## Development and analysis of prospective anti-HIV probiotic vaccines

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Major improvements have been made in the treatment and prevention of HIV/AIDS. However, a prophylactic vaccine is still unavailable, and several vaccine-candidate trials yielded less than favourable results. Given that the HIV pandemic has not slowed down significantly, there is an urgent need for the development of an effective vaccine. The HIV-1 Gag protein, a key player in HIV particle assembly, is a suitable antigen for use in HIV vaccine development since antibodies targeting HIV-1 Gag will interfere with the replication of the virus. In our vaccine development strategy, it was important for us to develop a candidate for mucosal administration. This is because the mucosal route is the major site for HIV transmission and early viral replication, which is associated with extensive and rapid depletion of CD4+ T-cells in the Gut-Associated Lymphoid Tissue (GALT). Here, we transformed probiotic strains of Lactobacillus plantarum and Lactobacillus fermentum with the recombinant plasmid vectors pSIP409 and pSIP411 harbouring the HIV-1 GagM gene. Following electroporation, HIV-1 GagM expression was induced in the probiotics using peptide pheromone. Via PCR and sequencing, the presence of GagM was confirmed in the L. plantarum+ pSIP409-GagM and L. fermentum+ pSIP411-GagM clones. Protein expression was induced with peptide pheromone. Then, protein expression was confirmed by western blotting with goat anti-HIV p24 primary antibody and anti-goat secondary antibody. ELISA was also performed to confirm the antigenicity of the HIV-1 Gag antigen and to also quantify the antigen in the two Lactobacilli clones. Our results show that 1.5×109 CFU of L. plantarum+ pSIP409-GagM expressed 125µg of HIV-1 Gag and 1.9×109 CFU of L. fermentum+ pSIP411-GagM clones expressed 125µg of HIV-1 Gag respectively. In vitro digestion with pepsin, pancreatin and bile salts suggested that partial digestion of the probiotic vaccine candidates may occur when administered orally. Taken together, our probiotic HIV-1 vaccine candidates showed good prospects for further immunological analysis via animal trial.