Review Article

Lactococcus lactis as a live delivery vector

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ABSTRACT

Mucosal surfaces of the body provide a universal entry portal for all known and emerging infectious pathogenic microbes. Therefore, it seems that special vaccination strategies are needed for vaccines that can hinder the entry capability of pathogenic microbes through the mucosal surfaces. Lactic acid bacteria are widely used in the food industry and are presently applied as delivery vehicles in many biological investigations. Among these bacteria, *Lactococcus lactis* is considered as a promising candidate for mucosal live vaccines to be used as an antigen delivery vector. This is an attractive alternative and a safer vaccination strategy against the pathogens, compared to other conventional methods. In this review, we summarized the applications of *L. lactis* as a mucosal vector of vaccine delivery for heterologous expression of proteins and its applications in biotechnology.

KEYWORDS: *Lactococcus lactis*, Live vector, Live delivery vaccines, Mucosal vaccines.

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INTRODUCTION

In 1980, Walter Schaffner demonstrated that bacteria are capable of transferring the genetic material into mammalian cells in vitro. Since then, bacteria have been suggested to be used as new transferring vectors for the plasmid vaccines [1-3]. Later, it was shown that Gram positive bacteria, such as Listeria monocytogenes, were also able to deliver plasmid DNA [4]. Moreover, attenuated or artificially engineered invasive bacteria have been tested as a vehicle for the transgene delivery [5].

For centuries, people had recognized that the consumption of ferm entedproducts can have a positive effect on their health. Over recent decades, these probiotics such as lactic acid bacteria (LAB) have been classified as "generally recognized as safe" (GRAS) by the United States Food and Drug Administration (USFDA) [6]. Moreover, some LAB were shown to be able to stimulate the immune system of the hosts like adjuvants because of their probiotic properties and immunomodulation capacities [7]. While both pathogenic and commensal bacteria have benefits and drawbacks as mucosal delivery vehicles, LAB are more desirable for their safety and fewer side effects [8].

Lactococcus lactis has a good record of safe usage in food fermentation industries and is able to survive the passage through the gastrointestinal tract of humans and animals [9]. Moreover, it does not invade or colonize the mucosal surfaces Of the host with a retention time of 2 to 3 days. Interestingly,*L. lactis* has no lipopolysaccharide and for this reason, it does not induce strong host immune responses [10-12]. Thanks to the genetic tools which have been developed in recent years and the availability of the nucleotide sequence of the complete genome of L. lactis, it would be easier now for researchers to manipulate the genes of interest and to produce and deliver proteins to the host mucosal surfaces, through the oral, genital or intranasal routes using *L. lactis* delivery systems. [13, 12, 14, 15]. Presently, a number of studies are being planned to use recombinant *L. lactis* for induction of immune responses against antigens [9].

In this review, we will focus on the potential of *L. lactis* as a vehicle for the delivery of oral vaccines. The first part of the review concentrates primarily with the interactions between *L. lactis* and the mucosal tissues of the host. In the second part, an overview regarding the new molecular biology studies for efficient expression of antigens from pathogenic organisms by *L. lactis* will be given. In the next parts, some early outcomes of such antigen producing bacteria as well as the available commercial expression systems and safety concerns will be summarized.

PART 1: *L. lactis* and interaction with the mucosal tissues of the host

Microfold (M) cells play an important role in the beginning of the mucosal immune response and perpetuity of the mucosal surface barrier. M cells transport pathogens and foreign molecules from the apical lumen side to the basal side via transcytosis. M cells do not have a mucus layer on their apical

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side [16, 5]. This character allows M cells to uptake antigens efficiently from the luminal space. The basal side of M cells which is formed from invaginated membranes has pockets and house dendritic cells (DCs; Fig. 1). These DCs take up transported pathogens and molecules and help to manage the adaptive immune responses [17]. This close vicinity of DCs to M cells is especially remarkable due to the rapid process of the transcytosed antigens and the presentation of the antigenic peptides to B and T cells for induction of the immune responses. Germinal center, contains a network of follicular DCs and many B cells, including IgA-producing B cells [16]. These B cells can migrate into the intestinal lamina propria and secrete IgA (sIgA). The space between neighboring follicles in the Peyer's patches (PPs) is called intrafollicular region (IFR). Intrafollicular region is full of T cells and DCs which help to administer the adaptive immune responses in the PPs [18].

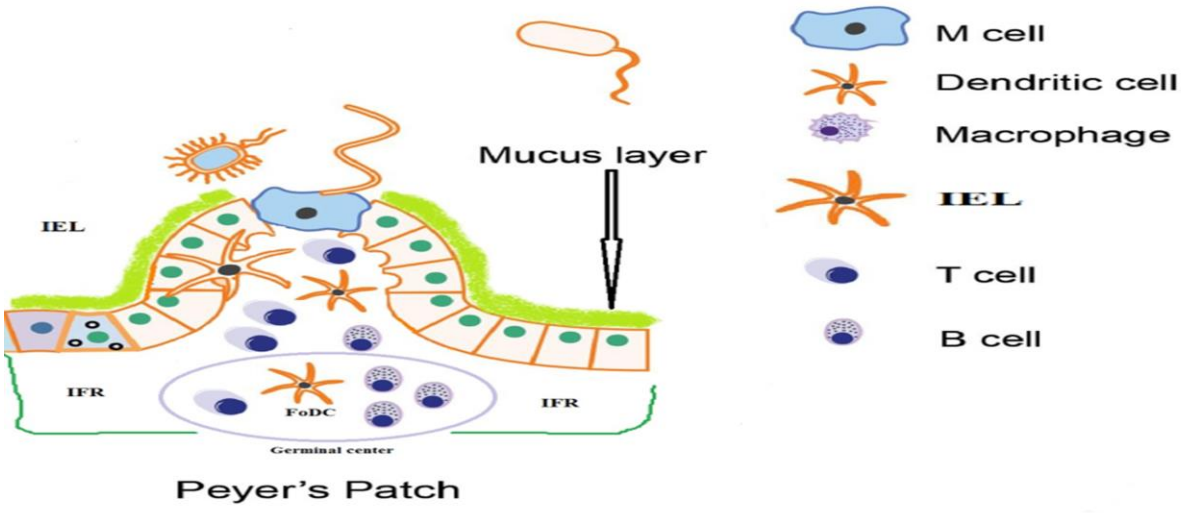

Fig. 1. Schematic representation of a Peyer's patches follicle, M cells and the different immune cell populations. M cells have no mucus. IFR: intra-follicular region, B: B cells, IEL: intraepithelial lymphocyte, T: T cells, FoDC: follicular dendritic cell, DC: dendritic cells.

L. lactis can enter through the intestinal epithelial cells (IECs) or M cells. Following internalization, it reproduces in the phagocytic cells, and causes cellular death mechanism to spread to deeper layers. In a usual manner, inflammatory response are induced and infiltration of polymorphonuclear cells causes the activation of inflammatory cascades and production of pro-inflammatory cytokines and severe tissue damages. Therefore, the microbes from the infected lesions are cleared and the production of antimicrobial neutralizing antibodies occurs. This will eventually tiger a dynamic immune response, engaging the native and acquired mucosal responses [19-21].

PART 2: LAB as a live vesicle for mucosal vaccine delivery

The development of the molecular methods and genetic manipulation have helped to effectively produce antigens and curative molecules at different cellular localizations in LAB and to deliver DNA and protein to eukaryotic cells, making these bacteria very useful as live vaccines. A remarkable property of genetically-engineered LAB is that their mucosal administration elicits both systemic and mucosal immunity [12]. Among the LAB, *L. lactis* is a promising candidate for development of the future vaccines because: (i) many genetic tools have so far been developed for it, (ii) its genome is completely sequenced, (iii) its safety properties have been revealed.

Iwaki and collaborators in 1990 were one of the first researcher s who attempted to use *L. lactis* as live vaccines [22]. Many investigations with recombinant *L. lactis* strains were later performed and either protection or incomplete protection have been observed in this regard [23]. lately, the use of LAB LAB DNA vaccine delivery vehicles has been studied as an vaccination [24-26]. A few recent studies in which LAB have been used as a vaccine, are reviewed in the next part.

PART 3: Early outcomes of *L. lactis* for vaccine delivery

The first investigation for a L. lactis-based mucosal vaccine was against Streptococcus mutans surface protein (Pac). It has been documented that when killed recombinant *L. lactis* which had cytoplasmic expression of surface protein antigen (PAc) was supplied orally, IgA and IgG responses against the antigen were observed [22]. Moreover, Clostridium tetani toxin fragment C (TTFC– tetanus toxin fragment C), expressed by *L. lactis* strain has shown highly immunogenic properties. [27, 28]. Studies have revealed that the nasal route of surface displayed recombinant TTFC was preferred [29]. Moreover, the intracellularly-expressed T3SS (type III secretory system protein) vaccines against EspB which was used orally has shown no specific serum and fecal antibodies after ten days and intraperitoneal vaccination of the EspB protein in BALB/c mice has increased serum IgG and fecal IgA levels [30].

The comparative efficacy of a FaeG (fimbria adhesion)-based vaccine has been explored by oral and intramuscular administration in piglets [31]. The intramuscular inoculation with recombinant *L. lactis* expressing FaeG has shown to induce specific systemic responses. In another study, nasal inoculation with recombinant *L. lactis* expressing conserved stretch peptide of the avian influenza M2 antigen in birds, has resulted in increased survival times against high pathogenic avian influenza virus A subtype H5N2 [32]. In another challenge in mice, nasal and bronchoalveolar lavages (BAL) inoculated with recombinant *L. lactis* expressing Brucella abortus Cu–Zn superoxide dismutase (SOD), has shown SOD–specific IgM and SOD–specific sIgA antibodies which could

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protect the mice against virulent B. abortus strain [9]. It has been shown that orally and intranasally vaccination *with L. lactis* strain expressing Rhodococcus equi VapA (virulence–associated protein A) in mice, have led to a specific mucosal immune response against VapA in challenge with a virulent strain of R. equi [33].

In another investigation, intragastric route vaccination with recombinant *L. lactis* producing VP7 has been shown to induce systemic IgG antibody response against rotavirus [34]. Here, when mice were orally immunized with recombinant *L. lactis* producing intracellularly rotavirus spike–protein subunit VP8, significant levels of intestinal IgA antibodies were produced while the secreted, cytoplasmic expressed protein or surface anchored-antigen induced anti–VP8 antibodies at both mucosal and systemic levels [34]. Orally administration of recombinant *L. lactis* producing enterotoxin B of Staphylococcus aureus in mice has been demonstrated to elicit cellular or systemic immune responses and increased survival rate in vaccinated mice against S. aureus [35]. Moreover, vaccinated animal with *L. lactis* expressing papillomavirus type16 (HPV16) E7 protein, persuasion humoral and cellular immune responses have been able to protect the animals against HPV-16 induced tumors [36].

In mice, intranasal administration of recombinant *L. lactis* strain expressing Yersinia pseudotuberculosis low-calcium response V (LcrV) antigen has been able to elicit specific systemic and mucosal antibody and cellular immune responses against Yersinia infection. LcrV is a major bacterial pathogenicity determinant that induces the production of interleukin-10 (IL-10) and takes part in the secretion and translocation of Yersinia toxin proteins into the phagocytes. This investigation has shown that the antigen and the administration route of vaccine are very important and can influence antigen-specific immune responses [37, 38]. These studies emphasize the practicality of vaccination or therapy with recombinant *L. lactis* due to their capacity for inducing mucosal and systemic immune responses [39, 40].

PART 4: *L. lactis* delivery systems:

The strains, plasmids and plasmid properties of *L. lactis* are summarized in Table 1. All used strains are obtained from *L. lactis* subspecies cremoris MG1363, a plasmid-free progeny of the dairy starter strain NCDO712.

NZ9000 is the most commonly used host strain and the standard host strain for nisin-regulated gene expression (NICE®). Moreover in this strain, nisK and nisR genes were cloned into the pepN gene of MG1363 [41]. In NZ9100 strain, nisin genes were inserted into a neutral locus. The replicon of the vectors pNZ8008, pNZ8148, pNZ8149 and pNZ8150 are the same and are resulted from the *L. lactis* plasmid pSH71. These Plasmids can replicate in many Gram-positive bacteria such as Streptococcus thermophilus and Lactobacillus plantarum as well as E. coli with a recA+ strain such as MC1061.

The pNZ8149 vector has the lacF gene as a food grade selection marker. For the transformation process, this vector needs a host strain with the lactose operon and without lacF gene, such as *L. lactis* NZ3900 [42, 43]. In pNZ9530, the replication genes have come from Enterococcus faecalis pAMß1plasmid which replicate only in Gram-positive host strains, for example *L. lactis* and Lactobacillus plantarum [44, 45].

Table 1. *L. lactis* strains and plasmids for expression.

Strains	Plasmids	Plasmids property	Reference
L. lactis NZ9000/NZ9100	pNZ8008	Reference plasmid for nisin, intracellular expression	[41, 46]
L. lactis NZ9000/NZ9100	pNZ8148	$Cm^{R,}$ intracellular expression	[41]
L. lactis NZ9000/NZ9100	pNZ8150	$Cm^{R,}$ intracellular expression	[41]
L. lactis NZ9000/NZ9100	pNZ9530	low copy plasmid, intracellular expression	[41, 45]
L. lactis NZ3000	pNZ8149	$lacF^{+,}$ food grade, intracellular expression	[43, 47]
L. lactis NZ3900	pNZ8149	$lacF^{+,}$ food grade, intracellular expression	[43, 47]
L. lactis NZ3910	pNZ8149	$lacF^{+,}$ food grade, intracellular expression	[48, 47]
L. lactis NZ9000/NZ9100	pNZ8120	$Cm^{R,}$ NICE Secretion vectors	[49]
L. lactis NZ9000/NZ9100	pNZ8121	$Cm^{R,}$ NICE Secretion vectors	[49], unpublished
L. lactis NZ9000/NZ9100	pNZ8122	$Cm^{R,}$ NICE Secretion vectors	[50]
L. lactis NZ9000/NZ9100	pNZ8123	$Cm^{R,}$ NICE Secretion vectors	unpublished
L. lactis NZ9000/NZ9100	pNZ8124	$Cm^{R,}$ NICE Secretion vectors	[51], unpublished
L. lactis NZ3900/NZ3910	pNZ8151	$lacF^{+,}$ food grade, intracellular expression	[41]
L. lactis NZ9130	pNZ8152	$lacF^{+,}$ food grade, intracellular expression	[48, 41]

Cm^{R}: Chloramphenicol resistance

PART 5: Safety Concerns

A grave concern about the use of live LAB mucosal vaccines has been the risk of releasing genetically modified organisms to the environment. Such manipulated bacteria which produce antigens and antibiotic markers, may lead to horizontal transfer of the plasmid to other bacteria Therefore, the use of auxotrophic mutants can prevent the reproduction of such organisms in the environment. Furthermore, food-grade plasmids and auxotrophic strains can be used to solve the problem about horizontal transfer of plasmids which carry antibiotic resistance markers to the environmental and host's microflora.

For this reason, scientists have replaced the thyA gene (coding for thymidylate synthase) with the gene for human IL–10 in *L. lactis* , thereby they have made an auxotrophic strain dependent on thymidine which cannot survive in the environment [52]. Moreover, a recombinant *L. lactis* has been made which contains the LLO (Listeriolysin O of Listeria monocytogenes) gene. This was considered to reduce the use of antibiotic markers and also, the probability of horizontal gene transfer to other bacteria in the natural environment was highly minimized [53].

In this paper, we reviewed some LAB mucosal vaccines which have shown some advantages compared to injected vaccines, which could be listed as: (a) their ability to induce the systemic and mucosal immune responses in the host, (b) their ease of manipulating, (c). their lack of requirement to be handled by expert personnel. Moreover, the safety concerns about the

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release of recombinant plasmids and chromosomally-modified bacterial strains in the environment can be controlled as we discussed in text. For these reasons, LAB are considered as suitable mucosal delivery vectors for heterologous antigens and can be used in clinical trials.

CONFLICT OF INTEREST

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