Display of an influenza virus multi-epitope on *B. subtilis* spores

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Bacterial spores are promising tools for the mucosal delivery for the development of new oral/nasal vaccines, offering stability, safety, and the ability to stimulate both humoral and cellular immune responses. This project explores the use of probiotic strains of intestinal origin of *B. subtilis* to display influenza epitopes and test them as mucosal vaccine on animal models. Compared to laboratory strains, probiotic strains may provide enhanced performance due to their adaptation to the gastrointestinal environment and to the positive effects of probiotics on the efficacy of mucosal immunizations. A probiotic strain of intestinal origin of *B. subtilis,* MV24*,* was selected according to the EFSA guidelines for safety. A chimeric Influenza A multi-epitope (ME-IVA) was designed, using IEDB, CALIBER and BepiPred 3.0 epitope prediction tools and a synthetic gene coding for ME-IVA produced. Specifically, the construct includes conserved T-cell epitopes from Influenza A Nucleoprotein (NP) and Matrix Protein 1 (M1), along with B-cell epitopes from Hemagglutinin (HA), Neuraminidase (NA), and the M2 ectodomain (M2e). These domains were connected with appropriate linkers to optimize processing, folding, and immunogenicity. The final sequence was codon-optimized for *E. coli* expression, allowing efficient overexpression and purification via affinity chromatography. The purified protein is being used for non-recombinant surface display through adsorption onto purified MV24 spores. In parallel, recombinant approaches are being developed by generating gene fusions between the ME-IVA sequence and spore coat proteins (CotB and CotC), enabling antigen display directly on the spore surface. Ongoing experiments are optimizing protein expression and surface display, which will allow future purification and immunological studies *in vivo*. This work focuses on developing a safe and stable probiotic spore-based platform for broad, cross-protective influenza vaccines.