New reference genomes of Mycobacterium bovis adapted to French genotype diversity

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Bovine tuberculosis (bTB) cattle outbreaks in France are present in **specific regions** and circulate in a multi-host system that includes not only domestic but also wild animals. The transmission link between infected animals remains difficult to establish given that they are **locally** infected ***M. bovis*** strains with identical **genotypes (spoligotype + MIRU-VNTR)** (1). **Whole genome SNP** (single nucleotide polymorphisms) compared to appropriate reference genomes can precisely differentiate strains (2). However, the previous reference genome (AF2122/97) belongs to the European 1 clonal complex mainly found in the British Isles but **less in France or in other European countries** (3). **New reference genomes genetically close to French field strains, such as** Mb3601 (4) that allowed us to describe the **Eu3 clonal complex, are required** to perform precise field molecular epidemiological studies.

Goal

The aim of the study was to obtain ten new complete *M. bovis* genomes closer to French field strains belonging to main different *M. bovis* clonal groups present in France.

Methods

Ten strains representing the *M. bovis* French genotypic diversity were selected taking into account *French M. bovis* clonal groups and their epidemiological importance (1-2).

Whole genome sequence were obtained with **MinION (Oxford Nanopore)** and **MiSeq (Illumina)** technologies and were *de novo* assembled using a hybrid method with Flye, Raven and Unicycler assemblers and Trycycler to generate consensus genomes.

86 genomes obtained by Illumina technology in a previous study (2) and 4 complete genomes (Mb3601, AF2122/97, BCG Pasteur and BCG Tokyo) were added annotation was performed using Prokka tool and Mb3601 as reference. Pangenomic studies were carried out with **Panaroo** tool.

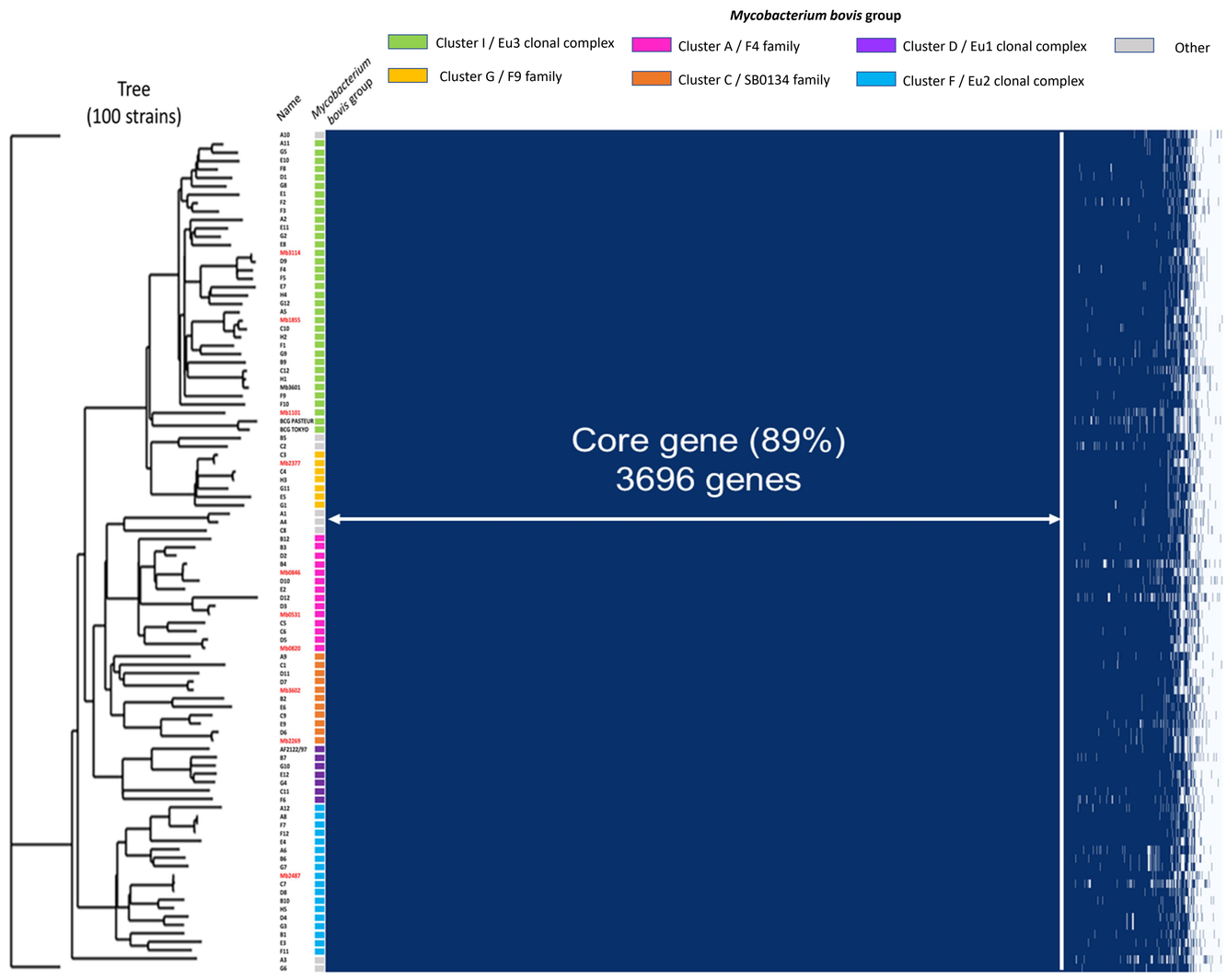

Fig 1. *Gene presence and absence in 100* strains*. The phylogenetic tree on the left is based on pan-genome core gene using 1708 genes. Phylogenetic tree use A10 strain is drawn at root with K3PU+F+I model. The new complete genomes are marked in red. The heatmap on the right shows gene presence in dark blue or gene absence in light blue. 4143 orthologous genes are identified. Core gene represents gene found in 100 or 99 genomes. The strains are grouped in 7 clusters which have been previously defined (2).*

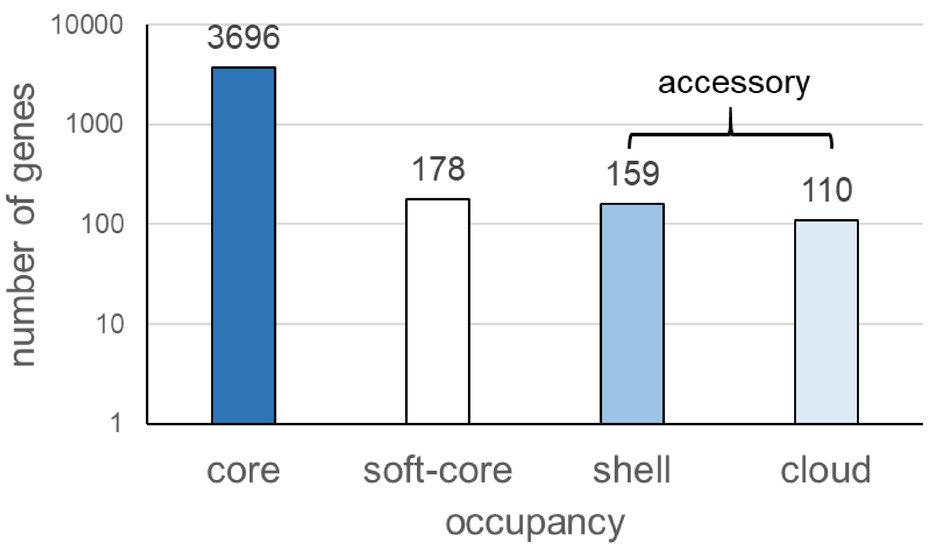

Fig 2. *Pangenomic histogram of 100* M. bovis *strains. The figure shows the core, soft-core, shell and cloud genes proportion in the genome's panel.*

Results and discussions

- **Ten new complete genomes of French *M. bovis*** strains were obtained with unique contigs for each assembly.
- Their characteristics are **very similar** and pangenomic studies show a high core gene similarity between genomes (Figure 1 and 2). However some differences can be observed such as the genome length. These differences are partially explained by differences in IS*6110* copy numbers (5) but also by the presence of deletions of different sizes (of more than 10 bp) that constitute **regions of difference** for these genomes.
- **Partial or complete gene deletion** can be involved by these genetic polymorphisms, some of them **specific to *M. bovis* groups**. E.g., sub-group of F4 family presents a specific deletion of the *bisc* gene (Mb0486, B4 and D10) (Figure 1).
- Further analyses on the complete genomes show **very few strain specific** genes and an accessory-genome average of 12% (Figure 3). These results show the presence of closed pangenomes in our panel in agreement with the already described high clonality of *M. bovis*.

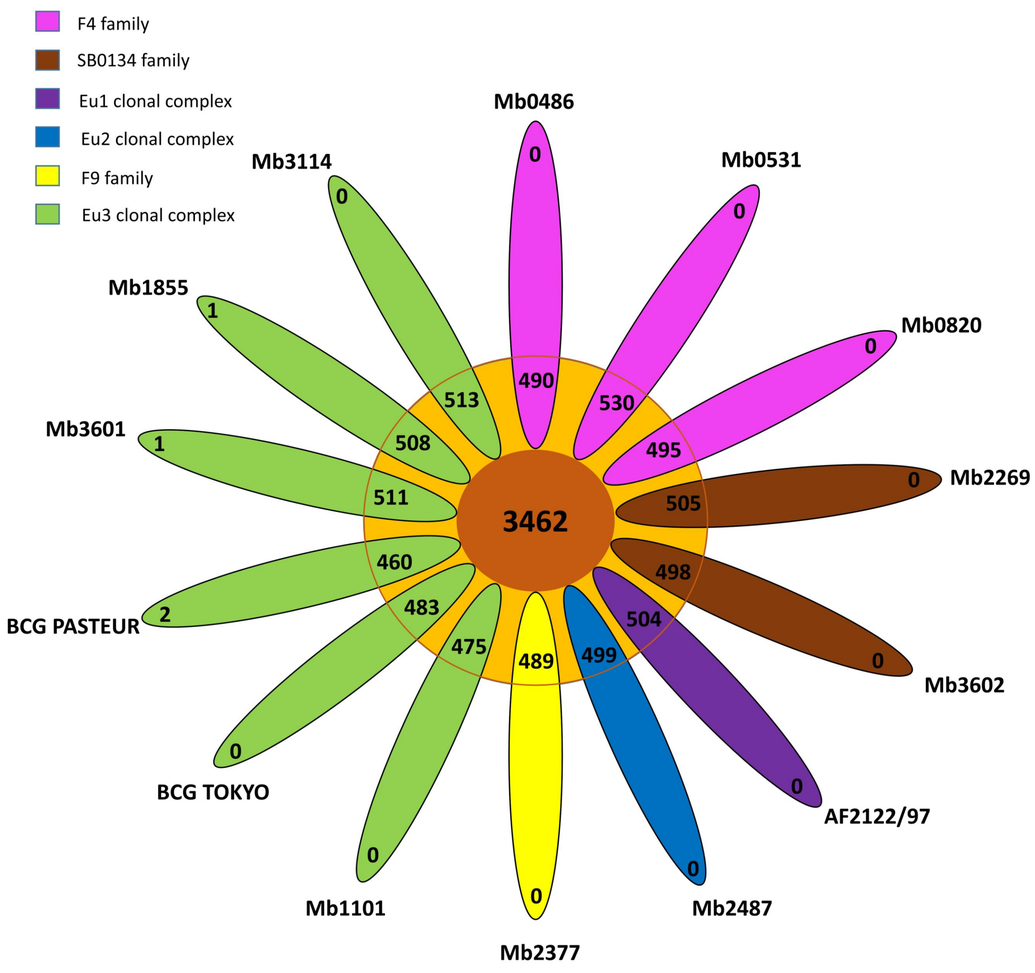

Fig 3. *Flower plot showing in the center genes present in all strains, genes present in some strains (dispensable) in the annulus, and strain specific genes of the 14* M. bovis *complete genomes in the petals. The analysis isolated the 14 complete genomes from the 100* M. bovis *strains presented in figure 1. Genomes are grouped in 6 groups previously described (Figure1) (2).*

Conclusions

Comparison of these complete genomes confirmed that the global genome organisation of *M. bovis* is remarkably stable. Research of large sequence polymorphisms will allow us to specify some genomic traits and absence of certain genes characterising each clonal group. These genomes will be useful to better understand the dynamic of bTB transmission between cattle and wild animals through precise molecular epidemiological studies and therefore to implement more effective control measures to eradicate bTB in these areas.

References

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