University of Groningen

Targeting diseases with genetically engineered *Lactococcus lactis* and its course towards medical translation

Villatoro-Hernandez, Julio; Montes-de-Oca-Luna, Roberto; Kuipers, Oscar P.

Published in:
Expert Opinion on Biological Therapy

DOI:
[10.1517/14712598.2011.542138](https://doi.org/10.1517/14712598.2011.542138)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2011

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Villatoro-Hernandez, J., Montes-de-Oca-Luna, R., & Kuipers, O. P. (2011). Targeting diseases with genetically engineered *Lactococcus lactis* and its course towards medical translation. *Expert Opinion on Biological Therapy*, 11(3), 261-267. <https://doi.org/10.1517/14712598.2011.542138>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Expert Opinion

1. Introduction
2. Applying the technology of *L. lactis* for current health issues
3. Expert opinion and future perspective

Targeting diseases with genetically engineered *Lactococcus lactis* and its course towards medical translation

Julio Villatoro-Hernandez[†], Roberto Montes-de-Oca-Luna & Oscar P Kuipers

[†]University of Groningen, Groningen Biomolecular Sciences and Biotechnology Institute, Molecular Genetics Group, Haren, The Netherlands

The use of the lactic acid bacterium *Lactococcus lactis*, primarily used in food fermentations, as therapeutic agent is no longer speculative but an imminent reality. After the successful completion of Phase I and II clinical trials in humans for the treatment of inflammatory bowel disease, an ongoing clinical trial to alleviate oral mucositis as well as the development of a pneumococcal and a flu vaccine using genetically modified *L. lactis*, many exciting possibilities exist to develop novel therapeutic and prophylactic biopharmaceuticals to alleviate a wide range of diseases. Here, we discuss existing characteristics of the systems currently employed and the nature of the immune responses evoked. We also discuss the criteria that are fundamental to making the systems feasible and efficient which should ultimately translate into human therapies. Finally, we examine the prospects for *L. lactis* to become a commercially viable therapeutic agent.

Keywords: biopharmaceuticals, human therapy, *Lactococcus lactis*, vaccine

Expert Opin. Biol. Ther. (2011) 11(3):261–267

1. Introduction

Recent reviews have described early and contemporary achievements in the use of the lactic acid bacterium (LAB) *Lactococcus lactis* as a therapeutic agent for different human diseases and for the delivery of biologically active immunomodulating proteins *in vivo* [1–3]. The results presented in these reports provide solid evidence supporting the appropriate use of genetically engineered *L. lactis* to target diseases and modulate immune responses. Two distinctions must be made in this aspect. The first is the development of a *L. lactis* able to produce a specific antigen, hence, regarded as a ‘vaccine’. The second is the production of a non-antigenic immunomodulatory protein by *L. lactis* (e.g., cytokines) to stimulate the immune system.

One of the earliest reports regarding *L. lactis* as a vehicle to elicit immune responses describes the genetic manipulation of strain IL1403 (previously referred to as *Streptococcus lactis*) to express the surface protein antigen (Pac) of *Streptococcus mutans*. The resulting genetically modified organism (GMO) was then used to immunize mice. Analysis of salivary and serological samples revealed the presence of IgA and IgG in salivary secretions and in serum, which were specifically reactive against *S. mutans* [4]. These and other studies explored the suitability of *L. lactis* in the development of (live or dead) vaccines. Although other approaches using pathogenic microorganisms, for example, *Salmonella* or *Listeria* species [5], were

informa
healthcare

Article highlights.

- Genetically modified *Lactococcus lactis* are currently being used to treat a variety of diseases. With the increasing number of clinical trials and applications, *L. lactis* has the potential to become a commercially available biopharmaceutical in the coming years.
- Several aspects affect the efficacy of *L. lactis* vaccines. The choice of antigen to be expressed by *L. lactis*, the natural attributes and composition of the antigen, the final cellular localization in *L. lactis*, as well as the route of vaccine administration are all important factors which need to be considered.
- The safety of administration of genetically manipulated *L. lactis* to humans must be thoroughly assessed. Deletion of essential genes or auxotrophic strains prevent long-lasting viable cells and undesired cell proliferation.
- Oral administration of genetically engineered *L. lactis* able to produce IL-10 is currently being employed to treat inflammatory bowel disease in humans (Crohn's Disease). This same strain is currently in clinical trials to treat moderate ulcerative colitis and oral mucositis.
- Improving protein production by synthetic biology and facilitating the antigen reaching the immune system are viable options to create a better immune response of the therapeutic agent.

This box summarizes key points contained in the article.

providing promising results in the induction of immune responses, there was a much more keen interest in *Lactococcus* because of its natural non-pathogenic and non-colonizing properties [2]. Such an approach eliminated the drawbacks of using pathogenic or noxious attenuated microorganisms that is the high risk and infeasible use in humans and in clinical trials.

Presently, there is a vast collection of reports evidencing the safe and effective use of *L. lactis*, firstly to produce a wide variety of proteins and peptides efficiently, but most importantly, to deliver these biologically active molecules *in vivo* without disturbing their specific activity [2,3].

In 2006, the first breakthrough in the application of *L. lactis* in humans was reported, with Braat and colleagues revealing a clinical approach to treat Crohn's Disease with an IL-10-secreting *L. lactis*. In the investigation they showed that the oral administration of IL-10-secreting *L. lactis* in patients suffering from this disease resulted in a reduction of the intestinal lesions and a consequent alleviation of the illness [6]. As an added safeguard to ensure limited survival of the IL-10-expressing *L. lactis* once is released in the environment, the heterologous gene allowing for IL-10 expression substituted an essential gene, thymidylate synthase (*thyA*). The survival of this *thyA*-deficient GMO depends on the presence of thymidine or thymine in the growth medium. In this way, the microorganism can be regarded as 'biologically contained' [7]. This study, along with others, paved the path

for many other investigators into translational applications of existing strains of *L. lactis* for the treatment of other diseases.

While *L. lactis* possesses many characteristics such as easy genetic manipulation and a long history of safe use in food, that make it an attractive choice for further medical applications, the focus has been on fermenting LAB with the ability to produce heterologous antigens rather than the optimization of this bacterium for use as a vaccine.

1.1 Cellular antigen location in *L. lactis*

The location of the antigen is important for its immune-modulating activity. For certain proteins, such as cytokines or antibody fragments, extracellular secretion is necessary due to their activity [7,8], whereas for other antigens it is unclear whether the antigen must be produced extracellularly, retained in the cytoplasm or attached to the cell wall. Several factors influence whether intracellular or extracellular localization is desirable. These include the nature of the protein, that is its folding, accumulation and toxicity for the producing cell and unwanted processing by cellular/membrane-located proteases. However, most important is the capability of the produced antigen to undergo the crucial antigen processing by the immune machinery to ultimately cause the desired specific (humoral and/or cellular) immune response in the host. In this regard, there is an ongoing debate among researchers to select the final location of the *L. lactis* expressed-antigen. In several studies, immunization with *L. lactis* expressing an intracellularly localized antigen caused protection in mice when they were challenged with the respective pathogen. In one such study, Hannify *et al.* demonstrated that intranasal immunization with a strain of *L. lactis* producing intracellularly-localized pneumococcal surface protein A (PspA) from *Streptococcus pneumoniae*, was able to cause protection in the immunized group when they were challenged with *S. pneumoniae*, the causal agent of pneumonia [9]. In another study, three strains of *L. lactis* (all derived from *L. lactis* NZ9000 [10]) were developed that were able to produce the E7 antigen of the human papilloma virus type 16 (HPV-16) at different cellular locations, that is intracellular, secreted and attached to the cell wall. After immunization of three groups of mice with the three strains, it was demonstrated that *L. lactis* with the E7 antigen attached to the cell wall displayed the highest immunogenicity as compared with the other two strains, even though equal amounts of E7 were produced by each strain [11]. There are many other examples of the effect of antigen location and immunogenicity and the reader is referred to a recent review [3].

Vaccination using different mucosal surfaces may cause a systemic production of IgA at different mucosal locations. In this regard it seems remarkable that nasal mucosal immunization not only stimulates an immune response in the respiratory tract, but can also give rise to a strong genital-vaginal mucosal immune response [12].

Therefore, it is highly relevant to study the nature of the antigen to be expressed by *L. lactis* (the candidate to create the vaccine) to determine the most suitable cellular compartment where it will be delivered and the most adequate route of administration.

While antigen localization and immunization routes are important aspects in eliciting a good immune response by the host, the correct presentation of the antigen by *L. lactis* to the antigen-presenting cells (APCs) is crucial if an effective vaccine is to be developed.

1.2 Antigen modeling to optimize MHC priming

It has been calculated that from 10^4 degraded proteins only 1 peptide binds to the MHC class I molecule [13]. This is a very modest amount of peptide compared with the massive amounts of antigen available when *L. lactis* is administered and yet it is sufficient to cause the prophylactic and therapeutic effects [9,11]. Considering the ease with which genetic manipulations can be made in *L. lactis*, including the ability to express the antigen in different cellular locations, it may be possible to confer desirable characteristics to antigens to be produced by the bacterium, resulting in a more effective vaccine. This could involve the fusion of virus-derived or bacteria-derived proteins to the antigen to facilitate MHC I presentation. This can result in a better cellular immunity response, as in previous experiments that have demonstrated how fusing a protein from bacterial origin (e.g., a domain from a toxin) to a particular antigen, results in an increment in the immunogenicity and an augmentation in the immune response. These experiments nevertheless, were performed employing DNA vaccines to produce the chimeric antigen [14-17]. Using adenoviral vectors, we have demonstrated that the fusion of peptides commonly found in the endoplasmic reticulum (ER) (e.g., calreticulin) to an antigen, resulted in the retention of the antigen in the ER, improving its presentation to the MHC I [18]. These experiments indicate that enhanced antigen presentation to the MHC I results in a better cellular immune response [17-19]. Taking in account these results, it is possible to use the same strategy and, for example, fusing calreticulin to antigens expressed by *L. lactis* in order to increase the immunogenicity. Alternatively, also to increase the antigenicity and obtain a more robust immune response, molecules capable of tight binding to APCs like dendritic cells (e.g., bacterial heat shock proteins) may be fused to the antigen of interest, as has been shown [15,16]. Such approaches in the design of immunomodulatory proteins or antigens expressed by *L. lactis* would result in a more acute immune response since they would be tailor-made to the type of immunity, when either humoral or cellular immunity is desired.

Currently, the immunological responses evoked by genetically manipulated strains of *L. lactis* have achieved in the majority of the cases, humoral responses in animal models [3]. In these cases, the immunization, with strains of *L. lactis* able

to produce a specific antigen results in the development of specific antibodies against the pathogen (the causal agent) and the alleviation of the disease [9,20].

Upon immunization, the genetically engineered *L. lactis* should express the antigen, retain antigen already synthesized or both. At some point the antigens (or fragments of them) are internalized by APCs and loaded into the endosomal compartment to the MHC II, where they are later transported to the cell surface. The final outcome of the above processes induce the generation of specific antibodies, which later will recognize (and neutralize) the causal agent and continue with the antibody-mediated immune response. This humoral immunity resulting is suitable for extracellular pathogens (e.g., bacteria, fungi and protozoa).

But what about for other diseases where cell-mediated immunity plays an essential role, like for example in the case of virus infection and even more importantly, for tumors? Is it possible to create a strategy using *L. lactis* that will target cancerous cells and will act as an anti-tumor agent?

In this context, it was demonstrated by Bermudez-Humarán and colleagues that immunization with a combination of two strains of *L. lactis* expressing either E7 or IL-12 resulted in both prophylactic and therapeutic effects in a cervical cancer model [21]. In this report, it was demonstrated how the E7 strain solely can confer (to a much lesser extent) antitumoral activity. Moreover, very few mice immunized with the non-expressing wild type strain of *L. lactis* had tumors of similar size compared with the group of mice immunized only with the strain expressing E7. This suggests that either the vaccination with *L. lactis* wild type alone may enhance at some level a non-specific immune response that affected the growth of the tumors, or that some of the mice vaccinated with *L. lactis*-E7 did not respond to the treatment and the tumor growth was similar as in the group vaccinated with the non-expressing wild type strain. Nevertheless, in this study it was demonstrated how an antigen-expressing *L. lactis* is capable of inducing a cellular immune response using simultaneously two strains of *L. lactis* expressing the E7 antigen of the HPV and cellular-immunity activating IL-12 [21].

2. Applying the technology of *L. lactis* for current health issues

In emerging diseases, like the recent epidemic caused by the H1N1 virus, the immediate availability (and distribution) of a vaccine against a pathogen would have a tremendous effect on the evolution and prevention of the epidemic. Unfortunately, currently, after identifying the causal agent of an emerging disease, the process to finally develop a safe and functional vaccine (and make it commercially available) is lengthy and tedious.

Table 1. Perspectives and criteria for safe and effective vaccines using *Lactococcus lactis*.

Criteria	Consideration	Ref.
Biological contingency of genetically manipulated LAB	Deletion of essential genes, auxotrophic strains	[7]
Expression systems employed	Constitutive expression, inducible systems (e.g., nisin-inducible system [NICE])	[10,22,28]
Augmentation of the immune response	Intracellular antigen targeting (antigen fusion with viral proteins, antigen-presenting cells binding proteins), sub-cellular antigen retention (e.g., in the rugged endoplasmic reticulum), immunomodulators (interleukins, viral or heat shock proteins)	[14-18,21,29]
Increasing protein expression	Codon optimization for <i>Lactococcus</i> , synthetic promoters, protease deficient strains	[30,31]
Route of administration	Dependent on the immune response most adequate for the targeted disease and the entry route of the causal agent	[3]
Antigen location in <i>L. lactis</i>	Cytoplasmic, extracellular or associated with the cell	[11]

For this reason, the existence of a 'plug-in and ready-to-produce' vaccine using *L. lactis* could expedite the production stage of a vaccine, and therefore, influence an ongoing epidemic. Moreover, because preparation of growth medium and cultures of *L. lactis* is relatively easy, the need for highly skilled personnel is minimal and makes it advantageous to proceed also in developing countries. Moreover, the route of administration, either intranasal or oral, avoids the use of infectious material, like needles and syringes commonly used to apply non-oral vaccines, decreasing the risk of consequent infections and reducing the cost of the vaccination. This becomes attractive in situations where substantial numbers of doses are needed in adverse conditions (environmental, geographical or limited human resources).

In a previous case we mentioned that the immunization with a genetically modified strain of *L. lactis* conferred protection to mice when they were challenged with the pathogen [9]. Using *L. lactis*, for example to develop a pneumococcal vaccine for humans, would prevent the need to use attenuated strains of the pathogen intended to be used as a vaccine, or lengthy and costly procedures to purify recombinant antigens used for vaccination.

At the present time, a patented technology called Actobiotics™ consists of biologically contained strains of *L. lactis* able to secrete biological active interleukins *in situ* [22]. These strains of *L. lactis* are being employed to alleviate inflammatory bowel disorders in humans (e.g., Crohn's disease and ulcerative colitis). Actobiotics are delivered to patients via oral administration (they are usually formulated in capsules) avoiding the use of syringes and needles. This technology is designed to selectively be delivered to receptors and cells localized in the gastrointestinal tract, reducing the exposure to non-target organs to a minimal (For detail information of Actobiotics and its characteristics the reader is referred to www.actogenix.com).

ActoGeniX, the company that exploits Actobiotics, has completed Phase IIA of clinical trial to treat ulcerative colitis in humans and is now in Phase I of a clinical trial to treat oral mucositis in cancer patients using Actobiotics [Data from ActoGeniX press release]. Safety is also an essential issue, for that reason, ActoGeniX has also taken measures to prevent undesired microbial endurance inside the host. This was achieved by substituting an essential gene of *L. lactis* (thymidylate synthase) with the gene of interest (IL-10) to prevent prevalence of the genetically manipulated *L. lactis* once they are released to the environment [7].

Another company focusing on the generation of vaccines using *L. lactis* is Mucosis, who recently developed FluGEM™, an intranasal vaccine to prevent influenza. Moreover, this company deals with a novel variety of a non-genetically modified strain of *L. lactis*, able to load antigens of viral, parasitic or bacterial origin on the cell envelope. The remarkable property of the system named Mimopath™ is that the *L. lactis* cells loaded with the antigen are administered dead, thus, the vaccine is regarded as a non-genetically modified non-live vaccine [23,24].

3. Expert opinion and future perspective

The use of *L. lactis* as a therapeutic agent has been documented as safe and efficient, giving good results in a variety of disease models. No side effects or undesired reactions have been detected up to now. There have been only two medical cases that reported *L. lactis* infection in humans, causing cardiovascular complications. The patients in these two cases suffered from previous cardiac abnormalities. In one of these patients the infection caused by *L. lactis* was firstly treated with antibiotics. Once the infection was cleared, the myxoma, a possible pre-existing condition, was surgically extirpated. After the surgery, the patient did not develop any further infection with *L. lactis* [25]. These isolated two

Table 2. List of clinical trials and diseases targeted with *Lactococcus lactis*.

Targeted disease	Route of administration	Physiological system targeted	Condition of <i>L. lactis</i> at administration	Model protein used	Completed clinical trial
Ulcerative colitis*	Oral	Digestive	Live (biologically contained)	IL-10	Phase II
Inflammatory bowel disease (Crohn's disease)*	Oral	Digestive	Live (biologically contained)	IL-10	Phase I
Mucositis*	Oral	Digestive	Live (biologically contained)	Human Trefoil factor 1 (hTFF1)	Ongoing Phase IB
Influenza [‡]	Oral or intranasal	Respiratory	Dead particles loaded with antigen	Diverse influenza antigens	Unavailable

*Obtained from ActoGeniX press release.

[‡]Obtained from Mucosis press release.

cases probably represent opportunistic infection of (in this case) *L. lactis*, due to the weakened general health of the patients [25,26]. To our knowledge no cases of infection by *L. lactis* have been reported in healthy individuals. This two incidents involving opportunistic infection by *L. lactis* in patients with deteriorated health should not discourage further use of *L. lactis* in clinical trials or in any other medically related research.

The application of *L. lactis* as a vaccine and therapeutic element is a viable option that can be used safely, and even more importantly, that can be developed rapidly to provide functional and effective vaccines in newly emerging diseases and world wide epidemics. The use of *L. lactis* as an established vaccine could be achieved by the creation of a 'ready-to-use' reliable system that allows the rapid integration of the antigen-coding DNA in *L. lactis* to provide an expeditious vaccine whenever is required, for example in emerging epidemics or when large amounts of vaccines are required in very little time.

Presently it is possible to optimize expression levels and overall protein production using synthetic biology for example with dual codon optimization, strong synthetic promoters or synthetic genes. This and other considerations are reviewed in Table 1, where the different present alternatives to achieve an efficient vaccine using *L. lactis* are also highlighted.

The low cost of the overall production of the vaccine when *L. lactis* is employed is an advantage that would allow developing countries to have access to vaccines and therapies that usually are expensive and scarce. A few examples are the availability of vaccines for tuberculosis, against HPV or for pneumonia using *L. lactis* in countries where the prevalence of these diseases is high (usually in countries with a large population with low income per capita), could decrease the

number of current pandemics that have persisted for decades due to the lack of commercial available vaccines that are readily accessible for the population.

In less than 5 years the use of *L. lactis* in clinical trials progressed from Phase I to Phase IIA (Table 2). There is also an ongoing clinical trial Phase IB to treat oral mucositis in cancer patients, using this same therapeutic agent. Other pre-clinical trials are currently being prepared to treat celiac disease, type 1 and 2 diabetes, and allergic diseases (Data from ActoGeniX press release), as well as the development of a pneumococcal vaccine (Mucosis) [27]. With this, there are precedents that encourage and support further applications of *L. lactis* to alleviate other diseases.

In the coming years it will be important that scientists supporting the use of *L. lactis* as a therapeutic agent transfer their achievements to endorsed proposals for clinical trials in humans to provide more evidence for future successful applications, ultimately culminating in the use of *L. lactis* as a commercial available biopharmaceutical against any disease of relevance.

Acknowledgements

The authors thank CE Price for critically reviewing this manuscript. J Villatoro-Hernandez would like to thank E Garza-Salinas for the constructive advice and support.

Declaration of interest

J Villatoro-Hernandez and OP Kuipers are supported by the research programme of the Kluyver Centre for Genomics of Industrial Fermentation, which is part of the Netherlands Genomics Initiative/Netherlands Organization for Scientific Research.

Bibliography

Paper of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.

1. Bermudez-Humaran LG. *Lactococcus lactis* as a live vector for mucosal delivery of therapeutic proteins. Hum Vaccin 2009;4:264-7
2. Van Huynegem K, Loos M, Steidler L. Immunomodulation by genetically engineered lactic acid bacteria. Front Biosci 2009;14:4825-35
3. Wells JM, Mercenier A. Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. Nat Rev Microbiol 2008;5:349-62
4. Iwaki M, Okahashi N, Takahashi I, et al. Oral immunization with recombinant *Streptococcus lactis* carrying the *Streptococcus mutans* surface protein antigen gene. Infect Immun 1990;58:2929-34
5. Mielcarek N, Alonso S, Loch C. Nasal vaccination using live bacterial vectors. Adv Drug Deliv Rev 2001;51:55-69
6. Braat H, Rottiers P, Hommes DW, et al. A Phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol 2006;4:754-9
- This report, to our knowledge, is the first clinical trial using a genetically modified strain of *L. lactis* in humans.
7. Steidler L, Neirynck S, Huyghebaert N, et al. Biological containment of genetically modified *Lactococcus lactis* for intestinal delivery of human interleukin 10. Nat Biotechnol 2003;21:785-9
8. Vandenbroucke K, de Haard H, Beirnaert E, et al. Orally administered *L. lactis* secreting an anti-TNF Nanobody demonstrate efficacy in chronic colitis. Mucosal Immunol 2009;1:49-56
9. Hanniffy SB, Carter AT, Hitchin E, et al. Mucosal delivery of a pneumococcal vaccine using *Lactococcus lactis* affords protection against respiratory infection. J Infect Dis 2007;195:185-93
10. de Ruyter PG, Kuipers OP, de Vos WM. Controlled gene expression systems for *Lactococcus lactis* with the food-grade inducer nisin. Appl Environ Microbiol 1996;62:3662-7
11. Bermudez-Humaran LG, Cortes-Perez NG, Le Loir Y, et al. 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;53:427-33
12. Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. Nat Med 2005;11:45-53
13. Yewdell JW. Not such a dismal science: the economics of protein synthesis, folding, degradation and antigen processing. Trends Cell Biol 2001;11:294-7
14. Cuesta AM, Suarez E, Larsen M, et al. Enhancement of DNA vaccine potency through linkage of antigen to filamentous bacteriophage coat protein III domain I. Immunology 2006;117:502-6
15. Chen CH, Wang TL, Hung CF, et al. Enhancement of DNA vaccine potency by linkage of antigen gene to an HSP70 gene. Cancer Res 2000;60:1035-42
16. Kim JW, Hung CF, Juang J, et al. Comparison of HPV DNA vaccines employing intracellular targeting strategies. Gene Ther 2004;11:1011-18
17. Liao CW, Chen CA, Lee CN, et al. Fusion protein vaccine by domains of bacterial exotoxin linked with a tumor antigen generates potent immunologic responses and antitumor effects. Cancer Res 2005;65:9089-98
18. Loera-Arias MJ, Martinez-Perez AG, Barrera-Hernandez A, et al. Targeting and retention of HPV16 E7 to the endoplasmic reticulum enhances immune tumour protection. J Cell Mol Med 2010;14:890-4
19. Hung CF, Monie A, Alvarez RD, et al. DNA vaccines for cervical cancer: from bench to bedside. Exp Mol Med 2007;39:679-89
20. Lee P, Faubert GM. Expression of the *Giardia lamblia* cyst wall protein 2 in *Lactococcus lactis*. Microbiology 2006;152:1981-90
21. Bermudez-Humaran LG, Cortes-Perez NG, Lefevre F, 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;175:7297-302
22. Steidler L, Rottiers P, Coulie B. Actobiotics as a novel method for cytokine delivery. Ann NY Acad Sci 2009;1182:135-45
23. Bosma T, Kanninga R, Neef J, et al. Novel surface display system for proteins on non-genetically modified Gram-positive bacteria. Appl Environ Microbiol 2006;72:880-9
24. van Roosmalen ML, Kanninga R, El Khattabi M, et al. Mucosal vaccine delivery of antigens tightly bound to an adjuvant particle made from food-grade bacteria. Methods 2006;38:144-9
25. Zechini B, Cipriani P, Papadopoulou S, et al. Endocarditis caused by *Lactococcus lactis* subsp. *lactis* in a patient with atrial myxoma: a case report. Diagn Microbiol Infect Dis 2006;56:325-8
26. Pellizzer G, Benedetti P, Biavasco F, et al. Bacterial endocarditis due to *Lactococcus lactis* subsp. *cremoris*: case report. Clin Microbiol Infect 1996;2:230-2
27. Audouy SA, van Selm S, van Roosmalen ML, et al. Development of lactococcal GEM-based pneumococcal vaccines. Vaccine 2007;25:2497-506
28. Mierau I, Kleerebezem M. 10 years of the nisin-controlled gene expression system (NICE) in *Lactococcus lactis*. Appl Microbiol Biotechnol 2005;68:705-17
29. Kang TH, Kim KW, Bae HC, et al. Enhancement of DNA vaccine potency by antigen linkage to IFN-gamma-inducible protein-10. Int J Cancer 2010, doi: 10.1002/ijc.25391
30. Fuglsang A. Lactic acid bacteria as prime candidates for codon optimization. Biochem Biophys Res Commun 2003;312:285-91

31. Mijakovic I, Petranovic D, Jensen PR.
Tunable promoters in systems biology.
Curr Opin Biotechnol 2005;16:329-35

Affiliation

Julio Villatoro-Hernandez^{†1,2},
Roberto Montes-de-Oca-Luna³ &
Oscar P Kuipers^{1,2}

[†]Author for correspondence

¹University of Groningen,
Groningen Biomolecular Sciences
and Biotechnology Institute,
Molecular Genetics Group,
Kerklaan 30, 9751 NN, Haren,
The Netherlands

Tel: +31 50 363 2107; Fax: +31 50 363 2348;

E-mail: j.villatoro.hernandez@rug.nl

²Kluyver Centre for Genomics of

Industrial Fermentation,
Delft, The Netherlands

³Facultad de Medicina,
Universidad Autónoma de Nuevo León,
Madero y Aguirre Pequeño S/N,
Monterrey, Nuevo León,
México 64460