Design of a potential attenuated vaccine strain of Mycoplasma sp. by precise modification of an essential gene using synthetic biology approaches

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Abstract

Mycoplasma species are responsible for a number of economically significant animal diseases for which there is a need for new and improved vaccine strains. Most of existing mycoplasma vaccines are attenuated strains that have been empirically obtained by blind serial passages or by chemical treatment. The recent development of synthetic biology approaches has considerably opened the way for the engineering of mycoplasma genomes. Using these new tools on *Mycoplasma mycoides* subsp. *capri* (Mmc) genome, the essential GTPase-encoding gene *obg* was modified directly on Mmc genome cloned in yeast. The targeted modifications reproduced some mutations that are known to be associated in various bacteria with a temperature-sensitive phenotype. Once transplanted back into a recipient cell, the phenotype of the resulting mutants was characterized. Their temperature sensitivity varied according to the position and the number of mutations produced in the *obg* gene. The mutant showing the most affected phenotype is characterized by 3 mutations within the *obg* gene, which considerably lowers the probability of reversion to a wild type phenotype. This study demonstrates the feasibility to build targeted attenuated strains of mycoplasma that could be used as vaccines with improved safety.

Keywords: mycoplasme, genome engineering, vaccine, attenuation, virulence

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