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Natural Occurrence of Horizontal Transfer of *Mycobacterium avium*-Specific Insertion Sequence IS*1245* to *Mycobacterium kansasii*▽

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***Mycobacterium kansasii* carrying IS*1245*, a highly prevalent insertion sequence among *Mycobacterium avium* isolates, was detected in a mixed culture of *M. avium* and *M. kansasii*. The insertion sequence was stable and able to transpose by a replicative mechanism in *M. kansasii*. These findings may have significant implications for molecular diagnosis and treatment outcome.**

Mycobacterium avium and *Mycobacterium kansasii* are major human mycobacterial pathogens, and both can cause pulmonary infections in immunocompetent individuals, as well as disseminated infections in the immunocompromised (6). Coinfection with *M. avium* and *M. kansasii* has also been documented in HIV-positive patients (8, 15). Current treatment of *M. avium* and *M. kansasii* infections is based on long-term antibiotic therapy and relies on different drug combinations, justifying all efforts directed to the correct identification of clinical isolates. Insertion sequences (IS) are mobile genetic elements within mycobacteria that are often species specific, a property that can be exploited for diagnosis and epidemiological studies (4). The host range of the IS*1245* element was considered to be limited to *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, and *M. avium* subsp. *silvaticum* (3, 9, 11–13, 17).

Detection of IS*1245* by PCR was used for confirmation of the presence of *M. avium* in a mixed culture from a bone marrow specimen from an HIV-positive patient. Fifteen slow-growing colonies were recovered from the bone marrow primary culture by plating dilutions on Middlebrook 7H10 solid medium supplemented with oleic acid, albumin, catalase, and dextrose (7H10-OADC). Four colonies (88.1 to 88.4) were nonpigmented, slow-growing mycobacteria identified as *M. avium* by PCR-restriction enzyme analysis (PRA) using the 16S-23S rRNA internal transcribed spacer (ITS) sequence as the target (14). Eleven colonies (88.5 to 88.15) produced yellow pigment after exposure to light and were identified as *M. kansasii* by PRA-ITS (Fig. 1A). For further characterization of these colonies, a 427-bp fragment from IS*1245* was amplified by PCR (3). Unexpectedly, IS*1245* amplicons were detected not only in the nonpigmented *M. avium* colonies but also in 8 out of 11 *M. kansasii* colonies (Fig. 1B). Amplicons generated with *M. kansasii* DNA were sequenced and showed 100% identity with the deposited IS*1245* sequence (accession number L33879) (data not shown). Final identification of the 15 colonies to the species level was obtained by sequencing a 440-bp fragment of the 5′ 16S rRNA gene (*Escherichia coli* positions 54 to 510) (16). Colonies 88.1 through 88.4 showed 100% sequence similarity to the corresponding sequence of *M. avium* type strain ATCC 25291 (accession number EF521895). Colonies 88.5 through 88.15 showed 100% similarity to the corresponding sequence of *M. kansasii* type strain CIP 104589 (accession number AF547940). The two sequences differed at 12 positions (97.3% similarity).

To confirm the presence of IS*1245* copies in the eight colonies of *M. kansasii*, restriction fragment length polymorphism (RFLP) experiments were performed by using IS*1245* as a probe (17). While the *M. avium* colonies (88.1 to 88.4) produced multiband RFLP patterns characteristic of *M. avium* subsp. *hominissuis*, the eight *M. kansasii* colonies that had produced amplicons by PCR-IS*1425* (88.8 to 88.15) showed a single IS*1245*-hybridizing band of approximately 4,750 bp. Moreover, a second IS*1245*-hybridizing band was detected in two of these colonies (88.11 and 88.14). Furthermore, the three PCR-IS*1245*-negative colonies of *M. kansasii* (88.5 to 88.7) lacked IS*1245* hybridization bands (Fig. 1C). Except for the presence of IS*1245*, pulsed-field gel electrophoresis patterns indicated that all of the 11 *M. kansasii* colonies were, in fact, the same strain (Fig. 1D).

The results obtained strongly suggest that *M. kansasii* acquired the IS*1245* element from the *M. avium* strain in a horizontal DNA transfer event which occurred either within the patient or during specimen or culture storage. This hypothesis was reinforced by the fact that both species were isolated from a unique specimen collected from one patient and by the finding of several *M. kansasii* colonies (88.5 to 88.7) of the same strain devoid of this insertion sequence element in that specimen (Fig. 1B, C, and D). The presence of the IS*1245* element in *M. kansasii* was not detected by the analysis of five additional *M. avium*-*M. kansasii* mixed cultures from different patients (data not shown), which agrees with the hypothesis that the event described here was the result of a particular horizontal DNA transfer episode.

Antimicrobial susceptibility testing was performed with three isolates using a microdilution method previously de-

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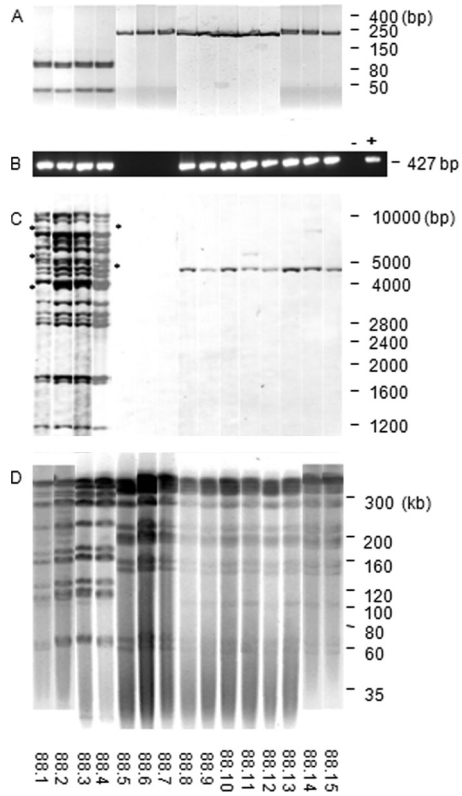

FIG. 1. Molecular characterization of 15 single colonies isolated from the original *M. avium-M. kansasii* mixed culture by PRA-ITS (A), PCR-IS*1245* (B), RFLP-IS*1245* (C), and pulsed-field gel electrophoresis (D). Lanes: colonies 88.1 to 88.4, *M. avium*; colonies 88.5 to 88.15, *M. kansasii*; −, PCR negative control (water); +, PCR positive control (DNA from *M. avium* ATCC 25291^T). Asterisks indicate RFLP-IS*1245* band polymorphisms in *M. avium* colonies. On the right are molecular size markers.

scribed (10). The drugs tested included streptomycin, isoniazid, ethambutol, rifampin, rifabutin, ciprofloxacin, amikacin, azithromycin, and clarithromycin. Interpretative criteria followed NCCLS recommendations (10). *M. avium* colony 88.3 was resistant to isoniazid (MIC, 1 μg/ml), ethambutol (MIC, 8 μg/ml), azithromycin (MIC, >8 μg/ml), and clarithromycin (MIC, >32 μg/ml). *M. kansasii* colonies 88.5 (IS*1245* negative) and 88.8 (IS*1245* positive) were susceptible to all of the drugs tested, showing that the acquisition of IS*1245* did not change the pattern of susceptibility of this *M. kansasii* strain to the drugs tested.

In order to examine the stability of IS*1245* in *M. kansasii*, two colonies (88.11 and 88.12) were grown in liquid Middlebrook 7H9-OADC on a shaker at 37°C until the optical density at 600 nm reached 0.6 to 0.8. Ten serial passages were then performed by diluting the cultures 1:10 in the same medium and incubating them again under the same conditions. After 10 passages, the cultures were added to solid 7H10-OADC by the spread plate method and 19 isolated colonies were analyzed by RFLP-IS*1245*. The original IS*1245* hybridization bands were maintained in all of the colonies isolated after 10 passages, demonstrating the stability of this insertion sequence in *M. kansasii*, at least during short-term (4 months) *in vitro* processing. However, one to three new hybridization bands were visualized in 11 out of 19 *M. kansasii* colonies analyzed, pointing to the occurrence of insertion sequence transposition by a replicative mechanism (Fig. 2).

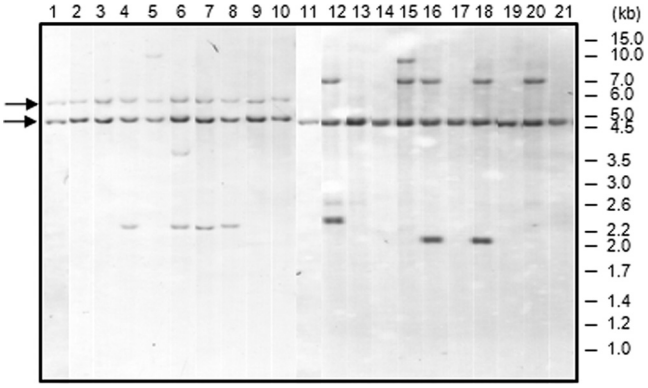

FIG. 2. RFLP-IS*1245* of colonies 88.11 and 88.12 and individual colonies isolated after 10 serial passages in 7H9-OADC liquid medium. Lanes: 1, original colony 88.11; 2 to 10, isolated colonies after subculture of colony 88.11; 11, original colony 88.12; 12 to 21, isolated colonies after subculture of colony 88.12. Black arrows indicate the hybridization bands present in the original colonies. On the right are molecular size markers.

Colonies of both species showed variations in RFLP-IS*1245* patterns during this study. Besides the IS*1245* transposition detected in *M. kansasii*, two or three RFLP-IS*1245* band polymorphisms were also detected in the *M. avium* colonies (Fig. 1C). Recent IS*1245* transposition events in *M. avium* have been observed in other studies with single colonies of the same isolate or isolates collected from individual patients over time (1, 3, 11, 12).

The IS*1245* insertion sequence was initially considered to be species specific for *M. avium*, but additional studies have shown that this element is sporadically present in *M. intracellulare*, *M. malmoense*, *M. scrofulaceum*, and *M. nonchromogenicum* (2, 7, 9). To our knowledge, the results obtained in this study confirm, for the first time, the presence of one or more copies of IS*1245* in a strain of *M. kansasii* by PCR, RFLP, and DNA sequencing.

As a consequence, the utilization of IS*1245* as a genetic marker for the identification of *M. avium* and its distinction from *M. kansasii* should be done with caution and combined with other genetic markers. The absence of IS*1245* copies in some *M. avium* strains must also be taken into account (5, 11, 13).

IS*1245* transposition events such as those described here can produce genome plasticity by the interruption or deletion of genes, affecting biological properties, which deserves further investigation.

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