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Development of *Escherichia coli* and *Mycobacterium smegmatis* recombinants expressing major *Mycobacterium tuberculosis*-specific antigenic proteins

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Objective/background: *Mycobacterium tuberculosis* is an obligate pathogenic bacterial species in the family Mycobacteriaceae and the causative agent of most tuberculosis (TB) cases. Until today, the only approved TB vaccine is Bacille Calmette Guerin (BCG), which has been used since 1921. While BCG provides fairly effective protection for infants and young children, its efficacy in adults is variable around the world. This could be due to several parameters including strains of the vaccine and exposure of individuals to different environmental bacterial infections. The situation is complicated by the emergence of multidrug resistant strains of *M. tuberculosis*. This urged the demand to develop new improved vaccines and immunotherapies against TB. Development of nonpathogenic recombinant constructs delivering *M. tuberculosis*-specific antigenic proteins provides the chance to evaluate candidates to be included in diagnostic tools and preventive vaccines. In our study, we are introducing some of the major *M. tuberculosis* genes in *Escherichia coli* and *Mycobacterium smegmatis*.
Methods: DNA corresponding to the genes Rv3891, Rv3020, Rv0287, Rv3875, Rv3874, Rv3872, Rv2346c, and Rv3619 were PCR-amplified from *M. tuberculosis* genomic DNA and visualized on gel electrophoresis at the expected DNA size. Products were subsequently ligated to the plasmid pGEMTeasy and used to transform TOP10 *E. coli*. Transformed colonies were selected on appropriate media. At the second stage, genes-DNA were subcultured in expression vectors pDE22 and pGESTH1; the recombinant plasmids were finally used to transform. *M. smegmatis* and *E. coli*, respectively. Expression of proteins in *E. coli* was confirmed by Western blotting and in *M. smegmatis* by reverse transcriptase polymerase chain reaction (RT-PCR).
Results: Amplified genes were successfully cloned and transformed in *E. coli* and *M. smegmatis*. Colonies of recombinant bacteria were detected on appropriate media. Western blotting and RT-PCR confirmed the expression of our corresponding proteins in both the bacterial vehicles.
Conclusion: Positive results of cloning and expression suggest that the constructed clones are ready tools for further assessment of their immunogenicity and can be included in improved diagnostic tools and vaccines against TB.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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