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Lactic acid bacteria as oral delivery systems for biomolecules

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Lactic acid bacteria (LAB) have become increasingly studied over the last two decades as potential delivery systems for various biological molecules to the gastrointestinal tract. This article presents an overview of characteristics of LAB as delivery systems and of the applications which have already been developed. The majority of LAB strains are able to survive the intestinal passage and some are also able to persist and colonize the intestine. Several strains were in fact described as members of the human commensal flora. They can interact with their host and are able to deliver large molecular weight biomolecules across the epithelium via M-cells or dendritic cells. The most widely applied LAB species has been *Lactococcus lactis*; however species from genus *Lactobacillus* are gaining popularity and the first examples from genus *Bifidobacterium* are starting to emerge. Bacteria are mostly applied live and enable continuous delivery of the biomolecules. However, killed bacteria (e.g. gram-positive enhancer matrix), with bound biomolecules or as adjuvants, are also being developed. The techniques for genetic modification of LAB are well known. This review focuses on the delivery of recombinant proteins and DNA, which can cause either local or systemic effects. We divide recombinant proteins into antigens and therapeutic proteins. Delivery of antigens for the purpose of vaccination represents the most abundant application with numerous successful demonstrations of the efficacy on the animal model. Therapeutic proteins have mostly been developed for the treatment of the inflammatory bowel disease, by the delivery of anti-inflammatory cytokines, or downregulation of pro-inflammatory cytokines. Delivery of allergens for the modulation of allergic disorders represents the second most popular application of therapeutic proteins. The delivery of DNA by LAB was demonstrated and offers exciting opportunities, especially as a vaccine. New discoveries may eventually lead to the transition of LAB as delivery systems in clinical practice.

1. Introduction

Oral drug delivery of macromolecules, including therapeutic peptides, oligosaccharides and nucleic acids is one of the main unprecedented challenges in modern pharmaceutical biotechnology. Its main advantage regarding biopharmaceuticals is avoidance of additional risk of immunogenicity, discomfort and pain that accompany the parenteral route of administration as well as the less expensive and less complex production of biodrugs that might be produced under non-sterile conditions. Although there have been major advances in delivering macromolecular drugs in humans by other non-invasive routes, including pulmonary or nasal delivery, only a few examples, in particular short peptides or some oral vaccines, have been developed successfully in the last ten years. The development of oral formulations for macromolecular drugs is therefore highly in demand and has been considered the "Holy Grail" of drug delivery. Oral delivery is patient friendly and enables good compliance. It is particularly attractive in the management of chronic diseases where prolonged parenteral administration is particularly debilitating. However, oral delivery is limited by the large molecular weight of the molecules, which limits their permeability. Low permeability, together with low stability, result in low and variable bioavailability. Several approaches were devised to overcome this problem. Penetration enhancers and protease inhibitors were included in formulations. Proteins were encapsulated in microparticles, nanoparticles and liposomes or conjugated with macromolecules, such as polyethylene glycol, transferrin or lectins. Their chemical modification, with e.g. cobalamin or lipids, was also tested (Shen 2003; Antosova et al. 2009).

One of the attractive, alternative ways for oral drug delivery is represented by the direct administration of recombinant bacteria, acting simultaneously as a cell factory and delivery system for pharmacologically active proteins. The most intensively investigated microorganisms for oral delivery of biopharmaceuticals are recombinant or biotechnologically-modified lactic acid bacteria (LAB).

2. Lactic acid bacteria

LAB are important industrial microorganisms and are mostly applied in the food industry. They are used for the fermentation and preservation of dairy, meat and vegetable products. Some of the dishes containing and prepared with LAB have been used for centuries. Due to their long safe usage they have been granted "generally recognized as safe" (GRAS) status by the Food and Drug Administration. LAB can also be found in the human gastrointestinal tract (GIT) as a component of its flora. LAB are

generally considered beneficial to human health. Some species, mostly from the genera *Lactobacillus* and *Bifidobacterium*, are considered probiotic and are added to the diet in the form of functional food supplements (de Vrese and Schrezenmeir 2008). Probiotics are defined as "live microorganisms which, when consumed in adequate amounts as part of food, confer a health benefit on the host".

LAB are Gram-positive bacteria with common metabolic and physiological characteristics. They are anaerobic or microaerophilic cocci or bacilli. They are phylogenetically related and comprise the following genera: *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weisela* (Stiles and Holzapfel 1997). Genus *Bifidobacterium* is also classified as LAB because it shares physiological characteristics and occupies the same environmental niches, even though it is phylogenetically more distant and is strictly anaerobic. LAB are also characterized by the low GC content of their genomes. A common feature of LAB is the production of lactic acid as the end product of carbohydrate metabolism (Kandler 1983). During their growth, the pH of the growth medium decreases due to the accumulation of lactic acid, which is exploited in the preservation of food. LAB are relatively resistant to growth in acid pH media. Their growth usually stops between pH 4.0 and 4.5. They have similar growth temperature tolerances and do not survive at temperatures above 45 °C. They are nutritiously demanding and are auxotrophic for several amino acids and other essential nutrients. They therefore occupy nutrient rich environments such as milk, meat, decomposing plant material and the mammalian GIT. Several LAB strains produce antibacterial peptides, bacteriocins, which serves as a competitive advantage in multibacterial environments.

LAB are readily amenable to genetic engineering (de Vos 1999). They can be used as hosts for the expression of recombinant proteins, which can be either constitutive or inducible. Secretion or surface binding of expressed proteins can be achieved. Introduction of appropriate enzymes can result in the establishment of new pathways for biosynthesis of small biomolecules (Berlec and Strukelj 2009).

3. Transition of lactic acid bacteria through the intestinal tract

The GIT is a harsh environment for bacteria, characterized by low pH in the stomach and intensive enzymatic activity and the presence of bile acids in the small intestine. This results in the degradation of ingested proteins, which prevents oral administration of therapeutic proteins. It also protects from ingested bacterial pathogens. However, it is known that the GIT is colonized by a large number of intestinal bacteria, which are well suited to intestinal conditions. Among them, several strains of LAB are able to survive the transition through the stomach and the small and large intestines in large numbers (Bezkorovainy 2001). This was demonstrated for *Lb. rhamnosus* GG, *Lb. johnsonii* La1, *Lb. casei* Shirota (Dicks and Botes 2010) and *Lb. casei* DN-114 001 (Oozeer et al. 2006). The degree of survival depends on the length of exposure to gastric acid and to bile acids, but is also strain dependent. Some of the bacteria remain unadhered in the lumen and move along with the intestinal contents while others get trapped in the intestinal mucous layer. Some are able to adhere *via* specific adherence molecules on their surface. Adherence is generally considered a prerequisite for colonization. Adhesion was often demonstrated on the intestinal epithelial cell models such as Caco-2 or HT-29. However, this only weakly reflects the *in vivo* conditions and does not consider the mucous layer which normally separates the colonic content from the epithelial lining (Johansson et al. 2008). Colonization was achieved by oral administration of several *Lactobacillus* strains to gnotobiotic animal models (Dicks and Botes 2010). *Lb. rhamnosus* GG was isolated from colonic biopsies of volunteers who consumed the bacteria (Alander et al. 1997). However, less data is available on the long-term colonization of human GIT, where bacteria are usually no longer detected after the end of the bacterial administration (Bezkorovainy 2001).

4. Interaction of lactic acid bacteria with the host

LAB interact with the host at the mucosal epithelium. The molecule which is being delivered by LAB can be secreted to the intestinal environment or is retained inside the bacterium. In the former case, the molecule exerts its activity in the GIT or is transported across the epithelial layer. In the latter case, the bacteria are degraded in the intestinal lumen, whereby the delivered molecule is released, or are first transported across the epithelial layer and then degraded. If the delivered molecule is displayed on the surface of bacteria, it can act both in the intestinal lumen and on transportation across the epithelium. A few different routes of transport across the epithelial layer have been described (Wells and Mercenier 2008; Hooper and Macpherson 2010). M cells, which are found in the Payer's patches in the small intestinal epithelium, are believed to be one of the most important entry points for the bacteria. They enable transport across the epithelial layer and present bacteria to underlying dendritic cells. The latter phagocytose the bacteria and migrate to the lymph nodes where they trigger the induction of the T cell response and differentiation of IgA^+ B cells into IgA^+ plasma cells. The latter produce IgAs which are translocated across the epithelial layer to the intestinal lumen where they control the bacterial population. Dendritic cells can also directly sample bacterial cells from the intestinal lumen by extending dendrites between epithelial cells, without disturbing tight junctions. Damage of the epithelium usually occurs due to infection or severe inflammation and can result in direct invasion of sub-epithelial tissues by bacterial cells. Invading bacteria are phagocytosed by macrophages which are abundant in human GIT. Macrophages in GIT do not trigger strong immune response physiologically which prevents potential deleterious effects. Further research on this field is needed to improve our understanding of these mechanisms.

5. Forms of lactic acid bacteria as delivery systems

5.1. Living bacteria

Living bacteria are an obvious choice for the delivery of biomolecules and have been used in most studies. Application of living bacteria is necessary if the biomolecule is produced *in situ* in the GIT. Several LAB have the ability to survive the passage through the GIT and therefore exert the activity in different parts of the intestine. Some bacteria are capable of colonization of the GIT which would result in the continuous production of biomolecules. This could be beneficial for certain applications, but limiting from the regulatory point of view. If a bacterium with intrinsic probiotic properties (Verna and Lucak 2010) is used as a host for biomolecule delivery, synergistic effects can be predicted. It can be assumed that live LAB could protect the surface displayed biomolecules from degradation.

5.2. Inactivated bacteria

LAB which deliver a given biomolecule can be killed prior to administration, if the biomolecule is produced prior to killing

and is retained in or on the bacterial remnants. This approach is suitable for the delivery of antigens for oral application, where the potential adjuvant activity of LAB is retained. The feasibility of this approach was demonstrated with successful intranasal immunization of mice with tetanus toxin fragment expressed in *L. lactis* which was killed prior to administration (Robinson et al. 1997). In contrast, pneumococcal antigen displayed on killed bacteria elicited lower immunoprotective activity than on live bacterial vectors (Hanniffy et al. 2007). The efficacy of the dead bacterial vectors depends on the quantity of delivered antigen, method of killing (chemical, thermal or radiation) and localization of produced biomolecule (Bahey-El-Din and Gahan 2010).

Another approach to the use of killed bacteria, which is gaining popularity, involves the use of gram-positive enhancer matrix (GEM) particles. These are cells of *L. lactis*, which are subjected to treatment with strong acids and organic solvents, which removes the entire intracellular content. The resulting GEM particles are composed of peptidoglycan and resemble the shape of lactococcal cells (van Roosmalen et al. 2006). They can serve as scaffolds for the attachment of biomolecules via peptidoglycan anchors, usually from AcmA protein (Steen et al. 2005). They are not problematic from the regulatory point of view since they contain no recombinant DNA and are not considered genetically modified. Additionally, they enable binding of multiple biomolecules to a single bacterial cell which can be very useful for the production of oral polyvalent vaccines, as in the case of a vaccine against *Streptococcus pneumoniae* which contains three pneumococcal proteins (Audouy et al. 2007) Another example is the delivery of low calcium response V protein (LcrV), which induced protection against *Yersinia pestis* infection (Ramirez et al. 2010). Saluja and co-workers reported strong adjuvant action of GEM particles which were mixed with influenza subunit vaccine (Saluja et al. 2010a, 2010b, 2010c) and demonstrated applicability of the system without anchoring the protein to the peptidoglycan.

6. Species and strains of lactic acid bacteria applied as delivery vectors

Of the several genera that belong to the group of LAB, genus *Lactococcus* and genus *Lactobacillus* have been most widely applied. *L. lactis* is one of the most studied species and has been considered a model lactic acid bacterium. A lot of tools for its genetic manipulation have been developed (van Hylckama Vlieg et al. 2006). According to the European Bioinformatic Institute (EBI) database (www.ebi.ac.uk/genomes), 6 species of *Lactococcus* have been sequenced, the first in 2001 (Bolotin et al. 2001). For this reason, *L. lactis* was commonly used as a delivery system. However, *L. lactis* is not considered probiotic and is not a regular inhabitant of human intestine. In contrast, several strains of genus *Lactobacillus* are considered probiotic and have therefore received a lot of research attention. According to the EBI database, genomes of 42 species from the genus *Lactobacillus* have been sequenced as of August 2011. Of these, several were used for the delivery of biomolecules to GIT, including *Lb. plantarum*, *Lb. helveticus*, *Lb. reuteri*, *Lb. casei* and *Lb. acidophilus* (Table 1, Table 2). *Bifidobacterium* is the second largest genus of probiotic bacteria and offers great potential in biomolecule delivery. However, the techniques for the genetic modification of bifidobacteria have only recently produced the first examples of such usage.

The choice of the appropriate species of LAB depends largely on the type of biomolecule delivered. With that in mind, the ability to colonize GIT may be either an advantage or a disadvantage. LAB are able to interact with the immune system through different pathways, resulting in complex immunostimulatory or tolerogenic effects which enable new therapeutic potentials (Fujiwara et al. 2004; Perdigon et al. 2001). The use of a probiotic bacterium (e. g. *Lactobacillus*) may be advantageous if synergistic or complementary effects can be achieved. A very important criterion for strain selection is the ability to ensure stable production of sufficient quantities of biomolecule in a physiologically active form.

7. Types of biomolecules delivered by lactic acid bacteria

7.1. Antigens for oral vaccination

Oral administration of antigens (oral vaccine) is desired for its patient friendly mode of application compared to the established parenteral way. LAB are suitable vectors for oral antigen delivery due to their "generally recognized as safe" (GRAS) status, low intrinsic immunogenicity, adjuvant properties and mucus adherence ability (Pouwels et al. 1998). They are able to provoke an adaptive rather than a tolerogenic immune response. The latter was usually obtained with the use of isolated antigens which has therefore been avoided (Lavelle and O'Hagan 2006). LAB-based vaccines would also have lower production costs than their parenteral counterparts. This could make them particularly attractive for less developed countries. Antigens delivered by LAB are too large to be absorbed by epithelial cells and do not enter the blood circulation. They are, however, able to reach the bowel lymphoid tissue through specialized M cells on Peyer's patches, as previously described. There they are presented to the basal immune cells, which can elicit local and distal mucosal, as well as systemic immune response (Davis 2001; Nouaille et al. 2003).

Reported LAB vaccines are targeted against bacterial pathogens, viral pathogens and against protozoa *(Plasmodium falciparum)* (Table 1). The majority of the studies were performed on mice, but pigs and chickens were also used. Tetanus toxin fragment C served as the model antigen due to its high immunogenicity (Robinson et al. 1997; Norton et al. 1996; Wells et al. 1993). It usually triggered a strong local immune response with IgA secretion; however a systemic immune response was also achieved. Several studies supported the efficacy of the vaccination and its ability to protect against pathogen challenge. Protective activity was observed with human papillomavirus HPV16 (Bermúdez-Humarán et al. 2005) (protection against viral antigen-expressing tumour cell line), enterotoxicogenic *E. coli* (Pouwels *et al.* 1998), group B *Streptococcus* (Bucatto et al. 2006) and *Streptococcus pyogenes* (Mannam et al. 2004).

Less successful studies reported only partial or not fully proven immune responses. These studies include the delivery of urease B of *Helicobacter pylori* by *Lb. plantarum* (Corthesy et al. 2005), antigen PspA of *Streptococcus pneumoniae* (Hannify et al. 2007; Oliveira et al. 2006), V2-V4 loop of gp120 protein from HIV-1 virus (Xin et al. 2003) and rotaviral protein VP7 (Perez et al. 2005).

The most recent studies include the expression of *Bacillus anthracis* protective antigen (PA) in *Lb. acidophilus*. The antigen was fused to a peptide that specifically targeted dendritic cells. This strategy enabled targeted antigen delivery and induced a strong protective response in mice (Mohamadzadeh et al. 2009). In another study, *Lb. plantarum* that was expressing oncofetal antigen (OFA) induced a specific anti-OFA immune response in mice (Fredriksen et al. 2010), which is an example of anti-cancer vaccine.

In some cases the efficacy of immunization was improved by simultaneous addition of interleukins 2 and 6 (Steidler et al.

Table 1: Examples of the use of LAB in antigen delivery

Pathogen	Delivered antigen	LAB	Ref.
Bacteria			
Streptococcus mutans (caries)	Surface protein PAc	*L. lactis*	Iwaki et al. (1990)
Clostridium tetani (tetanus)	Tetanus toxin Fragment C	*L. lactis, Lb. plantarum*	Robinson et al. (1997), Grangette et al. (2002)
Streptococccus pneumoniae	PsaA, PspA	*L. lactis, Lb. casei, Lb. plantarum, Lb. helveticus*	Oliveira et al. (2006)
Helicobacter pylori	UreB, Cag12	*L. lactis*, *Lb. plantarum*	Lee et al. (2001)
Brucella abortus	L7/L12 ribosomal protein, GroEL heat-shock protein	*L. lactis*	Pontes et al. (2003)
Streptococcus pyrogenes	C repeat region of M protein	*L. lactis*	Mannam et al. (2004)
enterotoxigenic *Escherichia coli*	Heat-stabile enterotoxin and heat-labile enterotoxin B; fimbrial protein	*Lb. reutei, Lb. casei*	Wu and Chung (2007), Wei et al. (2010)
Streptococci (wide spectrum)	GBS pilus (from *Streptococcus* group B)	*L. lactis*	Buccato et al. (2006)
Borrelia burgdorferi (Lyme disease)	OspA	*Lb. plantarum*	Del Rio et al. (2010)
Yersinia pseudotuberculosis	LcrV	*L. lactis*	Daniel et al. (2010)
Proteus mirabilis	Fimbrial protein MrpA	*L. lactis*	Scavone et al. (2007)
Bacillus anthracis (anthrax)	*B. anthracis* protective entigen	*Lb. acidophilus*	Mohamadzadeh et al. (2009)
Viruses			
Human papillomavirus type 16 (cervical cancer)	E7 protein, L1 protein	*L. lactis, Lb. plantarum, Lb. casei*	Bermudez-Humaran et al. (2005)
HIV	Envelope protein	*L. lactis*	Xin et al. (2003)
SARS-coronavirus	Nucleocapsid protein, peplomeric protein - segments SA and SB	*L. lactis, Lb. casei*	Lee et al. (2006)
Rotavirus	Protein VP7	*L. lactis*	Perez et al. (2005)
Coronavirus (transmissible gastroenteritis)	Peplomeric glycoprotein S	*Lb. casei*	Ho et al. (2005)
Transmissible gastroenteritis virus (TGEV)	D antigenic site of the spike protein	*Lb. pentosus*	Di-Qiu et al. (2011)
Avian influenza virus	HA1+cholera toxin subunit B	*L. lactis*	Lei et al. (2011)
Influenza virus H5N1	HA antigen	*L. lactis*	Lei et al. (2010)
Hepatitis B virus	PreS region of surface antigen	*L. lactis*	Zhang et al. (2011)
Protozoa			
Plasmodium falciparium (malaria)	GLURP-MSP3 fusion protein, MSP1, MSA2	*L. lactis*	Ramasamy et al. (2006)
Giardia lamblia	Cyst wall protein 2	*L. lactis, S. gordonii*	Lee et al. (2009)
Leishmania donovani	A2 antigen	*L. lactis*	Yam et al. (2011)

1998) or cholera toxin (Xin et al. 2003). Despite the relatively long history of animal experiments, none of the developed systems has to date entered human clinical trials.

7.2. Therapeutic proteins

LAB can be used for the delivery of therapeutic proteins to GIT (Table 2). Therapeutic proteins exert local action, either in the intestinal lumen or in the surrounding tissues. The majority of applications have been dedicated to the treatment of inflammatory bowel disease (IBD), which comprises Crohn's disease and ulcerative colitis. Probably the most successful application was the delivery of interleukin-10 (IL10). IL-10 plays an important role in the inflammation processes in IBD. Its deficiency leads to spontaneous development of colitis in IL-10-knockout mice (Paul et al. 2011). Administration of *L. lactis* expressing recombinant IL-10 significantly improved clinical outcome in mice with chemically induced or spontaneous colitis (Steidler et al. 2000). An improved system was later introduced by preparing a thymidylate kinase deficient clone of the bacterium (Steidler et al. 2003). Such a clone depends on external sources of thymidine and thymine, which prevents its survival in the environment and increases its safety. This clone was used in a phase I clinical study on 10 Crohn's disease patients and produced encouraging results (Braat et al. 2006). Improvement of ulcerative colitis in animal trial was also achieved by the delivery of trefoil factor (Vandenbroucke et al. 2004) and LcrV protein from *Yersinia pseudotuberculosis* (Foligne et al. 2007) which both stimulate the secretion of IL-10. Downregulation of proinflammatory cytokines was also achieved with the delivery of neuropeptide α-melanocyte stimulation hormone (α-MSH) by *Lb. casei* (Yoon et al. 2008) and the delivery of transforming growth factor β (TGF-β) by *Bacteroides ovatus* (Hamady et al. 2010a). Another approach to the treatment of IBD is to lower the level of pro-inflammatory cytokine TNFα. Single domain antibodies (nanobodies) against TNFα were expressed in *L. lactis* and proved to be effective in a murine model of colitis (Vandenbroucke et al. 2010). An alternative approach involved the expression and lactococcal surface display of TNFα-binding affibodies (Ravnikar et al. 2010). An approach which does not interfere with cytokine signalling was demonstrated by the delivery of superoxide dismutase (MnSOD). MnSOD is responsible for the de-activation of reactive oxygen compounds, which diminishes their pro-inflammatory properties (Caroll et al. 2007).

Table 2: Examples of the use of LAB in the delivery of therapeutic proteins

Condition	Delivered protein	LAB	Ref.
Inflammatory bowel disease	IL-10	*L. lactis*	Steidler et al. (2000), Braat et al. (2006)
Ulcerative colitis	mTFF trefoil factor	*L. lactis*	Vandenbroucke et al. (2004)
Ulcerative colitis	LcrV from *Yersinia pseudotuberculosis*	*L. lactis*	Foligne et al. (2007)
Ulcerative colitis	MnSOD from *Streptococcus thermophilus*	*Lb. gasseri*	Carrol et al. (2007)
Ulcerative colitis	alpha-melanocyte stimulating hormone	*Lb. casei*	Yoon et al. (2008)
Ulcerative colitis	transforming growth factor-β	*Bact. ovatus*	Hamady et al. (2010a)
Ulcerative colitis	Nanobody against TNFα	*L. lactis*	Vandenbroucke et al. (2010)
Ulcerative colitis	Affibody against TNFα	*L. lactis*	Ravnikar et al. (2010)
Allergy	Bet v 1 antigen from birch pollen	*L. lactis, Lb. plantarum*	Daniel et al. (2006), Schwarzer et al. (2010)
Allergy	Anti-idiotypic scFv or IgE mimotope	*Lb. johnsonii*	Scheppler et al. (2005)
Allergy	Mite allergens Der p 1, Der p 5	*Lb. plantarum*	Rigaux et al. (2009), Charng et al. (2006)
Allergy	β-lactoglobulin	*Lb. case*	Hazebrouck et al. (2006)
Allergy	ovalbumin	*L. lactis*	Huibregtse et al. (2007)
Celiac disease	Gliadin epitope	*L. lactis*	Huibregtse et al. (2009)
Caries	scFv against *S. mutans* (antigen I/II)	*Lb. zeae*	Kruger et al. (2005)
Lipase deficiency (pancreas insufficiency)	Lipase from *Staphylococcus hyicus*	*L. lactis*	Drouault et al. (2002)
Improvement of intestinal homeostasis	keratinocyte growth factor-2	*Bact. ovatus*	Hamady et al. (2010b)
Intestinal development	epidermal growth factor	*L. lactis*	Cheung et al. (2009)
Cancer	TNFα-related apoptosis inducing ligand	*B. longum*	Hu et al. (2009)
Viral infecion	IFN-α2b	*B. longum*	Yu et al. (2010)
Obesity	oxyntomodulin	*B. longum*	Long et al. (2010)

A considerable number of applications have been developed for immune modulation. Their goal was the induction of immune tolerance with the delivery of allergen. This approach is contrary to that for antigen delivery, in which the goal is the induction of an immune response. Sensitization with recombinant bacteria that produced recombinant birch pollen allergen significantly reduced the immune response against that allergen. It also induced Th1 and T-regulatory response after sensitization (Daniel et al. 2006; Schwarzer et al. 2011). Mite allergens were delivered in similar fashion (Rigaux et al. 2009, Charng et al. 2006). Egg ovalbumin allergen was expressed in *L. lactis* and its delivery demonstrated ovalbumin-specific tolerance in mice (Huibregtse et al. 2007). The major cow milk allergen, β-lactoglobulin, was also expressed in *Lb. casei* and tested on mice (Hazebrouck et al. 2006). *L. lactis* expressing the gliadin peptide epitope caused antigen-specific tolerance in transgenic mice that were gliadin sensitized (Huibregtse et al. 2009), representing a strategy for the treatment of celiac disease. Different approaches in hypersensitivity management that do not include allergen delivery were also presented. These include the delivery of single chain variable fragment (scFv) of antibody against IgE and the delivery of IgE mimotopes that stimulate formation of IgG against IgE (Scheppler et al. 2005). Both approaches result in the reduced levels of IgE which is responsible for excessive immune response.

LAB can also be used for the delivery of enzymes to supplement their deficiency in conditions like pancreatic insufficiency. An example is the delivery of lipase from *Staphylococcus hycus* by recombinant *L. lactis* which enhanced lipid digestion in pancreatic insufficient pigs (Drouault et al. 2002).

Another example of the delivery of a therapeutic protein by LAB was the expression of epidermal growth factor in *L. lactis*. Its administration was able to enhance intestinal development and growth of early weaned mice (Cheung et al. 2009) and could be applied in weaning transition in children. Similarly, *Bact. ovatus* was used for the delivery of human keratinocyte growth factor-2 to improve intestinal epithelial homeostasis (Hamady et al. 2010b).

Beside the intestine, the oral cavity has also been targeted. ScFv fragments against *Streptococcus mutans* were delivered using LAB with the aim of treating caries (Krüger et al. 2005). Recent applications of bifidobacteria as delivery vehicles offered some exciting new treatment options. *Bifidobacterium longum* expressing the extracellular domain of TNFα-related apoptosis inducing ligand (TRAIL) was successfully used in anti-tumour therapy of mouse osteosarcoma (Hu et al. 2009). The same species was applied for the delivery of IFN-α2b. Immunomodulatory effects in mice were shown and could be applied in the treatment of viral infections (Yu et al. 2010). *B. longum* was also used for the delivery of intestinal hormone oxyntomodulin for the treatment of obesity. A decrease in food intake, body weight and blood triglyceride was observed in an obese mouse model (Long et al. 2010).

7.3. DNA for oral vaccination

DNA is another biomolecule that can be delivered by LAB. For that purpose the bacteria have to enter cells, where they get degraded and their DNA released. Host transcription and translation mechanisms are used to form the required protein. A few applications were designed for the intracellular production of antigen for vaccination. This kind of immunization is able to trigger not only humoral, but also cell mediated immunity. This potentially enables treatment of chronic diseases and viral infections. So far, DNA transfer to cells and expression of model recombinant protein has been demonstrated. Internalin was used to facilitate cell permeability by LAB (Guimaraes et al. 2005; Guimaraes et al. 2006). Later, DNA transfer from *L. lactis* to murine epithelial cells was demonstrated without the presence of permeability enhancers. It resulted in the intracellular production of β-lactoglobulin (Chatel et al. 2008). There is also a report

of successful immunization of mice against foot-and-mouth disease after delivery of the gene for Vp1 viral protein (Li et al. 2007). Other applications of DNA delivery can be envisaged, such as transkingdom RNAi, which was developed for *E. coli* (Xiang et al. 2006). In this case, introduced bacterial DNA drives the production of RNAi and silencing of the disease associated genes.

8. Conclusion

LAB constitutes a novel vehicle for the delivery of biomolecules to the GIT. There, they are able to elicit both local and systemic action. The majority of applications are aimed at modulating the immune response. Induction or delivery of anti-inflammatory cytokines or downregulation of pro-inflammatory cytokines is desired in the treatment of inflammatory bowel disease, where excessive action of the immune system is one of the culprits of the disease symptoms. A specific antigen can be delivered to induce a cellular or humoral immune response for the purpose of vaccination. Alternatively, a similar approach can lead to the induction of tolerance in hypersensitivity disorders. Genera *Lactococcus* and *Lactobacillus* are most often used as hosts; however genus *Bifidobacterium* has also attracted attention and its popularity could increase in the future. Despite numerous applications which have been proposed and their proof-of-principle demonstrated on animal models, only a few have entered human trials. A major breakthrough has been the successful completion of Phase I clinical trial with IL-10 producing *L. lactis* and its advancement to Phase II. This has paved the way for future applications, as some regulatory constraints were identified and successfully met.

LAB are advantageous due to their safety and ability to deliver functional eukaryotic proteins to the intestinal tract. They serve as production hosts and as protective coatings at the same time. This can result in lower treatment costs, as there is less need for downstream processing (protein purification) and for intestinal formulation development. The ability to achieve systemic effects by oral administration is preferable in pharmacy, but is difficult to achieve with protein or DNA therapeutics. LAB can offer a solution, at least for a few applications. LAB can have an intrinsic physiological activity which can be synergistic e.g. adjuvant action in vaccines. The disadvantages of engineered LAB have so far prevented their wider use in therapy. In the case of *in vivo* production of therapeutic molecules, the precise dosage is difficult to control and the fate of the bacteria in the intestine and pharmacokinetics are also difficult to determine. The precise mechanism of action is uncertain. The major obstacle has been the fear of release of the genetically modified organism into the environment. Even though this has been successfully tackled by the development of a containment system for *L. lactis*, killed bacteria or GEM particles could be preferred by the regulatory agencies.

This review has been concerned mainly with polymeric biomolecules, such as proteins and DNA. However, delivery of small molecules such as vitamins (riboflavin (LeBlanc et al. 2005), folic acid (LeBlanc et al. 2010)) has also been achieved. Even though these applications are attractive they are not likely to be cost effective until regulatory issues are satisfactorily addressed. The first approval of a LAB-based therapy would open door for the incoming applications.

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