentiflavum* was thought to only cause lymphadenitis, but recently a few cases concerning pulmonary diseases has been reported in immunocompetent and immunocompromised patients as well[2-4, 13, 14].

The significance of this case is that an accurate identification of *M. lentiflavum* cannot be made by the *rpoB* gene, which is generally used as the molecular method for the identification of NTM species in Korea[7-12]. We previously compared, with single strand sequencing easily available in clinical laboratories[15], the usefulness of several genes (16S rRNA, *hsp65*, and *rpoB*) in identifying NTM to the species level. With *rpoB*, there were no comparable reference sequences of *M. lentiflavum* in BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>). With PCR-RFLP of another segment of *rpoB*, a method that is widely used for the species identification of NTM in Korea[7], *M. lentiflavum* was misidentified as *M. szulgai*, which is also a scotochromogen. It is not surprising that the misidentification occurred, because the methods of PCR-RFLP have inherent drawbacks, such as the problem of fragments shorter than 50 bp and a lack of standardization, in the identification of organisms. On the other hand, the availability of PCR and DNA restriction techniques in any laboratory is an undeniable advantage. The problems of identification, as shown in most clinical laboratories, are ignoring the fortuitous organism or giving wrong name with one of the more well known species frequently isolated. These may lead to significant biases in epidemiological and drug susceptibility studies. *M. lentiflavum* is generally resistant to antituberculous drugs (isoniazid, rifampin, ethambutol and streptomycin).

We emphasize the importance of accurate identification of NTM isolates in Korea so as not to underestimate the presence of the pathogen known as *M. lentiflavum*.

## 요 약

비결핵항산균 중 *Mycobacterium lentiflavum*은 최근 국외에서 숙주의 면역상태와 관계없이 질환을 일으키는 신생 병원균으

로 증례 보고되고 있다. 저자들은 호흡기 검체에서 분리된 *M. lentiflavum*을 1예를 경험하였다. 비록 본 증례에서는 과거의 결핵력으로 인해 파괴된 폐실질의 정착균일 가능성도 배제할 수는 없지만, 임상 검체에서 이렇게 드물게 분리되는 비결핵항산균을 정확히 동정하기 위해서는 현재의 일반적인 검사실 동정법에 대한 재평가가 필요하다.

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