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***Lactococcus lactis* strains delivering IL-10 protein or cDNA to the intestinal mucosa show anti-inflammatory properties in a TNBS-induced chronic colitis model**

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Interleukin-10 (IL-10) is the most important anti-inflammatory cytokine at intestinal level. Oral treatments with IL-10 are inefficient because of its sensitivity to the harsh conditions of the GI tract, and systemic treatments cause undesirable side effects. Our aim was to compare the protective effects of IL-10, delivered by recombinant lactoccoci using two novel expression systems, in a murine colitis model mimicking the relapsing nature of inflammatory bowel diseases. The first system is based on a stress-inducible promoter (pGroES/L) for the production and delivery of heterologous proteins *in situ* at mucosal surfaces, and the second allows the delivery to the host cells of an *il-10* cDNA, harbored in a eukaryotic DNA expression vector (pValac). Colitis was induced in female BALB/c mice by intrarectal injection of TNBS. Mice that survived the challenge and recovered their body weight received one of the bacterial treatments or saline solution orally during 14 days. Colitis was reactivated 25 days after the first TNBS challenge with a second injection. Three days after colitis reactivation, cytokine profiles and inflammation in colon samples were evaluated. Animals receiving *L. lactis* delivering pGroES/L: *il-10* or pValac: *il-10* plasmids showed lower body weight loss and damage scores in their large intestines compared to inflamed non-treated mice. Both treatments also increased IL-10 concentration in the intestine, compared to the controls without treatment and maintained an increased ratio of IL-10/ pro-inflammatory cytokines. Our results confirm the protective effect of IL-10 delivered by *L. lactis* either as a protein or as a cDNA in a TNBS-induced chronic colitis model, and provides a step further in the use of genetically engineered bacteria as a valid system to deliver therapeutic molecules at mucosal level.

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Characterization of a cell surface serine protease in *Lactobacillus acidophilus* involved in immunomodulation and intestinal epithelial barrier integrity

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Health-promoting aspects attributed to probiotic microorganisms, including adhesion to intestinal epithelia and modulation of the host mucosal immune system, are mediated by proteins found on the bacterial cell surface. Notably, certain probiotic and commensal bacteria contain a surface (S-) layer as the outermost stratum of the cell wall. S-layers are semi-porous, crystalline arrays of self-assembling, proteinaceous subunits called S-layer proteins (Slps). In *Lactobacillus acidophilus* NCFM, the S-layer protein, SlpA, has been implicated in both mucosal immunomodulation and adhesion to host intestinal epithelium. As such, it is critical to explore the properties of S-layers and secreted cell surface proteins as a functional interface for probiotic activity. Here, we describe a gene (*lba1578)* encoding an extracellular, S-layer associated serine protease. To functionally characterize this protein, *lba1578* was deleted from the chromosome of *L. acidophilus*. Following co-incubation with dendritic cells, NCK2282 (*Δlba1578)* demonstrated increased immunomodulation of TNF-α, IL-6, and IL-10 compared to wild-type. Furthermore, there was a significant increase in tight-junction permeability for Caco-2 monolayers exposed to NCK2282 (*Δlba1578)* compared to NCFM parental strain. These data suggest that the S-layer associated serine protease LBA1578 exhibits immunmodulatory properties and may contribute to maintenance and integrity of the intestinal epithelium.