Gene Knockout of Orotate Phosphoribosyltransferase (*pyrE*) from *Mycobacterium tuberculosis*

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Tuberculosis (TB) is a chronic infection mainly caused by *Mycobacterium tuberculosis* (MTB), killing over 2 million people annually. Patients' non-compliance to the current treatments, along with HIV infection, leads to the occurrence of multidrug-resistant and extensively drug-resistant TB strains, and to an increasing number of new cases. Novel TB treatments should overcome such drawbacks. Nucleotide metabolism is an essential pathway for microorganism viability; and pyrimidine biosynthesis has already been shown to be required for virulence of *T. gondii*. Orotate phosphoribosyltransferase (OPRT) catalyses the second reaction in the *de novo* synthesis of pyrimidine nucleotides, leading to OMP formation. The aim of this study was knockout the OPRT coding *pyrE* gene, through a two-step strategy. DNA sequences of 1 kb upstream and downstream of *pyrE* gene were individually amplified from the MTB genome and cloned into pCR-Blunt (Invitrogen) vectors. Both fragments were digested with appropriated enzymes and cloned into the p2NIL shuttle vector. A gene cassette containing resistance markers (*hyg, lacZ, sacB*) was then cloned into the p2NIL *PacI* restriction site, which allows selection on X-gal plates of proper suicide plasmids, as well as MTB cells where double recombination process has occurred. The obtained mutant MTB cells will be selected with uracil supplementation and tested for its infection and immunogenic capabilities. A MTB strain unable to catalyze *de novo* synthesis of pyrimidine nucleotides will probably constitute an attenuated strain. Such strain may have vaccinal properties, allowing an adequate immune response of the host to further wild-type infections. Keywords: tuberculosis, pyrimidine metabolism, orotate phosphoribosyltransferase. Financial support: BNDES, CAPES.