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New polymorphisms within the variable number tandem repeat (VNTR) 7 locus of *Mycobacterium avium* subsp. *paratuberculosis*

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ABSTRACT

Variable number tandem repeat (VNTR) is a frequently employed typing method of *Mycobacterium avium paratuberculosis* (MAP) isolates. Based on whole genome sequencing in a previous study, allelic diversity at some VNTR loci seems to over- or under-estimate the actual phylogenetic variance among isolates. Interestingly, two closely related isolates on one farm showed polymorphism at the VNTR 7 locus, raising concerns about the misleading role that it might play in genotyping. We aimed to investigate the underlying basis of VNTR 7-polymorphism by analyzing sequence data for published genomes and field isolates of MAP and other *M. avium* complex (MAC) members. In contrast to MAP strains from cattle, strains from sheep displayed an "imperfect" repeat within VNTR 7, which was identical to respective allele types in other MAC genomes. Subspecies- and strain-specific single nucleotide polymorphisms (SNPs) and two novel (16 and 56 bp) repeats were detected. Given the combination of the three existing repeats, there are at least five different patterns for VNTR 7. The present findings highlight a higher polymorphism and probable instability of VNTR 7 locus that needs to be considered and challenged in future studies. Until then, sequencing of this locus in future studies is important to correctly assign the underlying allele types.[1]

1. Introduction

Mycobacterium avium paratuberculosis (MAP) is a member of the *M. avium* complex (MAC) and the causative agent of Johne's disease, one of the most economically important diseases in ruminants [1]. The disease is characterized by emaciation and chronic granulomatous enteritis and is suspected to play a role in Crohn's disease in humans [2]. Genotyping of these pathogens helps to better understand the epidemiology of disease and allows sources of infection to be identified, with an ultimate goal of designing more efficient control programs [3]. According to host preference, MAP was originally classified as cattle (C) and sheep (S) types. Later, pulsed-field gel electrophoresis (PFGE) was able to further classify S strains into type I and type III, while assigning solely type II to all C strains [4].

Nowadays, mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) genotyping is one of the most widely used typing methods of MAP isolates. This is a rapid PCR-based method that provides higher typing resolution within the main strains, and its results can be readily compared among laboratories using an online database [5].

Ahlstrom et al. [6] described some limitations of MIRU-VNTR typing compared with SNP analysis based on whole genome sequencing data, where MIRU-VNTR over- or under-estimated the phylogenetic variance among MAP isolates. This necessitates a re-evaluation of the loci included in the MIRU-VNTR typing scheme. Among the MIRU-VNTR loci used, polymorphism at the VNTR 7 locus in two closely related isolates from the same farm raises a key question as to the suitability of VNTR 7 for molecular epidemiology. In order to gain a deeper insight into the underlying basis of polymorphism at this locus, we aimed to investigate respective sequence data.

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[1] *Note:* Partial VNTR 7 sequences reported here have been deposited in the GenBank database under accession numbers KT824780, KT833325, KT833326, KT833327 and KT833328.

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2. Materials and methods

2.1. Analysis of the available MAP genomes

We used an online tool (http://insilico.ehu.es/PCR/index.php?mo=Mycobacterium) for *in silico* amplification of the VNTR 7 locus in MAP strain k10 (GenBank accession number: NC_002944) using primers designed by Thibault et al. [7]. All homologous sequences of MAP (n = 31) and other MAC (n = 31) origins were obtained by BLAST N analysis and compared using an online multiple sequence alignment software (http://tcoffee.crg.cat/apps/tcoffee/do:regular) hosted by the Centre for Genomic Regulation, Barcelona [8].

Fig. 1. Multiple sequence alignment of published partial sequences of VNTR 7 locus of *Mycobacterium avium* complex (MAC) origin (GenBank accession numbers are provided next to each sequence). In contrast to (MAP) cattle (C) genomes (one asterisk), which always displayed either one or two 22 bp perfect repeats (highlighted with red and green colors), all MAP sheep (S) genomes (#) had a 22 bp imperfect repeat with a length of 12 bp (highlighted in blue), which was identical to all other MAC genomes (^). A (C > T) SNP at position 89 bp (three asterisks) clearly differentiates between MAP and all other MAC isolates, while a (G > A) SNP at position 100 bp (Two asterisks) differentiates between MAP C and S types (positions were calculated relative to a 203 bp PCR product amplified usinh primers described by Thibault et al. (2007).(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)