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# IS*6110* DNA fingerprinting analysis of individually separated colonies of *Mycobacterium tuberculosis*

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**Summary** There are no data so far that show IS*6110* restriction fragment length polymorphism (RFLP) patterns of individually separated tuberculosis bacilli from clinical isolates, and their alterations during follow-up surveys. We picked 20–60 tuberculosis clones from clinical isolates under anti-tuberculosis medication, and individually analysed their DNA fingerprinting patterns using IS*6110* RFLP as well as spoligotyping as a second typing.

The study using cloned bacilli of *Mycobacterium tuberculosis* showed that clinical isolates contained several clones with different DNA fingerprints and that their band patterns altered weakly but distinctly during follow-up surveys. However, there was no significant difference in the fingerprinting patterns when clinical isolates were to RFLP without separating to subjected/individual colonies.

In view of the IS*6110* RFLP of individually separated tuberculosis bacilli, we have now speculated several possibilities: (1) that clones with different DNA fingerprints exist in clinical isolates; (2) that IS*6110* RFLP patterns of the materials depend on the population of the original clone and the variants having DNA fingerprints different from the original pattern; and (3) that their band patterns are influenced not only by the stability of the original germ having its own fingerprint, but also by the fragility of the new clones.


## Introduction

IS*6110* DNA fingerprinting of *Mycobacterium tuberculosis* is one of the powerful tools for transmission analysis of tuberculosis.[1–4] Clinical isolates from patients with tuberculosis have been considered to contain clonally expanded *M. tuberculosis*.

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Therefore, it is generally accepted that their DNA fingerprint patterns are derived from their original clones. The estimated average half-life of the band of IS*6110* DNA fingerprinting is approximately 3.2–3.7 years.[5–7] Thus, the DNA band patterns of *M. tuberculosis* obtained from sputa may alter slowly but distinctly. There are no data so far that show the DNA fingerprinting of each clonally separated *M. tuberculosis* from sputa, and their alterations during follow-up surveys. In order to elucidate this point, we analysed the IS*6110* DNA fingerprinting, as well as spoligotyping as a second typing, of the single-cloned *M. tuberculosis*.

## Material and method

### Patients

Since 2001, all *M. tuberculosis* complex isolates in Osaka Prefectural Habikino Hospital (since October 2003, renamed as Osaka Prefectural Medical Center for Respiratory and Allergic Diseases) have been subjected to IS*6110* DNA fingerprinting.[8] Four patients with lung tuberculosis were selected in this study because their present illness and the drug susceptibilities of their bacilli were intensively investigated. The drug susceptibility profiles of cases 1–4 were recorded, and the corresponding clinical isolates were stored. Case 1 suffered from primary rifampicin-resistant tuberculosis. He was given a 6-month standard short course therapy and was initially cured. However, tuberculosis relapsed 30 months later. Cases 2 and 3 had multi-drug resistant (MDR) tuberculosis. Tuberculosis of case 4 was streptomycin (SM) resistant. After a standard short course of anti-tuberculosis therapy, the disease of case 4 relapsed again. In case 2, interestingly, the susceptibility to rifampicin transiently increased, and then decreased again. In cases 3 and 4, transient alterations were observed in IS*6110* restriction fragment length polymorphism (RFLP) patterns during follow-up surveys, which disappeared finally. Brief summaries of these patients are described in Table 1.

### Collection of bacilli of *M. tuberculosis*

The studied bacilli of *M. tuberculosis*, grown from sputa, were frozen and stored at −80 °C. The storage of *M. tuberculosis* and analysis of its DNA were performed after obtaining written informed consent.

### Single colony isolation

The frozen *M. tuberculosis* stocks were thawed and cultured on 7H11 agar medium. As many as possible resulting colonies were individually picked (up to 60 colonies) and cultured in 3 ml of liquid medium for 3–4 weeks. The growing cells were cultured onto the slopes of Ogawa medium.

### DNA extraction

Bacilli of *M. tuberculosis* were harvested from the slopes of Ogawa medium, placed into 0.75 ml of buffer (0.3 M Tris–HCl, 0.1 M NaCl, 6 mM EDTA) with 25% (v/v) of 0.1 mm glass beads, vortexed by a bead beater (Biospec products) for 200 s at 4600 rpm and centrifuged at 5000 rpm for 5 min. The supernatant was collected and the DNA was extracted by the standard phenol–chloroform extraction method.

### IS*6110* RFLP analysis

The IS*6110* DNA fingerprinting was performed according to a standardized protocol.[9] Briefly, genomic DNA was digested with restriction endonuclease *Pvu*II (Wako junyaku, Japan), electrophoresed through a 0.8% agarose gel, and vacuum blotted onto a nylon membrane.[10] The molecular size standard used was a mixture of *Hind*III-digested fragments of bacteriophage $\lambda$DNA and *Hae*III-digested $\phi$x174 DNA (5 ng of each digest). After blotting, the membranes were hybridized with a

**Table 1** Patient characteristics.

| | Sex | Age (years) | Drug resistance |
|---|---|---|---|
| Case 1 | Male | 39 | RFP |
| Case 2 | Female | 76 | INH, RFP, EB, KM, CS, TH, LVFX |
| Case 3 | Male | 61 | INH, RFP, EB, SM, KM, CS, TH, LVFX, EVM |
| Case 4 | Male | 61 | SM |

INH: isoniazid; RFP: rifampicin; EB: ebuthol; SM: streptomycin; KM: kanamycin; CS: cycloserine; TH: ethionamide; LVFX: levofloxacin; EVM: emviomycin.