ABSTRACT

The motivation for this study was to have an in-depth understanding of the biology of *Mycoplasma mycoides* (*Mm*) cluster organisms that is hitherto deficient. The main objective was to apply transposon mutagenesis and synthetic biology technologies to decipher host pathogen interactions. For transposon mutagenesis a plasmid (pMT85-tetM-PRS313-LacZ) was introduced into *Mycoplasma mycoides* cluster strains by electroporation and or chemical methods. The developed transposon mutagenesis libraries proved to be invaluable tools to further study these pathogens affecting livestock. However, the libraries generated among the *Mycoplasma mycoides* subsp. *mycoides* and *Mycoplasma mycoides* subsp. *capri* were not stable over time as compared to those of *Mycoplasma capricolum* subsp. *capricolum*.

Subsequently, genes earlier on known to play a role in the virulence of Mycoplasma such as (GlpF, GlpK, GlpO, gtsA, gtsB, gtsC & gtsD) were deleted using the Tandem Repeat Endonuclease cleavage (TREC) method. This was under the assumption that the generated mutant (GM12::YCpMmyc1.1-Δ68) would turn out to be less virulent. To investigate this postulation, an *in-vivo* experiment model was advanced as proof of principle. Using a *Mycoplasma mycoides* subsp. *capri* (*Mmc*) strain, a known goat pathogen, goats were each infected with 10^9 cfus live bacteria in two groups as follows; the first group (n=8) received the wild type strain *Mmc* GM12 whereas the second (n=6) received the presumably attenuated strain, GM12::YCpMmyc1.1-Δ68). Interestingly, all animals that received the wild type strain developed clinical disease characterized by pneumonia, coughing, a high fever (an average of 41^{o}C), inappetence and later anorexia. In addition, all candidates in this group never lived past day 5 post infection (pi). In comparison, all animals that received the mutant strain, though developed mild clinical disease initially characterized by slight depression, just after inoculation and recovered, reverted to normal behavior, living up-to day 28 pi, the intended end time for the experiment.

The Karplan Meyer survival curve exhibited a clear distinction between the two experimental groups (*p-value* < 0.001). Further analysis of the mutant GM12::YCpMmyc1.1-Δ68 revealed that it had completely lost its immune evasion mechanism of Immunoglobulin G cleavage a trait otherwise retained in the parent strain.

In addition, the mutant strain lost its ability to produce Hydrogen peroxide in the presence of glycerol *in-vitro*.

This is the first time transposon mutant libraries are generated in Africa among Mycoplasma pathogens that affect livestock, invaluable tools for in-depth studies. Proof of the true virulence factors in *Mmc* in both *in-vitro* and *in-vivo* experiments was demonstrated. These approaches are a gateway to additional research that shall contribute to improving the existing vaccine(s) or better still, new vaccine or drug candidates developed for Mycoplasmas in the *Mm* cluster.