MAC-INMV-SSR: a web application dedicated to genotyping members of Mycobacterium avium complex (MAC) including Mycobacterium avium subsp. paratuberculosis strains

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http://mac-inmv.tours.inra.fr/

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Abstract

Genotyping of *Mycobacterium avium* subsp. *paratuberculosis* (Map) is an indispensable tool for surveillance of this significant veterinary pathogen. For Map, multi-locus variable number tandem repeat analysis (MLVA) targeting mycobacterial interspersed repetitive units (MIRUs) and other variable number variable-number tandem repeats (VNTRs) was established using 8 markers. In the recent past this standard, portable, reproducible and discriminatory typing method has been frequently applied alone or in combinations with multi-locus short-sequence-repeat (MLSSR) sequencing. With the large diffusion of these genotyping methods, standardization between laboratories need to be managed, and knowledge of existing profiles and newly defined genotypes should be indexed and shared. To meet this need, a web application called "MAC-INMV-SSR database" http://mac-inmv.tours.inra.fr/ was developed. This freely accessible service allows users to compare MLVA and MLSSR subtype data of their strains with those of existing reference strains analyzed with the same genotyping methods.

Keywords: Genotyping, *Mycobacterium avium* subsp. *paratuberculosis*, MAC complex, database, MLVA, MLSSR.

1. Introduction

Paratuberculosis is a major ruminant infection that remains endemic worldwide, despite significant investments in the deployment of control programs. It is a chronic inflammatory

bowel disease of ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis* (Map) (Clarke, 1997). Mainly prevalent in dairy farming, it is incurable and causes persistent diarrhea at the clinical stage leading to the death of the animal (Clarke, 1997; Harris and Barletta, 2001). With a very slow evolution to symptomatic status in cattle, goat and sheep, the disease is systematically diagnosed too late (Mikkelsen et al., 2011; Nielsen and Toft, 2008). Vaccination is subject to authorization and cannot be widely expanded because it interferes with the detection of bovine tuberculosis (bTB) (Bastida and Juste, 2011).

Despite the implementation of control programs with significant financial efforts in most developed countries, the herd-level prevalence of paratuberculosis remains very high, likely >50% in most countries with a substantial dairy industry (Biet and Boschiroli, 2014). Molecular genotyping techniques used to track the strains and to detect their dispersion have therefore been intensely developed in recent decades. The particularly long and tedious growth of Map in culture is a limiting factor for restriction fragment length polymorphism (RFLP) methods (Pavlik et al., 1999) or whole genome sequencing (WGS) (Bryant et al., 2016), both requiring sufficient amounts of genomic DNA. Therefore, the most widespread genotyping tools are PCR-based, which do not require lengthy Map culture or high-quality DNA preparation. These include multi-locus variable number tandem repeat analysis (MLVA) targeting mycobacterial interspersed repetitive units (MIRUs) and variable-number tandem repeats (VNTRs) and multi-locus short-sequence-repeat (MLSSR) sequencing (Amonsin et al., 2004; Bannantine et al., 2013; Biet et al., 2012; Motiwala et al., 2004; Motiwala et al., 2005; Stevenson, 2015; Thibault et al., 2007; Thibault et al., 2008).

However, in contrast to the well-established situation e.g. for strains of the *M. tuberculosis* complex (Weniger et al., 2012; Weniger et al., 2010), tools to standardize genotyping nomenclature between laboratories and to manage and index existing and newly defined genotypes were lacking. Here we describe in details all functionalities of a web database

dedicated to the genotyping of Map isolates and closely related *Mycobacterium avium* complex MAC members. The purpose of this website is to provide a unique genotype nomenclature to favor international communication and sharing of these profiles.

2. Genotyping systems implemented in the database

The MLVA and MLSSR methods implemented in this web application have been developed for Map and include eight locus MIRU-VNTR (Thibault et al., 2007) and 11 loci-based SSR respectively (Amonsin et al., 2004). These methods can be used alone or in combination. Even if this database is dedicated to Map, these genotyping methods can also be used on strains of MAC complex members due to the high DNA sequence similarities between Map and MAC (Biet et al., 2012; Radomski et al., 2010; Thibault et al., 2007).

3. Database description and utilization.

3.1.1. MLVA and MLSSR-related tools

For each method, the principle of genotyping is described and illustrated (figure 1 and Table 1 and 2). The MIRU-VNTR and SSR markers are detailed and their position on the genome of the *M. paratuberculosis* K-10 strain are shown (figure 1). Corresponding primers and PCR conditions are detailed to favor standardized use of these genotyping methods (see pages INMV typing and MLSSR Typing website pages).

3.1.2. Strain identification

Once the genotyping data have been obtained, users can identify their strain profiles in different ways (figure 2). For MLVA typing, identification of an individual strain can be done by indicating the number of repeats or the size of the PCR fragment for each locus separately, or by entering the concatenate of the numbers of repeats identified in the eight loci. Identification can also be done simultaneously for multiple strains by using the "multiple

identification" functionality, via upload of corresponding MLVA data included in an MS Excel file. The use of this functionality is facilitated by automated recognition of column headings of the Excel file (figure 2). The profiles generated by MLVA are referred to as so-called “INMV” types with a number assigned according to the order of appearance in the database. Similar functionalities are implemented for identification of MLSSR types.

When the queries correspond to existing INMV or MLSSR profiles, the results that are returned comprise information such as the reference type code assigned, the prevalence of the profiles in the database and the publications that described the strains sharing these profiles. Results also indicate the closest INMV or MLSSR profiles.

If a profile is not yet known in the database, the user can request a new reference type code online via a dialog box, to increment the database. In this process, we allocate a unique code per genotype to guarantee unambiguous identification and standardized subsequent comparison with this genotype.

3.1.3. Database browsing

In this part, all the available MLVA and MLSSR profiles can be consulted. The MLVA profiles are from isolates of *M. paratuberculosis*, as well as of *Mycobacterium avium* subspecies *avium*, *hominissuis* and *silvaticum*, as MLVA has been validated for all these members of the MAC complex (Biet et al., 2012). Likewise, MLSSR profiles are from *M. paratuberculosis* and *Mycobacterium avium* subspecies *hominissuis* isolates, as MLSSR can be applied at least on both subspecies. As of July 2019, the database compiles a total of 239 different MLVA profiles for Map and MAC strains, and 57 MLSSR profiles of Map and *M. avium hominisuis* strains. The main window gives information on reference INMV and SSR types along with species/subspecies information (for MLVA), their respective copy numbers for each locus and date of submission. Each profile is clickable to access more detailed

information on corresponding isolates, comprising links to the publication(s) that described them, and for MLVA profiles, the frequency of the profile relatively to all other known profiles (Figure 3)..

3.2. Download tools, update and contact

The download section provides the users with different options to download detailed documentation on the genotyping methods, markers, and protocols under MS EXCEL or PDF format. All new profiles recently deposited in the web database or newly identified in the literature are indicated in this section, along with their reference profile code, their characteristics and their associated publications. Publications that do not use the same loci or partially are not included in the database i.e. articles (Adachi et al., 2016; Borrmann et al., 2011; Christianson et al., 2012; Ichikawa et al., 2010; Inagaki et al., 2009; Marquetoux et al., 2016; Mobius et al., 2009; Okuni et al., 2012; Ricchi et al., 2011; Ronai et al., 2016; Ronai et al., 2015; Uchiya et al., 2018; van Hulzen et al., 2011; Verdugo et al., 2014; Wojtasik et al., 2012).

Users can submit questions for technical support or comments to improve the database via a dialog box similar to that used for the submission of new profiles.

4. Conclusion

The Mac-INMV-PLUS web application is a freely accessible web service dedicated to the MLVA and MLSSR typing methods for Map strains. This web application compiling genotypic profiles classified according to a standardized and controlled nomenclature, has been created to facilitate inter-laboratory comparison and exchange of genotyping and epidemiological data on Map strains but also applicable to other MAC members. Future

development of this web application could include implementation of typing using SNP that is starting to emerge.

Acknowledgments

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Tables

Table 1. MLVA markers, positions, and primer sequences

N°	Locus name	Positions in genome[a]	PCR size	Forward primer	Reverse primer
1	292	3253590-3253889	300	CTTGAGCAGCTCGTAAAGCGT	GCTGTATGAGGAAGTCTATTCATGG
2	X3	4441875-4442070	196	AACGAGAGGAAGAACTAAGCCG	TTACGGAGCAGGAAGGCCAGCGGG
3	25	3665598-3665947	350	GTCAAGGGATCGGCGAGG	TGGACTTGAGCACGGTCAT
4	47	4128604-4128821	217	CGTTGCGATTTCTGCGTAGC	GGTGATGGTCGTGGTCATCC
5	3	131320-131527	208	CATATCTGGCATGGCTCCAG	ATCGTGTTGACCCCAAAGAAAT
6	7	3711417-3711619	203	GACAACGAAACCTACCTCGTC	GTGAGCTGGCGGCCTAAC
7	10	4279553-4279855	303	GACGAGCAGCTGTCCGAG	GAGAGCGTGGCCATCGAG
8	32	1125707-1126004	298	CCACAGGGTTTTTGGTGAAG	GGAAATCCAACAGCAAGGAC

[a]The positions in the genome are the coordinates of the considered MLVA locus in the *M. paratuberculosis* strain K10 genome (GenBank accession number AE016958).

Table 2. MLSSR markers, positions, and primer sequences

SSR N°	Positions in genome[a]	SSR sequence locus	Forward primer	Reverse primer
1	1793091-1793109	GGGGGGGGGGGG GGGGGGGG	TCAGACTGTGCGGTATGGAA	GTGTTCGGCAAAGTCGTTGT
2	2719085-2719094	GGGGGGGGGG	GTGACCAGTGTTTCCGTGTG	TGCACTTGCACGACTCTAGG
3	607784-607794	CG CG CG CG CG C	ATCCAACGCCATGTACTCGT	GAGCAGCATCGAGGTGAAAC
4	3406364-3406373	GC GC GC GC GC	GTTCTCGATGGACAGCTTGC	GGAGGACGAACCACACTCAT
5	3735342-3735351	GC GC GC GC GC	TGTCGAACTTGCTCTTGGTG	CGTCCTGCAACATCTTCTCC
6	4286068-4286084	GCG GCG GCG GCG GCG GC	GAATCGTCTTGCCTCACTGG	TCGAGCAACTGATCTCCACA
7	4310932-4310948	CCG CCG CCG CCG CCG CC	CGGCAATACCTCGAACAGAT	GCTGAAGAGGTCGTGCAGAT
8	1028129-1028145	GGT GGT GGT GGT GGT GG	AGATGTCGACCATCCTGACC	AAGTAGGCGTAACCCCGTTC
9	2955362-2955378	TGC TGC TGC TGC TGC TG	GACAAGTTCGGGTTGACCAC	AGTTCCTCGACCCAGTCGT
10	3558075-3558090	GCC GCC GCC GCC GCC G	CTGGAACGTGTCCGAATTG	GTATTCGGTGCGGATCTCCT
11	1536798-1536812	CCG CCG CCG CCG CCG	CTGGAGTGGAAGAGCAGTCC	GCTGCGTTACCTCAACACC

[a]The positions in the genome are the coordinates of the considered SSR locus in the *M. paratuberculosis* strain K10 genome (GenBank accession number AE016958).

293

294 Figure legends

295

296 Figure 1. MIRU-VNTR and SSR markers used in the genotyping of Map.

297 (A) Circular representation of the MIRU-VNTR, SSR and IS*900* markers on the genome of

298 the *M. paratuberculosis* K-10 strain. (B) schematic representation of the PCR fragments

299 obtained with the 8 MIRU-VNTR markers with the K-10 strain.

300

301 Figure 2. INMV and MLSSR nomenclature system and service for reference code assignation.

302 The user can consult and query all the existing INMV and MLSSR profiles in order to see if

303 profiles identified in his laboratory already exist. If profiles are not yet known, the user can

304 request attribution of a new reference genotype code online.

305

306 Figure 3. Information related to the strains and profiles.

307 For each profile, it is possible to know the source of the strain genotype, their corresponding

308 species and the link to the publication that described it. For MLVA, the associated graph

309 shows the frequency of the isolates showing this profile in the database, compared to the

310 frequencies of isolates with all the other profiles compiled in the database.

311