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Comparative analysis of endogenous CRISPR/Cas and DNA repair associated-genes in mycobacteria: towards genetic manipulation for development on TB vaccines.

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Introduction: Genetic manipulation presents an important tool used in the development of new vaccines against tuberculosis, a persistent threat to global health. CRISPR technology has revolutionized genetic manipulation enabling an easy and practical approach to modify the genome in several organisms, including mycobacteria. In that regard, studying the endogenous CRISPR and the CRISPR-associated DNA repair pathways is crucial to better understand its application in mycobacteria.

Methods: Genome sequences from M. tuberculosis (Mtb), M. bovis (Mb) and BCG were recovered from GenBank. Gene sequences linked to the endogenous CRISPR system and DNA repair pathways were aligned and compared. 3D structure was predicted by homology using the SWISS-MODEL.

Results: Mycobacteria possess a type III CRISPR/Cas system characterized by specific genes (Cas1, Cas2, Cas6, Cas10, Csm2-6). Mb and BCG present a mutation in Csm6 that introduces an early stop codon. The palindromic repeats (CRISPR loci) are separated into two parts by two coding sequences (Mtb: 2814c-2815c, Mb: 2838c-2839c). The size of spacers ranged from 34 to 41 bp across all mycobacterial strains. Analysis of NHEJ- and SSA-related genes revealed several SNPs: ligD (C344R), recC (G329E, V3512L), recD (T536A), and recB (R514H, V811F, and a deletion leading to an early stop codon). Of all DNA repair pathways, HR appears to be highly conserved exhibiting a single SNP (V748A) in adnB.

Discussion and conclusion: Minimal differences were observed in all genes related to the endogenous CRISPR system and the DNA repair pathways. Notably, Csm6 (shortened in Mb and BCG) plays a crucial role in the processing of spacer sequences. CRISPR is highly dependent on the host's DNA repair mechanisms. The overall similarity suggests that the genetic manipulation through CRISPR is feasible across all mycobacterial strains evaluated. When applied to BCG it may provide prospects towards the development of new and improved vaccines.

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Conflicts of Interest
None