



**HAL**  
open science

## Lactococci and lactobacilli as mucosal delivery vectors for therapeutic proteins and DNA vaccines

Luis Bermudez Humaran, Pascale Kharrat, Jean-Marc Chatel, Philippe  
Langella

► **To cite this version:**

Luis Bermudez Humaran, Pascale Kharrat, Jean-Marc Chatel, Philippe Langella. Lactococci and lactobacilli as mucosal delivery vectors for therapeutic proteins and DNA vaccines. *Microbial Cell Factories*, 2011, 10, 10.1186/1475-2859-10-S1-S4 . hal-01189725

**HAL Id: hal-01189725**

**<https://hal.science/hal-01189725>**

Submitted on 29 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

PROCEEDINGS

Open Access

# Lactococci and lactobacilli as mucosal delivery vectors for therapeutic proteins and DNA vaccines

Luis G Bermúdez-Humarán, Pascale Kharrat, Jean-Marc Chatel, Philippe Langella\*

From 10th Symposium on Lactic Acid Bacterium  
Egmond aan Zee, the Netherlands. 28 August - 1 September 2011

## Abstract

Food-grade Lactic Acid Bacteria (LAB) have been safely consumed for centuries by humans in fermented foods. Thus, they are good candidates to develop novel oral vectors, constituting attractive alternatives to attenuated pathogens, for mucosal delivery strategies. Herein, this review summarizes our research, up until now, on the use of LAB as mucosal delivery vectors for therapeutic proteins and DNA vaccines. Most of our work has been based on the model LAB *Lactococcus lactis*, for which we have developed efficient genetic tools, including expression signals and host strains, for the heterologous expression of therapeutic proteins such as antigens, cytokines and enzymes. Resulting recombinant lactococci strains have been tested successfully for their prophylactic and therapeutic effects in different animal models: i) against human papillomavirus type 16 (HPV-16)-induced tumors in mice, ii) to partially prevent a bovine  $\beta$ -lactoglobulin (BLG)-allergic reaction in mice and iii) to regulate body weight and food consumption in obese mice. Strikingly, all of these tools have been successfully transposed to the *Lactobacillus* genus, in recent years, within our laboratory. Notably, anti-oxidative *Lactobacillus casei* strains were constructed and tested in two chemically-induced colitis models. In parallel, we also developed a strategy based on the use of *L. lactis* to deliver DNA at the mucosal level, and were able to show that *L. lactis* is able to modulate the host response through DNA delivery. Today, we consider that all of our consistent data, together with those obtained by other groups, demonstrate and reinforce the interest of using LAB, particularly lactococci and lactobacilli strains, to develop novel therapeutic protein mucosal delivery vectors which should be tested now in human clinical trials.

## Introduction

The administration of therapeutic molecules via mucosal routes offers several important advantages over systemic delivery such as reduction of secondary effects, easy administration and the possibility to modulate both systemic and mucosal immune responses [1]. Moreover, direct delivery of the appropriate medical molecules to exert their effects at mucosal surfaces is a very efficient prophylactic and therapeutic strategy. Mucosal surfaces are the primary interaction sites between an organism and its environment and they thus represent the major portal

of entry for pathogens. In the last fifteen years, there have been several reports of successful immunisation with a variety of mucosal vector vaccines [2]. They can induce efficient systemic immune responses with less collateral side effects than systemic vaccines [1]. Additionally, mucosal immunisation is more easily performed, without the need for needles and syringes, thereby eliminating the requirement for trained personnel (important feature for mass vaccination programs) [3]. Nevertheless, a major disadvantage is that a large amount of protein needs to be administered, due to the fact that the majority of protein will degrade, with very small quantities surviving degradation at mucosal surfaces such as the gastro intestinal tract [1]. Therefore, the development of new vectors, able to

\* Correspondence: philippe.langella@jouy.inra.fr  
INRA, UMR1319 Micalis, Commensal and Probiotics-Host Interactions  
Laboratory, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France  
Full list of author information is available at the end of the article

efficiently deliver molecules to target tissues represents a technological challenge.

Today, sufficient data is available supporting the fact that lactic acid bacteria (LAB), notably lactococci and lactobacilli, are excellent candidates as delivery vectors of therapeutic proteins, in the development of novel preventive and therapeutic strategies for humans. LAB are non-pathogenic Gram-positive bacteria and they have an extraordinary safety profile, since they have been widely consumed for centuries by humans in fermented foods. Therefore, they constitute an attractive alternative to attenuated pathogens, which are the most popular live vectors being used currently. Attenuated pathogenic bacteria, such as derivatives of *Mycobacterium*, *Salmonella*, and *Bordetella* spp. are particularly well adapted to interact with mucosal surfaces as most of them they normally use to initiate the infection process. Unfortunately, these bacteria can recover their pathogenic potential and are therefore not entirely safe for use in humans, especially in children and immunosuppressed patients. Several detailed reviews of vector delivery strategies, based on LAB, have been published within the last five years, of which three are particularly exhaustive and convincing [4-6]. Herein, we will be summarizing our current research and advances on the use of lactococci and lactobacilli as live delivery vectors of proteins with health interest. We will also be describing the use of LAB as DNA-vaccine-delivery vehicles to deliver DNA directly to antigen-presenting cells of the immune system.

### 1. Lactic acid bacteria as mucosal delivery vectors of antigens and cytokines

The immunogenicity of soluble proteins administered orally or intranasally is generally low and can be significantly enhanced by either coupling the protein to a bacterial carrier or by genetic engineering of bacteria resulting in the production of the desired antigen. As previously mentioned, food-grade or commensal Gram-positive bacteria constitute an attractive alternative to attenuated pathogenic bacteria [6]. In particular, the model LAB *Lactococcus lactis* and certain species of lactobacilli possess a number of properties, making them attractive candidates for the development of mucosal vaccines [7]. Moreover, many antigens and/or cytokines have been successfully expressed in LAB, and mucosal administration of these genetically engineered LAB has been shown to elicit both systemic and mucosal immunity (see additional file 1).

The production of a desired antigen by LAB can occur in three different cellular locations: i) intracellular, which allows the protein to escape harsh external environmental conditions (such as gastric juices in the

stomach after oral administration of the recombinant strain) but requires cellular lysis for protein release and delivery, ii) extracellular, which allows the release of the protein into the external medium, resulting in direct interaction with the environment (food product or the digestive tract), and iii) cell wall-anchored, which combines the advantages of the other two locations (i.e., interaction between the cell wall-anchored protein and the environment, in addition to protection from proteolytic degradation). In this context, several studies have compared the production of different antigens in LAB, using all three locations and evaluated the subsequent immunological impact [Reviewed in Refs. [6] and [7]]. Even if the comparison between the localization is difficult due to the amount of protein depending on the localization, these studies demonstrated that the highest immune response was usually obtained with cell-wall anchored antigens exposed to the surface of LAB. Therefore, most of the recent LAB vaccination studies have selected surface exposure of the antigen of interest, rather than intra- or extracellular production.

#### ***Lactococcus lactis* as live delivery vector of proteins of health interest**

*L. lactis* is the most widely used LAB in the production of fermented milk products, and is considered as the model LAB because many genetic tools have been developed in particular for heterologous protein production [8]. Moreover, *L. lactis* is considered to be a good candidate for heterologous protein production because it secretes relatively few proteins and only one, Usp45, in detectable quantities [9,10]. In addition, the most commonly used laboratory *L. lactis* strain (MG1363) is plasmid-free and does not produce any extracellular proteases [11]. However, the major advantage of using *L. lactis* as a live vector for mucosal delivery of therapeutic proteins resides in its extraordinary safety profile, since this bacterium is catalogued as a non-invasive and non-pathogenic organism with a GRAS status. Finally, the capacity of *L. lactis* to produce many different proteins of health interest has been clearly demonstrated in the last two decades (see additional file 1). All of these features explain why most of the relevant studies focusing on the use of LAB as protein and DNA delivery vectors have been performed with *L. lactis*.

#### **Heterologous proteins production in *L. lactis***

Currently, several inducible and constitutive expression signals are available for *L. lactis*[2][3,6]. In our studies, we mainly used the nisin inducible promoter (PnisA), which is the major element of the NICE (Nisin Induced Controlled Expression) system [12]. Nisin is a bacteriocin produced by *L. lactis*, which contains eleven adjacent chromosomal genes (*nisABTCIPRKFEFG*) encoding for biosynthesis and immunity against nisin [13]. The *nisA* gene encodes for the structural nisin gene, whereas *nisRK* encode for the dual-component system responsible for

the induction of other genes within the cluster. All of our genes of interest were cloned downstream of  $P_{nisA}$  and the resulting plasmid was introduced in *L. lactis* NZ9000 (MG1363 strain carrying *nisRK* genes on its chromosome). Addition of sub-inhibitor nisin concentration levels, into the culture medium, induces the expression of the gene of interest proportionally to the dose of nisin used. This system is now considered as the most efficient one for heterologous expression in *L. lactis* [14]. We have thus developed an efficient heterologous protein production-secretion system in *L. lactis* based on  $P_{nisA}$  and a small stable and well-characterized protein, *Staphylococcus aureus* nuclease (Nuc) [15].

A family of three expression vectors: pCYT, pSEC, and pCWA was developed, to allow protein targeting to be either intracellular, secreted, or cell wall anchored, respectively. We also constructed a fourth expression vector called pSEC:LEISS, which contains a synthetic propeptide (LEISSTCDA) identified as a production-secretion booster [16]. These vectors, also functional in several other LAB species including lactobacilli, streptococci, enterococci and bifidobacteria, have been successfully used to produce approximately 50 different heterologous proteins in *L. lactis* up to date (see additional file 1). More recently, we also developed a new bile salts-inducible promoter, which is currently being tested in *in vivo* experiments (data not shown). Concerning the possible host factors affecting production-secretion in *L. lactis*, we have identified *ybdD* which, once inactivated, induces an overproduction of secreted protein [Morello *et al.*, unpublished data]. In addition, the secretion machinery for *L. lactis* has also been complemented with *B. subtilis* SecDF, which induced an increase in both production and secretion rates [17]. Within our panel of *L. lactis* strains, we also have three mutants: one inactivated in the unique extracellular housekeeping protease HtrA [18], one inactivated in the major intracellular protease ClpP, and one inactivated in both HtrA and Clp [19]. These strains are essential to reach controlled and stable production of highly degraded proteins in the wild type *L. lactis* strain [20].

## 2. Use of recombinant lactococci to induce mucosal and systemic immune responses against bacterial and viral pathogens

Currently, a number of studies support the use of recombinant *L. lactis* to induce mucosal and systemic immune responses against a desired antigen [2,7]. In 1990, the first attempt to use *L. lactis* as a mucosal vaccine was performed with killed recombinant lactococci producing a cell wall-attached form of a *Streptococcus mutans* protective antigen (PAC). Mice immunized orally with this killed *L. lactis* recombinant strain developed PAC-specific serum IgG and mucosal IgA antibodies [21]. These results demonstrated, for the first time, that *L. lactis* can

efficiently present an antigen to the immune system. In 1993, Wells *et al.* [22] then reported, for the first time, of the use of live recombinant *L. lactis*, producing tetanus fragment C (TTFC), to protect mice via subcutaneous injection against a lethal challenge with tetanus toxin. Afterwards, the same group evaluated the effect of immunization route (oral or nasal administration) on live recombinant lactococci producing TTFC in mice [23,24]. Oral immunization in mice resulted in a lower serum IgG and mucosal IgA antibody response as compared to nasal immunisation; whereas the protective efficacy (*i.e.* challenge with tetanus toxin) was similar between both routes. Many studies have been conducted to analyze the expression of viral, bacterial or eukaryotic heterologous proteins in *L. lactis* (see additional file 1).

The immunogenicity of the resulting recombinant strains has been evaluated, in mouse models in some cases, with very promising results. Amongst them, one of the best documented projects is based on the use of recombinant *L. lactis* producing human papillomavirus type 16 (HPV-16) E7 antigen. This viral protein is considered as a major candidate antigen for vaccines against HPV-related cervical cancer, the second cause of cancer death in women. The intracellular production of E7 antigen led to its rapid degradation in the cytoplasm of *L. lactis*, even when produced in a protease-free *clpP* strain [25]. In contrast, secreted and cell wall-anchored forms are rescued from proteolysis and produced a higher level of E7 in *L. lactis* [25,26]. Antigen-specific humoral (production of E7 antibodies) and cellular (secretion of IL-2 and IFN- $\gamma$  cytokines) responses were observed after intranasal administration of recombinant lactococci expressing E7 antigen at different levels and in cellular locations to mice. The responses were significantly higher in mice immunized with *L. lactis* expressing E7 as a cell wall-anchored form [27]. Subsequently, the protective effect of mucosally co-administered live *L. lactis* strains expressing cell wall-anchored E7 and a secreted form of interleukin-12 to treat HPV-16-induced tumors in a murine model was then evaluated [28]. When challenged with lethal levels of tumor cell line TC-1 expressing E7, 50% of pre-treated mice demonstrated complete prevention of TC-1-induced tumors. Therapeutic immunization with these recombinant strains, (*i.e.*, 7 days after TC-1 injection) induced regression of palpable tumors in 35% of treated mice. These preclinical results suggest the feasibility of mucosal vaccination and/or immunotherapy against HPV-related cervical cancer using genetically engineered lactococci. Although most immunological studies have been performed with *L. lactis* producing TTFC and E7 antigen, the reports supporting the use of recombinant lactococci as mucosal vaccines continue to grow, and approximately more than 50 peer-reviewed publications have validated this potential to date (see additional file 1).

#### **Use of recombinant lactococci in cow's milk allergy model**

Cow's milk allergy (CMA) is a complex disorder and is the most common allergy in young infants, with an incidence rate of 2-6%, decreasing to 0.1-0.5 % in adulthood. CMA develops early in infancy within 12 to 24 months of birth, but 80-90% of affected children recover by acquiring tolerance to cow's milk by the age of 5 years [29,30]. *L. lactis* has been engineered to produce  $\beta$ -lactoglobulin (BLG), one of the major allergens found in cow's milk, resulting in LL-BLG. The recombinant BLG was produced predominantly in a soluble, intracellular, and mostly denatured form. Mucosal administration of LL-BLG strain induced BLG specific fecal IgA, although allergen-specific IgE, IgA, IgG1 or IgG2a were not detected in mice sera [31]. A similar immune response was reported after oral administration of recombinant *L. lactis* secreting a T-cell determinant IgE epitope of BLG [32]. Adel-Patient et al [33] then demonstrated that oral administration of recombinant *L. lactis* strains producing different amounts of recombinant BLG partially prevents mice from sensitization. Oral pre-treatment with these strains prevented a Th2-type immune response elicited by systemic sensitization, via reduction of specific IgE and the induction of allergen-specific IgG2a and fecal IgA antibodies. The intensity of the Th1 immune response induced correlates with the amount of recombinant BLG produced, since the most effective strains were those producing the highest amount of BLG [33].

Similar to oral administration, intranasal delivery of recombinant *L. lactis* strains did not induce the secretion of BLG specific antibodies, but elicited IFN- $\gamma$  production in murine splenocytes after BLG re-stimulation. Intranasal pre-treatment of mice with LL-BLG reduced airway eosinophilia influx and IL-5 secretion in bronchoalveolar lavage (BAL) after intranasal allergen challenge. In the same study, intranasal co-administration of recombinant LL-BLG and LL-IL12 elicited a protective Th1 immune-response, inhibiting the allergic response in mice without affecting specific BLG IgE secretion [34]. Elsewhere, we also showed that intranasal administration of LL-IL12 strain decreased allergy symptoms in an asthma model induced by ovalbumin [35]. The effects of intranasal administration of LL-BLG strain were also tested in a therapeutic protocol. In orally sensitized mice, intranasal administration of recombinant strain reduced IgG1 production but did not influence specific BLG IgE or IgG2a secretion. After intranasal challenge, a mild decrease in IL-4 and IL-5 secreted into BAL was detected [36].

#### **Effects of intranasal administration of recombinant *L. lactis* strains secreting human leptin in *ob/ob* mice**

Leptin is a 16 kDa protein encoded by the obese (*ob*) gene, and is an adipocyte-derived pleiotropic hormone

that modulates a large number of physiological functions, including control of body weight and regulation of the immune system [37]. In humans, leptin plays a crucial role in regulation of body weight, as demonstrated by morbid obesity in patients with congenital mutations in either leptin or the leptin receptor gene [38-41]. When body fat increases, leptin inhibits food intake and stimulates energy expenditure to control body weight. Although leptin treatment induced remarkable weight-loss in patients with rare congenital leptin deficiency [42-45], it showed poor efficiency in most obese patients. Indeed, clinical trials involving subcutaneous administration of recombinant leptin to obese subjects indicated that a significant reduction of body weight was only observed if serum leptin concentrations were 20- to 30-fold higher than normal physiological levels [46]. This poor response was attributed in part to insufficient transport of leptin across the blood brain barrier in obese patients [47].

Since intranasal delivery is an efficient route for administration of drugs directly to the brain [48-50], we considered that intranasal leptin administration may be an interesting strategy to bypass the blood brain barrier in leptin resistant humans. Thus, the aim of our project was to measure the effect of intranasal administration of a recombinant *L. lactis* strain secreting a biologically active form of leptin (LL-LEP) in *ob/ob* mice. We first demonstrated that the secreted recombinant leptin is a fully biologically active hormone, by showing its capacity to stimulate a STAT3 reporter gene in HEK293 cells transfected with the Ob-Rb leptin receptor [51]. The immunomodulatory activity of the LL-LEP strain was then evaluated *in vivo* by co-expression with the *L. lactis* strain expressing human papillomavirus type-16 (HPV-16) E7 protein (LL-E7) [51]. In C57BL/6 mice immunized intranasally with LL-LEP and LL-E7 strains, the adaptive immune response was significantly higher than in mice immunized with LL-E7 only, demonstrating the adjuvanticity of leptin. We then analyzed the effect of daily intranasal administration of LL-LEP in *ob/ob* mice and thus observed that this treatment induced a significant reduction in body weight gain and food intake [51]. These results demonstrate that leptin can be produced and secreted in an active form by *L. lactis*, and that the LL-LEP strain regulated *in vivo* antigen-specific immune responses, as well as body weight and food consumption.

#### **Immune response to antigens delivered by *Lactobacillus* spp**

The use of genetically modified lactobacilli (i.e. *Lb. fermentum*, *Lb. acidophilus*, *Lb. casei* and *Lb. plantarum*) to produce heterologous proteins and to develop a new generation of mucosal vaccines was first proposed during the 90s decade [52,53]. By the end of the 90s and into the early 2000s, several laboratories had successfully utilized



recombinant strains of *Lb. casei* and *Lb. plantarum* as vehicles for delivery of medically relevant proteins to mucosal surfaces, with both strains stimulating strong local immune responses [6,54]. Approximately 50 peer-reviewed publications have already been published confirming the advantages of the *Lactobacillus* genus to serve as live mucosal vaccines, since lactobacilli can persist longer in the digestive tract and some strains have probiotic properties (i.e. show health-promoting activities for humans and animals) [54]. Similar to *L. lactis*, several studies analyzing the expression of a variety of viral, bacterial or eukaryotic origin proteins in *Lb. plantarum* and *Lb. casei* have been conducted (see additional file 1). We have evaluated the immunogenicity of E7 antigen producing recombinant *Lb. plantarum* in mouse models with promising results [55].

#### **Use of recombinant *Lb. casei* in cow's milk allergy model**

We recently developed a recombinant strain of *Lactobacillus casei* capable of producing BLG. The immunomodulatory potency of intranasal and oral administration of this recombinant lactobacilli on a subsequent sensitization of mice to BLG was investigated by Hazebrouck et al. [56], who analyzed the influence of the administration route on the immune response elicited by the recombinant BLG *Lb. casei* producing strain. Intranasal pre-administration of the BLG-producing *Lb. casei* enhanced BLG-specific IgG2a and IgG1 responses, but did not influence BLG-specific IgE production in sensitized mice. Unexpectedly, oral pre-administration led to a significant inhibition of BLG-specific IgE production, whereas IgG1 and IgG2a responses were not stimulated in sensitized mice. The production of IL-17 by BLG-reactivated splenocytes was similar between oral and intranasal route administrations. However in BLG-reactivated splenocytes from mice intranasally pretreated, a greater secretion level of Th1 cytokines (IFN- $\gamma$  and IL-12) and Th2 cytokines (IL-4 and IL-5) was detected, suggesting a mixed Th1/Th2 cell response; whereas only production of Th1 cytokines, but not Th2 cytokines, was enhanced in BLG-reactivated splenocytes from mice orally pretreated. Those results indicate that the mode of administration of recombinant LAB may be critical for their immunomodulatory properties [56].

#### **Anti-oxidative proteins delivery by *Lb. casei* in colitis-induced murine models**

Inflammatory bowel diseases (IBD) constitute a group of disorders characterized by chronic and relapsing inflammation of the gastrointestinal tract (GIT). The two most common forms of IBD are Crohn's disease and ulcerative colitis, which are associated with an influx of neutrophils and macrophages, resulting in the consequent production of inflammatory mediators such as proteases, cytokines and reactive oxygen species (ROS) [57]. ROS include the superoxide radical ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), and the hydroxyl radical ( $HO^{\cdot}$ ) [58], which have all been demonstrated to be both cytotoxic and mutagenic (i.e.

cause damage to cellular lipids, proteins and DNA) [59]. To detoxify ROS, cells have evolved protective mechanisms via antioxidant enzymes such as superoxide dismutases (SOD) and catalases (CAT), which degrade  $O_2^{\cdot-}$  and  $H_2O_2$ , respectively, thereby preventing the formation of  $HO^{\cdot}$  [60]. Thus, therapeutic use of antioxidant enzymes to decrease ROS amount level is a promising strategy to prevent and/or cure IBD. Several studies have shown that LAB, such as lactobacilli, may play a preventative role in IBD [61,62]. Under this context, we originally demonstrated that *Lb. casei* BL23 strain can attenuate moderate Dextran Sodium Sulfate (DSS) induced colitis in mice [63]. However, the use of a recombinant *Lb. casei* BL23 strain producing manganese CAT (MnCAT) did not improve the protective effect of inflammation reduction [63]. On the other hand, other recent studies have successfully reported the use of either recombinant *Lb. gasseri* or *Lb. plantarum* strains to produce and deliver *in situ* biologically active manganese SOD (MnSOD) to treat colitis in an interleukin-10 (IL-10) knockout mouse model and in a 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced colitis in rats [64][65]. We then cloned MnSOD from *Lactococcus lactis* into *Lb. casei* BL23 to evaluate the potential increase in the aforementioned protective effect towards ROS by delivery of MnSOD [66]. We therefore compared the effect of intragastric administration of *Lb. casei* BL23 MnSOD alone or in combination with *Lb. casei* BL23 MnCAT in the murine model of DSS 3%-induced colitis. Based on histological scores, a significant reduction of caecal and colonic inflammation was observed with either administration of *Lb. casei* BL23 MnSOD alone or the co-administration of *Lb. casei* BL23 MnCAT and *Lb. casei* BL23 MnSOD. However, there was no additional improvement in inflammation reduction with the administration of *Lb. casei* BL23 MnCAT as compared to administering *Lb. casei* BL23 MnSOD alone. These results suggest that *Lb. casei* BL23 MnSOD may have an anti-inflammatory effect on gut inflammation. More recently, we demonstrated that both *Lb. casei* BL23 MnSOD and MnCAT were able to significantly attenuate TNBS-induced inflammation damage in mice as shown by higher survival rates, decreased animal weight loss, lower bacterial translocation to the liver and the prevention of damage to the large intestine [66].

### **3. Recombinant lactic acid bacteria as DNA delivery vehicles**

The advantage of DNA vaccines relies in their ability to induce both cellular and humoral Th1 immune responses [67,68]. In contrast to bacteria-mediated delivery of protein antigens, bacteria-mediated delivery of DNA vaccines leads to the expression of post-translationally modified antigens by host cells resulting in presentation of conformationally restricted epitopes to the immune system [69].

As for protein delivery, the use of food-grade lactococci and lactobacilli as DNA delivery vehicles is a promising alternative to attenuated pathogens.

#### ***L. lactis* is able to modulate the host immune response through cDNA delivery**

Recombinant BLG is expressed mainly in denatured form with *E. coli* or *L. lactis*, whereas its production, in eukaryotic cells, is in the native conformation [31,70]. *L. lactis* strains have been used to deliver an expression cassette encoding BLG cDNA, under the transcriptional control of the CMV viral promoter, into the Caco-2 epithelial cell line. The expression cassette was inserted into a *L. lactis* replicating plasmid. Production and secretion of BLG was observed in Caco-2 cells after incubation with *L. lactis* carrying the expression plasmid, demonstrating that non-invasive *L. lactis* can deliver fully functional plasmids into epithelial cells. Interestingly, no production of BLG was observed when Caco-2 cells were co-incubated with purified plasmid alone or mixed with *L. lactis*, suggesting that the plasmid requires to be inside the bacterium in order to achieve transfer into epithelial cells with subsequent BLG production [71]. After oral administration of *L. lactis* in mice, carrying the eukaryotic expression cassette encoding for BLG, both BLG cDNA and protein were detected in the small intestine 72 hours after the final administration. No BLG (protein/dna or both) was detected 6 days after the last oral administration. Mice developed a BLG specific Th1 primary immune response, characterized by a weak and transitory IgG2a serum response. In sensitized pre-treated mice, IgE and IL-5 concentrations decreased by 70 and 40%, respectively as compared to sensitized naive mice. Moreover, only splenocytes from pre-treated mice secreted IFN- $\gamma$  after BLG specific re-activation [72]. The *in situ* production elicits a specific immune response protecting the mice from further sensitization with cow's milk proteins. To our knowledge, this is the first evidence of functional genetic material transfer from food-grade transiting bacteria to a host.

#### **Recombinant invasive lactococci as DNA delivery vehicles**

As demonstrated with recombinant *E. coli*, invasion of the host cell is a limiting step to achieve efficient DNA vaccine delivery [73]. To increase lactococcal DNA vaccine delivery efficiency, *L. lactis* was rendered invasive by expression of the *inlA* gene of *Listeria monocytogenes*, encoding for the Internalin A surface protein, which mediates the invasion of non phagocytic cells by *L. monocytogenes* [74,75]. Once expressed by *L. lactis*, InlA can promote the internalization of lactococci into the human epithelial line Caco-2 *in vitro* and into enterocytes *in vivo* after oral administration to guinea pigs. In addition, *L. lactis* InlA+ can deliver a functional plasmid encoding for GFP, and about 1% of Caco-2 cells express GFP after co-culture with this strain [76]. Recombinant invasive *L. lactis* strains expressing the *Staphylococcus aureus* Fibronectin Binding

Protein A encoding gene also showed a heightened ability to be internalized into mammalian cells as compared to the control strain. Consequently, both recombinant invasive strains were more efficient in eGFP expression plasmid delivery into Caco-2 cells, resulting in a higher number of GFP producing cells [77]. *In vivo*, *L. lactis* InlA+ was able to invade guinea pig enterocytes after oral administration [76].

#### **Conclusions and future challenges**

We consider that all of our consistent data, together with those obtained from other groups (see additional file 1), reinforce the interest in using lactococci and lactobacilli strains to develop novel therapeutic protein mucosal delivery vectors, which should be tested now in human clinical trials. Therefore, a biocontainment strategy to prevent the dissemination in the environment of these genetically modified LAB should be developed before they can be used in humans as it is mentioned in a recent review on these strategies [78]. Following the demonstration that an IL-10-producing *L. lactis* strain (LL-IL10) could treat colitis in mouse models [79], Steidler *et al* developed the first biocontainment system for LL-IL10 strain in order to be allowed to start the first human clinical study using this recombinant strain. To address these safety concerns with the use of LL-IL10 in humans, the chromosomal thymidylate synthase (*thyA*) gene was replaced by the gene encoding for IL-10 to generate a thymidine auxotroph phenotype. In the absence of thymidine or thymine, the viability of the *thyA* LL-IL10 strain was reduced by several orders of magnitude and containment was validated *in vivo* in pigs [80]. A phase I clinical trial was then conducted with the *thyA* LL-IL10 strain in human patients suffering from Crohn's disease, demonstrating that the containment strategy was effective [81]. Following those positive results, a phase IIA trial was performed and a press release was published by the end of 2009 revealing that all three primary endpoints of the study have been met: i) safety and tolerability; ii) environmental containment and iii) assessment of biomarkers associated with the strain. With respect to the secondary endpoint, the clinical results did not reveal a statistically significant difference in mucosal healing versus placebo. In view of these results, the authors of this pioneering human clinical trial and other teams involved in this promising field should now consider the optimization of some aspects of their LAB delivery strategy. The improvement should be done at different levels such as the nature i) of the delivered molecule; ii) of the LAB species as *Lb. casei* for example seems to show some advantages compared to *L. lactis* and iii) of the expression system to increase the quantities of the delivered molecule *in situ*. Such efforts should and need to be continued because the future of prophylactic and therapeutic strategies based on recombinant lactococci and

lactobacilli requires clear demonstration of their efficacy in such human clinical trials, which will lead to their better acceptance.

## Additional material

**Additional file 1: Summary of therapeutic proteins expressed by lactococci and lactobacilli and validated in animal studies.**

### Acknowledgements

This article has been published as part of *Microbial Cell Factories* Volume 10 Supplement 1, 2011: Proceedings of the 10th Symposium on Lactic Acid Bacterium. The full contents of the supplement are available online at <http://www.microbialcellfactories.com/supplements/10/S1>.

### Competing interests

The authors declare that they have no competing interests.

Published: 30 August 2011

### References

- Bermudez-Humarán LG: **Lactococcus lactis** as a live vector for mucosal delivery of therapeutic proteins. *Hum Vaccin* 2009, **5**(4):264-267.
- Holmgren J, Czerkinsky C: Mucosal immunity and vaccines. *Nat Med* 2005, **11**(4 Suppl):S45-53.
- Bermudez-Humarán LG, Langella P: Perspectives for the development of human papillomavirus vaccines and immunotherapy. *Expert Rev Vaccines* 2010, **9**(1):35-44.
- Bahey-El-Din M, Gahan CG, Griffin BT: **Lactococcus lactis** as a cell factory for delivery of therapeutic proteins. *Curr Gene Ther* 2010, **10**(1):34-45.
- Bahey-El-Din M, Gahan CG: **Lactococcus lactis**-based vaccines: Current status and future perspectives. *Hum Vaccin* 2011, **7**(1).
- Wells JM, Mercenier A: Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. *Nat Rev Microbiol* 2008, **6**(5):349-362.
- Bermudez-Humarán LG, Corthier G, Langella P: recent advances in the use of **Lactococcus lactis** as live recombinant vector for the development of new safe mucosal vaccines. *Recent Res Devel Microbiology* 2004, **8**:147-160.
- Morello E, Bermudez-Humarán LG, Llull D, Sole V, Miraglio N, Langella P, Poquet I: **Lactococcus lactis**, an efficient cell factory for recombinant protein production and secretion. *J Mol Microbiol Biotechnol* 2008, **14**(1-3):48-58.
- van Asseldonk M, Rutten G, Oteman M, Siezen RJ, de Vos WM, Simons G: Cloning of **usp45**, a gene encoding a secreted protein from **Lactococcus lactis** subsp. **lactis** MG1363. *Gene* 1990, **95**(1):155-160.
- van Asseldonk M, de Vos WM, Simons G: Functional analysis of the **Lactococcus lactis** **usp45** secretion signal in the secretion of a homologous proteinase and a heterologous **alpha**-amylase. *Mol Gen Genet* 1993, **240**(3):428-434.
- Gasson MJ: Plasmid complements of **Streptococcus lactis** NCDO 712 and other lactic streptococci after protoplast-induced curing. *J Bacteriol* 1983, **154**(1):1-9.
- de Ruyter PG, Kuipers OP, Beerthuyzen MM, van Alen-Boerrigter I, de Vos WM: Functional analysis of promoters in the **nisin** gene cluster of **Lactococcus lactis**. *J Bacteriol* 1996, **178**(12):3434-3439.
- Kuipers OP, Beerthuyzen MM, de Ruyter PG, Luesink EJ, de Vos WM: Autoregulation of **nisin** biosynthesis in **Lactococcus lactis** by signal transduction. *J Biol Chem* 1995, **270**(45):27299-27304.
- Mierau J, Kleerebezem M: 10 years of the **nisin**-controlled gene expression system (NICE) in **Lactococcus lactis**. *Appl Microbiol Biotechnol* 2005, **68**(6):705-717.
- Le Loir Y, Gruss A, Ehrlich SD, Langella P: Direct screening of recombinants in gram-positive bacteria using the secreted staphylococcal nuclease as a reporter. *J Bacteriol* 1996, **178**(14):4333.
- Le Loir Y, Gruss A, Ehrlich SD, Langella P: A nine-residue synthetic propeptide enhances secretion efficiency of heterologous proteins in **Lactococcus lactis**. *J Bacteriol* 1998, **180**(7):1895-1903.
- Nouaille S, Morello E, Cortez-Peres N, Le Loir Y, Commissaire J, Gratadoux JJ, Pomerol E, Gruss A, Langella P: **Complementation of the Lactococcus lactis** secretion machinery with **Bacillus subtilis** **SecDF** improves secretion of staphylococcal nuclease. *Appl Environ Microbiol* 2006, **72**(3):2272-2279.
- Poquet I, Saint V, Seznec E, Simoes N, Bolotin A, Gruss A: **HtrA** is the unique surface housekeeping protease in **Lactococcus lactis** and is required for natural protein processing. *Mol Microbiol* 2000, **35**(5):1042-1051.
- Frees D, Ingmer H: **ClpP** participates in the degradation of misfolded protein in **Lactococcus lactis**. *Mol Microbiol* 1999, **31**(1):79-87.
- Cortes-Perez NG, Poquet I, Oliveira M, Gratadoux JJ, Madsen SM, Miyoshi A, Corthier G, Azevedo V, Langella P, Bermudez-Humarán LG: **Construction and characterization of a Lactococcus lactis** strain deficient in intracellular **ClpP** and extracellular **HtrA** proteases. *Microbiology* 2006, **152**(Pt 9):2611-2618.
- Iwaki M, Okahashi N, Takahashi I, Kanamoto T, Sugita-Konishi Y, Aibara K, Koga T: **Oral immunization with recombinant Streptococcus lactis** carrying the **Streptococcus mutans** surface protein antigen gene. *Infect Immun* 1990, **58**(9):2929-2934.
- Wells JM, Wilson PW, Norton PM, Gasson MJ, Le Page RW: **Lactococcus lactis**: high-level expression of tetanus toxin fragment C and protection against lethal challenge. *Mol Microbiol* 1993, **8**(6):1155-1162.
- Norton PM, Wells JM, Brown HW, Macpherson AM, Le Page RW: **Protection against tetanus toxin in mice nasally immunized with recombinant Lactococcus lactis** expressing tetanus toxin fragment C. *Vaccine* 1997, **15**(6-7):616-619.
- Robinson K, Chamberlain LM, Schofield KM, Wells JM, Le Page RW: **Oral vaccination of mice against tetanus with recombinant Lactococcus lactis**. *Nat Biotechnol* 1997, **15**(7):653-657.
- Bermudez-Humarán LG, Langella P, Miyoshi A, Gruss A, Guerra RT, Montes-de-Oca-Luna R, Le Loir Y: **Production of human papillomavirus type 16 E7 protein in Lactococcus lactis**. *Appl Environ Microbiol* 2002, **68**(2):917-922.
- Cortes-Perez NG, Bermudez-Humarán LG, Le Loir Y, Rodriguez-Padilla C, Gruss A, Saucedo-Cardenas O, Langella P, Montes-de-Oca-Luna R: **Mice immunization with live lactococci displaying a surface anchored HPV-16 E7 oncoprotein**. *FEMS Microbiol Lett* 2003, **229**(1):37-42.
- Bermudez-Humarán LG, Cortes-Perez NG, Le Loir Y, Alcocer-Gonzalez JM, Tamez-Guerra RS, de Oca-Luna RM, Langella P: **An inducible surface presentation system improves cellular immunity against human papillomavirus type 16 E7 antigen in mice after nasal administration with recombinant lactococci**. *J Med Microbiol* 2004, **53**(Pt 5):427-433.
- Bermudez-Humarán LG, Cortes-Perez NG, Lefevre F, Guimaraes V, Rabot S, Alcocer-Gonzalez JM, Gratadoux JJ, Rodriguez-Padilla C, Tamez-Guerra RS, Corthier G, et al: **A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors**. *J Immunol* 2005, **175**(11):7297-7302.
- Exl BM, Fritsche R: **Cow's milk protein allergy and possible means for its prevention**. *Nutrition* 2001, **17**(7-8):642-651.
- Crittenden RG, Bennett LE: **Cow's milk allergy: a complex disorder**. *J Am Coll Nutr* 2005, **24**(6 Suppl):582S-591S.
- Chatel JM, Langella P, Adel-Patient K, Commissaire J, Wal JM, Corthier G: **Induction of mucosal immune response after intranasal or oral inoculation of mice with Lactococcus lactis producing bovine beta-lactoglobulin**. *Clin Diagn Lab Immunol* 2001, **8**(3):545-551.
- Chatel JM, Nouaille S, Adel-Patient K, Le Loir Y, Boe H, Gruss A, Wal JM, Langella P: **Characterization of a Lactococcus lactis** strain that secretes a major epitope of bovine beta-lactoglobulin and evaluation of its immunogenicity in mice. *Appl Environ Microbiol* 2003, **69**(11):6620-6627.
- Adel-Patient K, Ah-Leung S, Creminon C, Nouaille S, Chatel JM, Langella P, Wal JM: **Oral administration of recombinant Lactococcus lactis** expressing bovine beta-lactoglobulin partially prevents mice from sensitization. *Clin Exp Allergy* 2005, **35**(4):539-546.
- Cortes-Perez NG, Ah-Leung S, Bermudez-Humarán LG, Corthier G, Wal JM, Langella P, Adel-Patient K: **Intranasal coadministration of live lactococci producing interleukin-12 and a major cow's milk allergen inhibits allergic reaction in mice**. *Clin Vaccine Immunol* 2007, **14**(3):226-233.
- Wu C, Yang G, Bermudez-Humarán LG, Pang Q, Zeng Y, Wang J, Gao X: **Immunomodulatory effects of IL-12 secreted by Lactococcus lactis** on



- Th1/Th2 balance in ovalbumin (OVA)-induced asthma model mice. *Int Immunopharmacol* 2006, **6**(4):610-615.
36. Cortes-Perez NG, Ah-Leung S, Bermudez-Humaran LG, Corthier G, Langella P, Wal JM, Adel-Patient K: **Allergy therapy by intranasal administration with recombinant *Lactococcus lactis* Producing bovine beta-lactoglobulin.** *Int Arch Allergy Immunol* 2009, **150**(1):25-31.
37. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: **Positional cloning of the mouse obese gene and its human homologue.** *Nature* 1994, **372**(6505):425-432.
38. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, *et al*: **Congenital leptin deficiency is associated with severe early-onset obesity in humans.** *Nature* 1997, **387**(6636):903-908.
39. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD: **A leptin missense mutation associated with hypogonadism and morbid obesity.** *Nat Genet* 1998, **18**(3):213-215.
40. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, *et al*: **A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction.** *Nature* 1998, **392**(6674):398-401.
41. Strosberg AD, Issad T: **The involvement of leptin in humans revealed by mutations in leptin and leptin receptor genes.** *Trends Pharmacol Sci* 1999, **20**(6):227-230.
42. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S: **Effects of recombinant leptin therapy in a child with congenital leptin deficiency.** *N Engl J Med* 1999, **341**(12):879-884.
43. Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, *et al*: **Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency.** *J Clin Invest* 2002, **110**(8):1093-1103.
44. Gibson WT, Farooqi IS, Moreau M, DePaoli AM, Lawrence E, O'Rahilly S, Trussell RA: **Congenital leptin deficiency due to homozygosity for the Delta133G mutation: report of another case and evaluation of response to four years of leptin therapy.** *J Clin Endocrinol Metab* 2004, **89**(10):4821-4826.
45. Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, *et al*: **Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults.** *Proc Natl Acad Sci U S A* 2004, **101**(13):4531-4536.
46. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, *et al*: **Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial.** *JAMA* 1999, **282**(16):1568-1575.
47. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, Considine RV: **Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance.** *Lancet* 1996, **348**(9021):159-161.
48. Lawrence D: **Intranasal delivery could be used to administer drugs directly to the brain.** *Lancet* 2002, **359**(9318):1674.
49. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL: **Sniffing neuropeptides: a transnasal approach to the human brain.** *Nat Neurosci* 2002, **5**(6):514-516.
50. Hallschmid M, Benedict C, Born J, Fehm HL, Kern W: **Manipulating central nervous mechanisms of food intake and body weight regulation by intranasal administration of neuropeptides in man.** *Physiol Behav* 2004, **83**(1):55-64.
51. Hazebrouck S, Oozeer R, Adel-Patient K, Langella P, Rabot S, Wal JM, Corthier G: **Constitutive delivery of bovine beta-lactoglobulin to the digestive tracts of gnotobiotic mice by engineered *Lactobacillus casei*.** *Appl Environ Microbiol* 2006, **72**(12):7460-7467.
52. Rush CM, Hafner LM, Timms P: **Lactobacilli: vehicles for antigen delivery to the female urogenital tract.** *Adv Exp Med Biol* 1995, **371B**:1547-1552.
53. Pouwels PH, Leer RJ, Boersma WJ: **The potential of *Lactobacillus* as a carrier for oral immunization: development and preliminary characterization of vector systems for targeted delivery of antigens.** *J Biotechnol* 1996, **44**(1-3):183-192.
54. Seegers JF: **Lactobacilli as live vaccine delivery vectors: progress and prospects.** *Trends Biotechnol* 2002, **20**(12):508-515.
55. Cortes-Perez NG, Lefevre F, Corthier G, Adel-Patient K, Langella P, Bermudez-Humaran LG: **Influence of the route of immunization and the nature of the bacterial vector on immunogenicity of mucosal vaccines based on lactic acid bacteria.** *Vaccine* 2007, **25**(36):6581-6588.
56. Hazebrouck S, Przybylski-Nicaise L, Ah-Leung S, Adel-Patient K, Corthier G, Langella P, Wal JM: **Influence of the route of administration on immunomodulatory properties of bovine beta-lactoglobulin-producing *Lactobacillus casei*.** *Vaccine* 2009, **27**(42):5800-5805.
57. Segui J, Gironella M, Sans M, Granell S, Gil F, Gimeno M, Coronel P, Pique JM, Panes J: **Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine.** *J Leukoc Biol* 2004, **76**(3):537-544.
58. Keshavarzian A, Banan A, Farhadi A, Komanduri S, Mutlu E, Zhang Y, Fields JZ: **Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease.** *Gut* 2003, **52**(5):720-728.
59. Grisham MB, Gaginella TS, von Ritter C, Tamai H, Be RM, Granger DN: **Effects of neutrophil-derived oxidants on intestinal permeability, electrolyte transport, and epithelial cell viability.** *Inflammation* 1990, **14**(5):531-542.
60. Rochat T, Gratadoux JJ, Gruss A, Corthier G, Maguin E, Langella P, van de Guchte M: **Production of a heterologous nonheme catalase by *Lactobacillus casei*: an efficient tool for removal of H<sub>2</sub>O<sub>2</sub> and protection of *Lactobacillus bulgaricus* from oxidative stress in milk.** *Appl Environ Microbiol* 2006, **72**(8):5143-5149.
61. Gosselink MP, Schouten WR, van Lieshout LM, Hop WC, Laman JD, Ruseler-van Embden JG: **Delay of the first onset of pouchitis by oral intake of the probiotic strain *Lactobacillus rhamnosus* GG.** *Dis Colon Rectum* 2004, **47**(6):876-884.
62. Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA: **Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis.** *Gut* 2004, **53**(1):108-114.
63. Rochat T, Bermudez-Humaran L, Gratadoux JJ, Fourage C, Hoebler C, Corthier G, Langella P: **Anti-inflammatory effects of *Lactobacillus casei* BL23 producing or not a manganese-dependant catalase on DSS-induced colitis in mice.** *Microb Cell Fact* 2007, **6**:22.
64. Han W, Mercenier A, Ait-Belgnaoui A, Pavan S, Lamine F, van S II, Kleerebezem M, Salvador-Cartier C, Hisbergues M, Bueno L, *et al*: **Improvement of an experimental colitis in rats by lactic acid bacteria producing superoxide dismutase.** *Inflamm Bowel Dis* 2006, **12**(11):1044-1052.
65. Carroll IM, Andrus JM, Bruno-Barcena JM, Klaenhammer TR, Hassan HM, Threadgill DS: **Anti-inflammatory properties of *Lactobacillus gasseri* expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis.** *Am J Physiol Gastrointest Liver Physiol* 2007, **293**(4):G729-738.
66. LeBlanc JG, del Carmen S, Miyoshi A, Azevedo V, Sesma F, Langella P, Bermudez-Humaran LG, Watterlot L, Perdigon G, de Moreno de LeBlanc A: **Use of superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's disease in mice.** *J Biotechnol* 151(3):287-293.
67. Tang DC, DeVit M, Johnston SA: **Genetic immunization is a simple method for eliciting an immune response.** *Nature* 1992, **356**(6365):152-154.
68. Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner PL, Dworki VJ, Gromkowski SH, Deck RR, DeWitt CM, Friedman A, *et al*: **Heterologous protection against influenza by injection of DNA encoding a viral protein.** *Science* 1993, **259**(5102):1745-1749.
69. Fouts TR, DeVico AL, Onyabe DY, Shata MT, Bagley KC, Lewis GK, Hone DM: **Progress toward the development of a bacterial vaccine vector that induces high-titer long-lived broadly neutralizing antibodies against HIV-1.** *FEMS Immunol Med Microbiol* 2003, **37**(2-3):129-134.
70. Chatel JM, Adel-Patient K, Creminon C, Wal JM: **Expression of a lipocalin in prokaryote and eukaryote cells: quantification and structural characterization of recombinant bovine beta-lactoglobulin.** *Protein Expr Purif* 1999, **16**(1):70-75.
71. Guimaraes VD, Innocentin S, Lefevre F, Azevedo V, Wal JM, Langella P, Chatel JM: **Use of native lactococci as vehicles for delivery of DNA into mammalian epithelial cells.** *Appl Environ Microbiol* 2006, **72**(11):7091-7097.

72. Chatel JM, Pothelune L, Ah-Leung S, Corthier G, Wal JM, Langella P: **In vivo transfer of plasmid from food-grade transiting lactococci to murine epithelial cells.** *Gene Ther* 2008, **15**(16):1184-1190.
73. Grillot-Courvalin C, Goussard S, Huetz F, Ojcius DM, Courvalin P: **Functional gene transfer from intracellular bacteria to mammalian cells.** *Nat Biotechnol* 1998, **16**(9):862-866.
74. Gaillard JL, Berche P, Frehel C, Gouin E, Cossart P: **Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci.** *Cell* 1991, **65**(7):1127-1141.
75. Mengaud J, Ohayon H, Gounon P, Mege RM, Cossart P: **E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells.** *Cell* 1996, **84**(6):923-932.
76. Guimaraes VD, Gabriel JE, Lefevre F, Cabanes D, Gruss A, Cossart P, Azevedo V, Langella P: **Internalin-expressing *Lactococcus lactis* is able to invade small intestine of guinea pigs and deliver DNA into mammalian epithelial cells.** *Microbes Infect* 2005, **7**(5-6):836-844.
77. Innocent S, Guimaraes V, Miyoshi A, Azevedo V, Langella P, Chatel JM, Lefevre F: ***Lactococcus lactis* expressing either *Staphylococcus aureus* fibronectin-binding protein A or *Listeria monocytogenes* internalin A can efficiently internalize and deliver DNA in human epithelial cells.** *Appl Environ Microbiol* 2009, **75**(14):4870-4878.
78. Lee P: **Biocontainment strategies for live lactic acid bacteria vaccine vectors.** *Bioengineered Bugs* 2010, **1**(1):75-77.
79. Steidler L, Hans W, Schotte L, Neirynek S, Obermeier F, Falk W, Fiers W, Remaut E: **Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10.** *Science* 2000, **289**(5483):1352-1355.
80. Steidler L, Neirynek S, Huyghebaert N, Snoeck V, Vermeire A, Goddeeris B, Cox E, Remon JP, Remaut E: **Biological containment of genetically modified *Lactococcus lactis* for intestinal delivery of human interleukin 10.** *Nat Biotechnol* 2003, **21**(7):785-789.
81. Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, van Deventer SJ, Neirynek S, Peppelenbosch MP, Steidler L: **A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease.** *Clin Gastroenterol Hepatol* 2006, **4**(6):754-759.
82. Buccato S, Maione D, Rinaudo CD, Volpini G, Taddei AR, Rosini R, Telford JL, Grandi G, Margarit I: **Use of *Lactococcus lactis* expressing pili from group B *Streptococcus* as a broad-coverage vaccine against streptococcal disease.** *J Infect Dis* 2006, **194**(3):331-340.
83. del Rio B, Dattwyler RJ, Aroso M, Neves V, Meirelles L, Seegers JF, Gomes-Solecki M: **Oral immunization with recombinant *Lactobacillus plantarum* induces a protective immune response in mice with Lyme disease.** *Clin Vaccine Immunol* 2008, **15**(9):1429-1435.
84. Grangette C, Muller-Alouf H, Goudercourt D, Geoffroy MC, Turneer M, Mercenier A: **Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant *Lactobacillus plantarum*.** *Infect Immun* 2001, **69**(3):1547-1553.
85. Maassen CB, Laman JD, den Bak-Glashouwer MJ, Tielen FJ, van Holten-Neelen JC, Hoogteijling L, Antonissen C, Leer RJ, Pouwels PH, Boersma WJ, et al: **Instruments for oral disease-intervention strategies: recombinant *Lactobacillus casei* expressing tetanus toxin fragment C for vaccination or myelin proteins for oral tolerance induction in multiple sclerosis.** *Vaccine* 1999, **17**(17):2117-2128.
86. Cheun HI, Kawamoto K, Hiramatsu M, Tamaoki H, Shirahata T, Igimi S, Makino SI: **Protective immunity of SpaA-antigen producing *Lactococcus lactis* against *Erysipelothrix rhusiopathiae* infection.** *J Appl Microbiol* 2004, **96**(6):1347-1353.
87. Lee MH, Roussel Y, Wilks M, Tabaqchali S: **Expression of *Helicobacter pylori* urease subunit B gene in *Lactococcus lactis* MG1363 and its use as a vaccine delivery system against *H. pylori* infection in mice.** *Vaccine* 2001, **19**(28-29):3927-3935.
88. Corthesy B, Boris S, Isler P, Grangette C, Mercenier A: **Oral immunization of mice with lactic acid bacteria producing *Helicobacter pylori* urease B subunit partially protects against challenge with *Helicobacter felis*.** *J Infect Dis* 2005, **192**(8):1441-1449.
89. Kim SJ, Jun DY, Yang CH, Kim YH: **Expression of *Helicobacter pylori* cag12 gene in *Lactococcus lactis* MG1363 and its oral administration to induce systemic anti-Cag12 immune response in mice.** *Appl Microbiol Biotechnol* 2006, **72**(3):462-470.
90. Kajikawa A, Satoh E, Leer RJ, Yamamoto S, Igimi S: **Intragastric immunization with recombinant *Lactobacillus casei* expressing flagellar antigen confers antibody-independent protective immunity against *Salmonella enterica* serovar Enteritidis.** *Vaccine* 2007, **25**(18):3599-3605.
91. Mannam P, Jones KF, Geller BL: **Mucosal vaccine made from live, recombinant *Lactococcus lactis* protects mice against pharyngeal infection with *Streptococcus pyogenes*.** *Infect Immun* 2004, **72**(6):3444-3450.
92. Hanniffy SB, Carter AT, Hitchin E, Wells JM: **Mucosal delivery of a pneumococcal vaccine using *Lactococcus lactis* affords protection against respiratory infection.** *J Infect Dis* 2007, **195**(2):185-193.
93. Medina M, Villena J, Vintini E, Hebert EM, Raya R, Alvarez S: **Nasal immunization with *Lactococcus lactis* expressing the pneumococcal protective protein A induces protective immunity in mice.** *Infect Immun* 2008, **76**(6):2696-2705.
94. Bahey-El-Din M, Casey PG, Griffin BT, Gahan CG: ***Lactococcus lactis*-expressing listeriolysin O (LLO) provides protection and specific CD8(+) T cells against *Listeria monocytogenes* in the murine infection model.** *Vaccine* 2008, **26**(41):5304-5314.
95. Enouf V, Langella P, Commissaire J, Cohen J, Corthier G: **Bovine rotavirus nonstructural protein 4 produced by *Lactococcus lactis* is antigenic and immunogenic.** *Appl Environ Microbiol* 2001, **67**(4):1423-1428.
96. Lee JS, Poo H, Han DP, Hong SP, Kim K, Cho MW, Kim E, Sung MH, Kim CJ: **Mucosal immunization with surface-displayed severe acute respiratory syndrome coronavirus spike protein on *Lactobacillus casei* induces neutralizing antibodies in mice.** *J Virol* 2006, **80**(8):4079-4087.
97. Ho PS, Kwang J, Lee YK: **Intragastric administration of *Lactobacillus casei* expressing transmissible gastroenteritis coronavirus spike glycoprotein induced specific antibody production.** *Vaccine* 2005, **23**(11):1335-1342.
98. Sim AC, Lin W, Tan GK, Sim MS, Chow VT, Alonso S: **Induction of neutralizing antibodies against dengue virus type 2 upon mucosal administration of a recombinant *Lactococcus lactis* strain expressing envelope domain III antigen.** *Vaccine* 2008, **26**(9):1145-1154.
99. Xin KQ, Hoshino Y, Toda Y, Igimi S, Kojima Y, Jounai N, Ohba K, Kushihiro A, Kiwaki M, Hamajima K, et al: **Immunogenicity and protective efficacy of orally administered recombinant *Lactococcus lactis* expressing surface-bound HIV Env.** *Blood* 2003, **102**(1):223-228.
100. Poo H, Pyo HM, Lee TY, Yoon SW, Lee JS, Kim CJ, Sung MH, Lee SH: **Oral administration of human papillomavirus type 16 E7 displayed on *Lactobacillus casei* induces E7-specific antitumor effects in C57/BL6 mice.** *Int J Cancer* 2006, **119**(7):1702-1709.
101. Cortes-Perez NG, Lefevre F, Corthier G, Adel-Patient K, Langella P, Bermudez-Humarán L: **Influence of the route of immunization and the nature of the bacterial vector on immunogenicity of mucosal vaccines based on lactic acid bacteria.** *vaccine* 2007, **25**:6581-6588.
102. Lee TY, Kim YH, Lee KS, Kim JK, Lee IH, Yang JM, Sung MH, Park JS, Poo H: **Human papillomavirus type 16 E6-specific antitumor immunity is induced by oral administration of HPV16 E6-expressing *Lactobacillus casei* in C57BL/6 mice.** *Cancer Immunol Immunother* 2010, **59**(11):1727-1737.
103. Cho HJ, Shin HJ, Han IK, Jung WW, Kim YB, Sul D, Oh YK: **Induction of mucosal and systemic immune responses following oral immunization of mice with *Lactococcus lactis* expressing human papillomavirus type 16 L1.** *Vaccine* 2007, **25**(47):8049-8057.
104. Aires KA, Cianciarullo AM, Carneiro SM, Villa LL, Boccardo E, Perez-Martinez G, Perez-Arellano I, Oliveira ML, Ho PL: **Production of human papillomavirus type 16 L1 virus-like particles by recombinant *Lactobacillus casei* cells.** *Appl Environ Microbiol* 2006, **72**(1):745-752.
105. Lei H, Xu Y, Chen J, Wei X, Lam DM: **Immunoprotection against influenza H5N1 virus by oral administration of enteric-coated recombinant *Lactococcus lactis* mini-capsules.** *Virology* 2010, **407**(2):319-324.
106. Dieye Y, Hoekman AJ, Clier F, Juillard V, Boot HJ, Piard JC: **Ability of *Lactococcus lactis* to export viral capsid antigens: a crucial step for development of live vaccines.** *Appl Environ Microbiol* 2003, **69**(12):7281-7288.
107. Moeini H, Rahim RA, Omar AR, Shafee N, Yusoff K: ***Lactobacillus acidophilus* as a live vehicle for oral immunization against chicken anemia virus.** *Appl Microbiol Biotechnol* 2011, **90**(1):77-88.
108. Xu Y, Li Y: **Induction of immune responses in mice after intragastric administration of *Lactobacillus casei* producing porcine parvovirus VP2 protein.** *Appl Environ Microbiol* 2007, **73**(21):7041-7047.

109. Perez CA, Eichwald C, Burrone O, Mendoza D: **Rotavirus vp7 antigen produced by *Lactococcus lactis* induces neutralizing antibodies in mice.** *J Appl Microbiol* 2005, **99**(5):1158-1164.
110. Zhang Q, Zhong J, Huan L: **Expression of hepatitis B virus surface antigen determinants in *Lactococcus lactis* for oral vaccination.** *Microbiol Res* 2011, **166**(2):111-120.
111. Steidler L, Robinson K, Chamberlain L, Schofield KM, Remaut E, Le Page RW, Wells JM: **Mucosal delivery of murine interleukin-2 (IL-2) and IL-6 by recombinant strains of *Lactococcus lactis* coexpressing antigen and cytokine.** *Infect Immun* 1998, **66**(7):3183-3189.
112. Bermudez-Humaran LG, Langella P, Cortes-Perez NG, Gruss A, Tamez-Guerra RS, Oliveira SC, Saucedo-Cardenas O, Montes de Oca-Luna R, Le Loir Y: **Intranasal immunization with recombinant *Lactococcus lactis* secreting murine interleukin-12 enhances antigen-specific Th1 cytokine production.** *Infect Immun* 2003, **71**(4):1887-1896.
113. Cortes-Perez NG, da Costa Medina LF, Lefevre F, Langella P, Bermudez-Humaran LG: **Production of biologically active CXC chemokines by *Lactococcus lactis*: evaluation of its potential as a novel mucosal vaccine adjuvant.** *Vaccine* 2008, **26**(46):5778-5783.
114. Bermudez-Humaran LG, Nouaille S, Zilberfarb V, Corthier G, Gruss A, Langella P, Issad T: **Effects of intranasal administration of a leptin-secreting *Lactococcus lactis* recombinant on food intake, body weight, and immune response of mice.** *Appl Environ Microbiol* 2007, **73**(16):5300-5307.
115. Zhang ZH, Jiang PH, Li NJ, Shi M, Huang W: **Oral vaccination of mice against rodent malaria with recombinant *Lactococcus lactis* expressing MSP-1(19).** *World J Gastroenterol* 2005, **11**(44):6975-6980.
116. Ramasamy R, Yasawardena S, Zomer A, Venema G, Kok J, Leenhouts K: **Immunogenicity of a malaria parasite antigen displayed by *Lactococcus lactis* in oral immunisations.** *Vaccine* 2006, **24**(18):3900-3908.
117. Charng YC, Lin CC, Hsu CH: **Inhibition of allergen-induced airway inflammation and hyperreactivity by recombinant lactic-acid bacteria.** *Vaccine* 2006, **24**(33-34):5931-5936.
118. Lee P, Faubert GM: **Oral immunization of BALB/c mice by intragastric delivery of *Streptococcus gordonii*-expressing *Giardia* cyst wall protein 2 decreases cyst shedding in challenged mice.** *FEMS Microbiol Lett* 2006, **265**(2):225-236.
119. Anuradha K, Foo HL, Mariana NS, Loh TC, Yusoff K, Hassan MD, Sasan H, Raha AR: **Live recombinant *Lactococcus lactis* vaccine expressing aerolysin genes D1 and D4 for protection against *Aeromonas hydrophila* in tilapia (*Oreochromis niloticus*).** *J Appl Microbiol* 2010, **109**(5):1632-1642.
120. Watterlot L, Rochat T, Sokol H, Cherbuy C, Bouloufa I, Lefevre F, Gratadoux JJ, Honvo-Hueto E, Chilmonczyk S, Blugeon S, *et al*: **Intragastric administration of a superoxide dismutase-producing recombinant *Lactobacillus casei* BL23 strain attenuates DSS colitis in mice.** *Int J Food Microbiol* 2010, **144**(1):35-41.

doi:10.1186/1475-2859-10-S1-S4

**Cite this article as:** Bermúdez-Humarán *et al.*: Lactococci and lactobacilli as mucosal delivery vectors for therapeutic proteins and DNA vaccines. *Microbial Cell Factories* 2011 **10**(Suppl 1):S4.

**Submit your next manuscript to BioMed Central  
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
www.biomedcentral.com/submit

