MULTILOCUS VARIABLE-NUMBER TANDEM REPEAT ANALYSIS OF *MYCOBACTERIUM KANSASII*

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AIM

Mycobacterium kansasii is one of the most virulent nontuberculous mycobacteria (NTM). It is also one of the six most commonly isolated NTM species around the world. The observed genetic homogeneity of *M. kansasii* strains results in poorly understood epidemiology of infections due to this pathogen. The aim of this study was to search the genome of *M. kansasii* for tandem repeat sequences, similar to variable number tandem repeat (VNTR) sequences in *M. tuberculosis* allowing for intraspecies differentiation.

METHODS

The *M. kansasii* genome (GenBank, NCBI, Reference Sequence: NC_022663.1) was screened for the occurrence of TR loci with the Vector NTI Software (Thermo Fisher Scientific, Waltham, MA, USA). Primers were designed to anneal to the flanking region of VNTRs utilizing same software as for the VNTR screening. PCR protocols were tested on a collection of 67 strains representing six *M. kansasii* subtypes (I-VI).

RESULTS

Among 1,447 VRTRs found in the genome of the *M. kansasii* ATCC 12478 reference strain, 24 were selected based on predefined criteria. Seven loci (VNTR 5, 9, 10, 12, 13, 16, 22) were excluded from the analysis due to multiple-band patterns generated upon electrophoresis. Different VNTR loci showed different ranges of allelic variability. For three loci (14, 17, 24) only two allelic variants were detected. Eight loci (3, 4, 7, 11, 15, 18, 21, 23) were demonstrated as three-allele types, and another two (1, 6) and three (8, 19, 20) loci as four-and five-allele types, respectively. The highest level of variation was evidenced for VNTR locus 2, with seven allelic types. The schematic representation of VNTR locus 8 allelic variants is depicted in **Figure 1**.

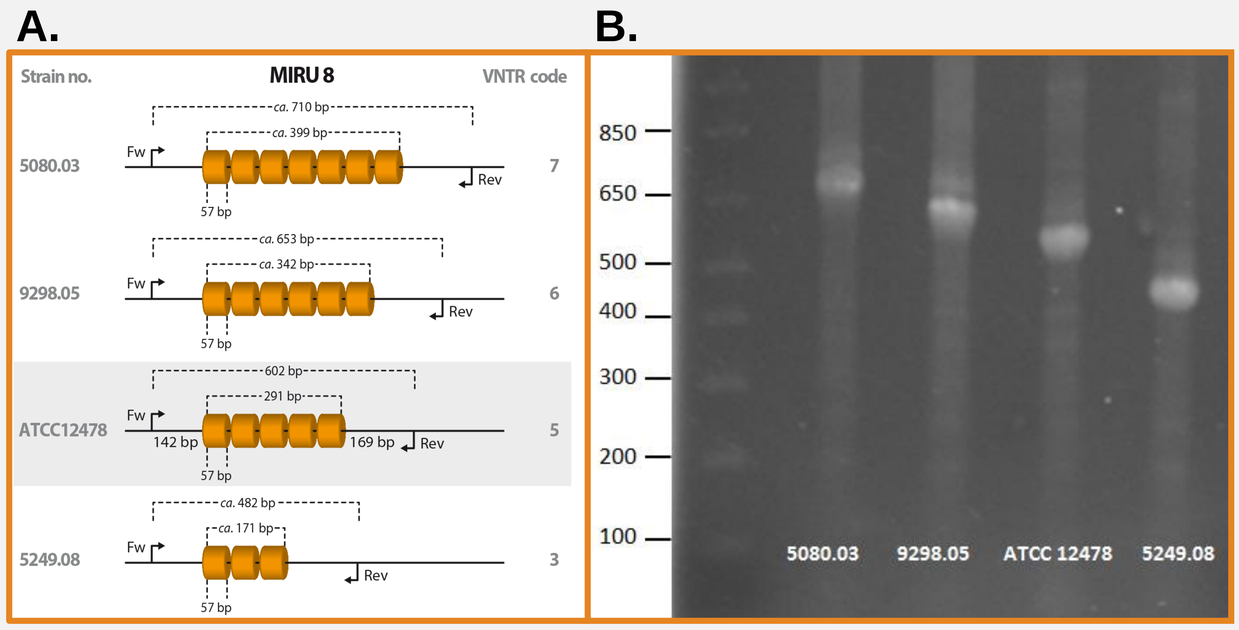

Figure 1. Diversity of VNTR 8 among analyzed *M. kansasii* isolates. A. Schematic representation of allelic diversity of VNTR 8 among genomes of isolates under the study. **B**. Corresponding PCR products obtained on agarose gels.

Gaston Discriminatory Index (HGDI=0.66) (**Table 1**).

Among 54 *M. kansasii* subtype I isolates only VNTR 1, 2, 8, and 20 showed variation, generating 11 different VNTR codes, split into 3 clusters and 8 unique profiles (HGDI=0.47). Cluster “a” contained almost two-thirds of the isolates (39/54; 72.2%), whereas cluster “b” and “c” included five (5/54; 9.2%) and two (2/54; 3.7%) isolates, respectively.

Six VNTRs (1-4, 14, and 23) were polymorphic among 7 *M. kansasii* subtype II isolates translating into four different profiles – a four-isolate (4/7; 57%) cluster „l” and three unique profiles (HGDI=0.71).

Profiles „p”, „r”, „s”, and „t” were specific for *M. kansasii* type III (*n*=2), IV (*n*=1) V (*n*=2) and VI (*n*=1), respectively.

Table 1. VNTR patterns of analyzed isolates.

VNTR code	VNTR locus																	No. of strains	Total no. of strains (subtype)
	1	2	3	4	6	7	8	11	14	15	17	18	19	20	21	23	24		
a	13	7	7	4	6	6	5	7	5	6	5	5	11	13	6	3	9	39	54 (I)
b	13	6	7	4	6	6	5	7	5	6	5	5	11	13	6	3	9	5	
c	13	8	7	4	6	6	5	7	5	6	5	5	11	13	6	3	9	2	
d	13	5	7	4	6	6	5	7	5	6	5	5	11	13	6	3	9	1	
e	13	7	7	4	6	6	7	7	5	6	5	5	11	13	6	3	9	1	
f	13	8	7	4	6	6	5	7	5	6	5	5	11	12	6	3	9	1	
g	13	7	7	4	6	6	6	7	5	6	5	5	11	13	6	3	9	1	
h	13	7	7	4	6	6	3	7	5	6	5	5	11	13	6	3	9	1	
i	13	7	7	4	6	6	5	7	5	6	5	5	11	12	6	3	9	1	
j	13	4	7	4	6	6	5	7	5	6	5	5	11	13	6	3	9	1	
k	12	7	7	4	6	6	5	7	5	6	5	5	11	13	6	3	9	1	
l	13	7	2	3	1	0	3	2	5	6	1	0	12	12	4	3	3	4	7 (II)
m	13	7	2	3	1	0	3	2	NP	6	1	0	12	12	4	3	3	1	
n	13	7	2	3	1	0	3	2	NP	6	1	0	12	12	4	0	3	1	
o	0	2	4	0	1	0	3	2	4	6	1	0	12	12	4	3	3	1	
p	0	7	2	0	4	1	NP	7	5	MP	1	1	NP	8	NP	5	9	2	2 (III)
r	MP[a]	3	2	0	1	0	0	1	5	6	NP	0	6	NP	NP	0	9	1	1 (IV)
s	14	7	2	0	6	0	3	2	5	14	1	0	7	2	NP	MP	9	2	2 (V)
t	12	7	MP	0	5	NP[b]	5	1	5	0	1	0	10	0	12	0	9	1	1 (VI)

Grey color indicates VNTR locus which shows variability in number of tandem repeats among subtypes (I and II).
[a] MP, multiple products obtained on agarose gel.
[b] NP, no PCR product obtained on agarose gel.

CONCLUSIONS

The newly designed VNTR-based typing method of *M. kansasii* appears to be a promising tool for *M. kansasii* fingerprinting and thus may help to better explore the transmission routes of *M. kansasii* infections.

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