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Delivery of Therapeutic Proteins through *Lactococcus lactis*

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Introduction

Food-grade bacteria, many of which are known as lactic acid bacteria (LAB), have, over the centuries, been extensively consumed, both by humans and livestock. Impact of these organisms on health is very well documented and, with the industrialization of food and feed production, is monitored systematically. As pathologies associated with their consumption are extremely scarce, their use in nutrition is therefore rarely disputed. One intrinsic advantage of food-grade LAB therefore lies in the knowledge that they are not pathogenic, not even when given overt opportunity, as would be the case during an ongoing disease.

Many LAB are normal constituents of the microflora in the intestine of mammals. It was not until recently that the effects of the enteric microflora on the well-being of the host have been recognized. Much effort is now undertaken to isolate specific strains of LAB that can be utilized to re-establish the normal function of the intestinal tract, both on the digestive as well as on the immunological level. *Lactococcus lactis*, a non-colonizing LAB, has also been used in industry for many years, where it is essential in a large number of dairy and other food fermentations.

LAB in vaccination and allergy

The 'safe' nature of LAB on the one hand, and the gradual availability of tools for genetic modification on the other, have prompted many speculations on novel

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Abbreviations: CD, Crohn's disease; cfu, colony-forming units; DSS, dextran sodium sulfate; GM, genetically modified; GMO, genetically modified organism; HIV, Human Immunodeficiency Virus; IBD, inflammatory bowel disease; IFN, interferon; IgA, immunoglobulin A; IL, interleukin; KT, killer toxin; LAB, lactic acid bacteria; mIFN, murine interferon; mIL, murine interleukin; mTFF, murine trefoil factor; Ptgs, prostaglandin-endoperoxide synthase; ScFv, single-chain Fv antibody; TFF, trefoil factor; Th, T helper cell; TTFC, tetanus toxin fragment C; UC, ulcerative colitis.

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medical applications. For example, Drouault and colleagues used GM *L. lactis* to try to enhance lipid digestion (Drouault *et al.*, 2002). They expressed *Staphylococcus hyicus* lipase in the cytoplasm of *L. lactis* and demonstrated that fat absorption in pigs was higher when treated with this strain. This approach has the potential to improve the treatment of pancreatic insufficiency in humans. In addition, Chang and colleagues have shown, *in vitro*, that *Lactobacillus jensenii*, a natural human vaginal isolate engineered to secret two-domain CD4 proteins, can inhibit HIV-1 entry into HeLa cells (Chang *et al.*, 2003).

In this field, however, directing and redirecting immunity has received a lot of attention. Over the past decade, much effort has been made to use LAB as delivery vehicles for foreign antigens at mucosal surfaces (for a review see Seegers, 2002; Nouaille *et al.*, 2003). The most striking example was found after oral or intranasal immunization with *L. lactis* expressing tetanus toxin fragment C (TTFC), where high antibody titres could be detected in the serum (Wells *et al.*, 1993; Norton *et al.*, 1997; Robinson *et al.*, 1997). The main interest of our group lies in the use of *L. lactis* for the expression and secretion of cytokines (Steidler *et al.*, 1995, 1998, 2000; Schotte *et al.*, 2000). Cytokines, such as interleukins and interferons, are small molecules that are important in the communication between and regulation of different cells of the immune system. By inducing the correct cytokines, the immune system is either activated or suppressed. We noticed that the expression of interleukins in *L. lactis* had no negative effects on the growth of the bacterium itself, which makes it an ideal tool for the delivery of therapeutic proteins at mucosal surfaces. Steidler and colleagues described, for the first time, the potential to boost the immune system by vaccinating mice with *L. lactis* that coexpressed an antigen with murine interleukin-2 (mIL-2) or mIL-6 (Steidler *et al.*, 1998). The antigen and the cytokine genes were placed in an operon-like structure. TTFC was expressed in the cytoplasm, while the interleukin was secreted into the environment. Intranasal immunization of mice with the strains expressing either mIL-2 or mIL-6 together with TTFC produced significantly higher antibody titres against the antigen than did the parental strain expressing TTFC alone. Bacteria expressing TTFC in combination with mIL-6 were also capable of eliciting serum anti-TTFC IgA responses. The boosting effect was lost when the bacteria were killed before vaccination by a pre-treatment with mitomycin C. This means that viable bacteria, which secrete the cytokine *in situ*, are essential to enhance the immune response.

Bermúdez-Humarán and colleagues were able to successfully secrete a heterodimeric cytokine, mIL-12, in *L. lactis* (Bermúdez-Humarán *et al.*, 2003). The researchers expressed mIL-12 as two separate polypeptides (p35 and p40) or as a single polypeptide by linking the p35 to the p40 subunit. The single-chain mIL-12 showed a higher biological activity. Expression of the two genes separately resulted in the formation of the heterodimeric polypeptide, p70, as well as the antagonistic homodimer p80 (p40–p40) (Ling *et al.*, 1995). Mice were immunized intranasally with the single-chain mIL-12-secreting strain and *L. lactis* that produced the E7 antigen of human papillomavirus type 16. *In vitro* stimulation of splenocytes isolated from the immunized mice with the antigen resulted in enhanced levels of the Th1 cytokines, mIL-2 and mIFNγ. This opens up the possibility of switching the Th2 response, the preferred default route after intranasal immunization with the E7 antigen, towards a Th1 response. Immune modulating capacity can thus be

ingeniously designed by the appropriate choice of host organism. In a mouse model for birch pollen allergy, Repa and co-workers demonstrated that coadministration of either one of two wild-type LAB, *L. lactis* or *Lactobacillus plantarum*, and the main allergen of birch pollen allergy prior to or after sensitization induced a shift towards Th1 immune responses (Repa *et al.*, 2003). This offers the possibility of vaccinating against type I allergy. The capacity of these wild-type LAB, as described by Repa and co-workers, may be further enhanced by designing them to secrete IL-12, as described by Bermúdez-Humarán and colleagues (Bermúdez-Humarán *et al.*, 2003; Repa *et al.*, 2003).

Single-chain antibodies

Neutralizing antibodies that are directed towards a pathogen, toxin, cytokine, or other agent have proved to be very valuable and specific tools in medicine. With the emergence of single-chain (ScFv) antibody technology, it has now become possible to produce neutralizing antibodies from recombinant bacteria. Most of the work in this area relates to the production of the antibody for downstream processing and use as a purified protein. Due to their structure, these peptides suffer from a very short half-life *in vivo,* therefore suitable delivery systems are required to allow for their use as therapeutics. A number of applications are now emerging in which the expressor strain itself is used for the *in situ* production of the antibody fragment, especially to control colonization by pathogens. It has been observed that the oral administration of antibodies that recognize *Streptococcus mutans* reduce caries (Lehner *et al.*, 1985; Michalek *et al.*, 1987). Therefore, *Lactobacillus zeae* was engineered to produce a single-chain antibody fragment against streptococcal antigen I/II adhesion molecule of *S. mutans* (Krüger *et al.*, 2002). When these bacteria were used in a rat model for caries, *S. mutans* counts and caries scores were markedly reduced. This approach may be of interest for *in vivo* immunotherapy.

An even more elaborate approach has been used to combat *Candida albicans*, one of the most frequent causative agents of mucosal inflammation in humans (Fidel and Sobel, 1996). Infections are seen in the mouth and oesophagus of immune compromised persons, such as HIV-infected subjects. *C. albicans* also causes acute vaginitis in otherwise healthy women. There exists a real need for new therapeutics in this area as few adequate drugs are known, resistance is increasing, and vaccines are not available. Beninati and co-workers (Beninati *et al.*, 2000) have made a recombinant *Streptococcus gordonii*, a species with good vaginal colonization and heterologous expression potential *in vivo* (Medaglini *et al.*, 1997, 1998; di Fabio *et al.*, 1998), for the eradication of *C. albicans* infections. Anti-idiotypic ScFv was produced with structural surface similarity to a wide spectrum killer toxin (KT) of *Pichia anomala* (Polonelli and Morace, 1987; Magliani *et al.*, 1997). Two *S. gordonii* strains were constructed: one that expressed the ScFv at its surface and a second that secreted the ScFv. Structural similarity to KT could be shown by cross-reactivity with specific monoclonal antibodies. Both surface-bound and secreted ScFv showed candidacidal activity over a wide concentration range *in vitro*. Both *S. gordonii* strains successfully colonized the vagina and cleared experimental *C. albicans* infection in rats, dependent on the presence of this ScFv. The secretor, however, showed a faster reduction of the pathogenic load, a result comparable with a full course treatment

with the antifungal fluconazole. This work shows that local production of a designer microbicide is a valid approach for the treatment of a very common mucosal pathology. Thus, the expression of single-chain antibodies by bacteria has the potential to be applied to a wide range of diseases.

Interleukin-10 secreting *L. lactis* for treating IBD

We demonstrated the immune modulating capacity of a recombinant mouse interleukin-10 secreting *L. lactis* strain in murine models for inflammatory bowel disease (IBD) (Steidler *et al.*, 2000). IBD, characterized by intestinal inflammation, comprises two distinct disorders, Crohn's disease (CD) and ulcerative colitis (UC). The symptoms of both diseases include diarrhoea, abdominal pain, rectal bleeding, weight loss, fever, lethargy, and loss of appetite. IBD is a lifelong and chronic disease characterized by periods of exacerbation and remission. Although the disease is usually not lethal, it has major impact on all aspects of quality of life. IBD affects 1–2 in 1000 individuals in Western societies, and the number is still increasing. Although both diseases show many similarities, they are clearly two distinct disorders. The inflammation in UC patients is restricted to the colon and only the mucosa is affected. CD can affect the whole intestinal tract, from mouth to anus, and the inflammation can be transmural. Both diseases are characterized by an imbalance of Th1 versus Th2 cells. CD shows predominant Th1 cytokines, such as IL-12 and IFNγ, whereas UC shows mainly Th2 cytokines, IL-4 and IL-5 (Niessner and Volk, 1995; Fuss *et al.*, 1996; Kakazu *et al.*, 1999). The aetiology is not known, but genetic predisposition (Hugot *et al.*, 2001; Ogura *et al.*, 2001) and the intestinal microflora are thought to play an important role (Sartor, 1997).

Many animal models have shown that the resident microflora play an important role in the development of IBD (Biancone *et al.*, 2002; Guarner *et al.*, 2002; Cummings *et al.*, 2003). The number of bacteria in the gut ranges from 10^8 organisms/g in the ileum to 10^{11}/g in the colon. It is believed that during inflammation in the gut, there is an increased influx of antigen derived from the luminal content or from pathogenic bacteria. This immune response is not properly shut down and induces immunity, rather than tolerance, towards the commensal antigens. Breach of tolerance towards the normal intestinal microflora may be the driving force behind IBD. Therefore, therapeutic manipulation of the intestinal microflora with antibiotics, pre- or probiotics offers possibilities to cure IBD (reviewed by Sartor, 2004). Another possibility is to shut down the improper immune response and re-establish tolerance. IL-10 is a key mediator in this process (Duchmann *et al.*, 1996). Attempts have been made to administer recombinant IL-10 to IBD patients. The results were not completely successful (van Deventer *et al.*, 1997). Giving IL-10 through the systemic route led to moderate side effects (Fedorak *et al.*, 2000; Schreiber, 2000; Tilg *et al.*, 2002). This impeded the long-term use of IL-10 at high doses. Moreover, oral administration is hampered by the extremely acid sensitivity of IL-10. We tried to circumvent all these drawbacks by constructing a mIL-10 secreting *L. lactis* strain (Steidler *et al.*, 2000). Daily oral doses of this strain could efficiently cure colitis in a DSS-induced murine model and prevent the onset of colitis in IL-10 knockout mice. When the bacteria were killed by UV-treatment, the positive result was abrogated. This again shows that the *in situ* production of IL-10 is essential for

curing colitis. After oral administration, these recombinant bacteria arrive at the inflicted area and produce their therapeutic agent. Because of this localized production, it can be speculated that side effects associated with systemic administration can be reduced or avoided.

Treatment of colitis with TFF secreting *L. lactis*

In a recent paper, Vandenbroucke and co-workers further expanded upon the principle of intestinal delivery of therapeutic proteins by *in situ* synthesis from *L. lactis* (Vandenbroucke *et al.*, 2004). They reported on a remarkably effective new prophylactic and therapeutic approach for acute and chronic colitis in mice, involving *in situ* secretion of murine trefoil factors 1, 2, and 3 (mTFF1, 2, and 3) by orally administered *L. lactis*.

Acute intestinal inflammation (acute colitis) is characterized by extensive epithelial ruptures. Their treatment may be a means to prevent the onset of IBD but, overall, the number of therapeutic strategies for acute colitis is rather limited. Accordingly, the need for new methods is evident.

TFF form a class of non-mitogenic peptides that are important in the protection and repair of the intestinal epithelium (recent review: Taupin and Podolsky, 2003) and, accordingly, are promising tools for treatment of acute colitis. However, no trefoil-based, orally administered drug for colitis has been reported. The main reason for this is that luminal administered TFF stick to the mucus of the small bowel and are removed from the lumen at the caecum (Poulsen *et al.*, 1999). Active *in situ* delivery of TFFs in the colon by localized synthesis from *L. lactis* circumvents this problem.

L. lactis is able to produce and secrete biologically active murine TFF in suitable quantities to allow for oral application and subsequent adequate production *in situ*. Physical delivery of TFFs by *L. lactis* at the colon could therefore be demonstrated and quantified. Daily intragastric administration of the TFF secreting strains, prior to or during disease induction, resulted in significant protection against DSS-colitis, as observed from reduced mortality and reduced loss of body weight. Furthermore, substantial improvement of the colon histology and reduction of inflammatory infiltrate were observed. Mice treated with UV-killed mTFF1 secreting *L. lactis* were not protected against DSS-induced acute colitis. This, once again, demonstrates that the protective effect requires *de novo* synthesis by live *L. lactis*.

Oral administration of high amounts of purified mTFF1 did not ameliorate acute colitis, whereas rectal administration did. Rectally administered mTFF1, however, was much less effective than orally administered mTFF secreting *L. lactis*. To explain this, the authors proposed a strategy in which intimate basolateral contact between colonocytes and *L. lactis* cells would enable TFF to accumulate out of reach of complexing mucins and allow them to interact with the putative basolateral TFF receptors on enterocytes (Thim and Mortz, 2000).

The paper by Vandenbroucke and colleagues is seminal in its scrutiny of the method of action of their novel approach (Vandenbroucke *et al.*, 2004). Ptgs2, the product of a known TFF target gene (Tan *et al.*, 2000; Rodrigues *et al.*, 2001), is strongly induced in the intestines of mice treated with mTFF secreting *L. lactis*, which proves that recombinant TFF was biologically active *in situ* in the colon.

Ptgs2 contributes to the healing and down-regulation of the inflammatory responses in the gastrointestinal tract (Mizuno *et al.*, 1997; Ehrlich *et al.*, 1998). Tan and co-workers showed that Ptgs2, and subsequently produced prostaglandins, mediate the cytoprotective action of TFF3 against oxidant epithelial cell injury (Tan *et al.*, 2000). Inhibition of Ptgs2 by meloxicam substantially abrogated the prophylactic effect of mTFF producing *L. lactis*. This indicates that, although induction of Ptgs2 is probably not the only pathway for TFF-mediated protection, the upregulation of Ptgs2 is of considerable importance in treatment of acute colitis.

This approach may provide effective medical applications for the treatment of acute and chronic phases of inflammatory bowel disease in humans, and may offer an alternative immune intervention strategy in the critical care of Crohn's disease patients. This work demonstrates that the strategy that was developed for the delivery of IL-10 (Steidler *et al.*, 2000) can be extended to therapeutics of a completely different structure, which allows us to speculate on the development of a wide array of new applications.

Biological containment

Systems such as the ones described above offer fascinating possibilities for future use as prophylactics or therapeutics in humans, as well as in animals. However, before they can be applied as such, they have to be redesigned in a way that tightly controls the spreading of genetic modifications into the environment. This involves three aspects: avoidance of antibiotic selection markers, control on the spread of the GMO, and control of the dissemination of the genetic modification to other hosts.

In all of the above-discussed applications, the therapeutic traits were inserted into plasmids that carry antibiotic resistance genes. With the current knowledge at hand that (multi)-drug resistant bacteria, non-pathogenic as well as pathogenic, arise quickly, an acceptable fear exists that antibiotic selection markers can become even more widespread when GMOs carrying antibiotic resistance genes are released in the environment. The first attempt to avoid the use of antibiotic resistance genes can be found in the design of food-grade selection markers (Platteeuw *et al.*, 1996; Leenhouts *et al.*, 1998; Sorensen *et al.*, 2000; Bron *et al.*, 2002). In these studies, most often a metabolically essential gene of the bacterium is inactivated or removed from the genome and complemented for on a plasmid. One intrinsic disadvantage of such food-grade vectors is accordingly the prerequisite for mutant strains. Furthermore, because the introduced foreign gene is still present on a plasmid, the possibility exists that the plasmid may disappear from the host, or may eventually be transferred to a new host. Therefore, a design in which the gene of interest is stably integrated into the bacterial genome is more desirable.

Additional to the single-chain antibodies to ameliorate the effects of *S. mutans* in dental caries as discussed earlier, Hillman and colleagues described a very elegant system, which used a genetically modified *S. mutans* strain in a replacement therapy (Hillman *et al.*, 2000). In dental caries, mutations that affect acid production by mutans streptococci have long been known to reduce their cariogenicity (reviewed by Anderson, 1992). To reduce lactic acid production of *S. mutans*, Hillman and colleagues replaced the lactate dehydrogenase gene by an extra alcohol dehydro-

genase gene isolated from *Zymomonas mobilis* (Hillman *et al.*, 2000). The gene replacement was made in a naturally occurring *S. mutans* strain that produces a lantibiotic named mutacin 1140 (Hillman *et al.*, 1984, 1998). The parent, non-GM mutacin-producing strain can persistently colonize the human oral cavity and proactively replace the indigenous strains (Hillman *et al.*, 1987). At least two of the three subjects that had been inoculated with 10^{11} bacteria onto their cleaned tooth surfaces remained colonized when tested 15 years later. When the GM strain was tested in rodent models, it was shown to be less cariogenic (Hillman *et al.*, 2000). This GMO does not carry any antibiotic resistance genes, the heterologous gene is integrated in the genome and the bacterium only replaces the naturally occurring *S. mutans* without affecting other closely related species (Hillman, 2002).

Other containment systems use suicide genes, such as nucleases or small toxins like Gef and Hok, that are induced once the GMO has finished exerting its action (for a review see Molin *et al.*, 1993). These so-called active biological containment systems have been developed mainly in Pseudomonas and are used in a process called bioremediation (Jensen *et al.*, 1993). This soil bacterium can be engineered in such a way that its growth becomes dependent on the presence of a pollutant. Once the pollutant is used up, the killing genes are induced. The suicide systems can be plasmid-borne (Contreras *et al.*, 1991; Knudsen *et al.*, 1995) or integrated into the genome (Recorbet *et al.*, 1993; Molina *et al.*, 1998). Active containment systems are dependent on the induction of the suicide genes by removing repression, e.g. addition of isopropylthio-ß-D-galactoside, which is not very likely outside the laboratory, or by depletion of growth components, such as proline (van Dillewijn *et al.*, 2004), tryptophan, or a xenobiotic compound (Jensen *et al.*, 1993). A drawback of the plasmid-borne system is that they can eventually escape to a host, which carries the repressor of the suicide system so that the suicide genes cannot be transcribed or translated. Since antibiotic resistance markers are present on these plasmid-borne systems, they are not suitable for use outside a laboratory situation. If the suicide genes are integrated into the genome, the efficiency may drop by several orders of magnitude. The success of the active suicide system is also dependent on the mutation rate. Typical numbers for the mutation rate are 10^{-6} per cell per generation (Knudsen and Karlstrom, 1991) for a single suicide, and 10^{-8} for a dual suicide, plasmid-borne system (Knudsen *et al.*, 1995), and less than 10^{-8} per cell per generation for a single suicide, chromosomally located system (Molina *et al.*, 1998).

When this system was tested in the outside world, it was shown that the bacteria died much slower than during the laboratory assays (Molina *et al.*, 1998). An attempt was made to ameliorate the system by using a mutant *Pseudomonas* strain. This strain had a deletion of a gene involved in synthesis of essential metabolites (Ronchel and Ramos, 2001). It was shown that this mutant strain was cleared faster from soil than the parental strain, and also that the mutation rate dropped to less than 10^{-9}. A further consideration on the use of active biological containment systems is that all suffer from leakage of the promoters used to drive the expression of the killing gene. This leads to a reduced growth rate and can give a selective growth advantage to cells with mutated suicide functions (Knudsen and Karlstrom, 1991).

We described a containment system in which an essential gene, *thymidylate*

synthase (*thyA*), in the genome of *L. lactis* is replaced by the gene of interest, i.e. human IL-10 (Steidler *et al.*, 2003). This GM strain, Thy12, is strictly dependent on the presence of thymidine or thymine for its growth and survival. In contrast to deprivation of any other metabolic mutant of its complementing metabolite, thymidine starvation of Thy12 leads to a sudden drop, 7 orders of magnitude in 72 hrs (see *Figure 12.1a*), in the number of colony-forming units (cfu). Depletion of other nutritional requirements, such as glucose, are usually bacteriostatic and not bacteriocidal (see *Figure 12.1b*). Depleting *thyA* deficient strains of thymidine or thymine results in cell death, due to increased DNA damage and subsequent induction of SOS-repair genes and fragmentation of the DNA. This phenomenon had long been reported and is known as thymineless death (reviewed by Ahmad *et al.*, 1998). However, readily amenable systems based on this phenomenon have not been described up to now.

Containment of Thy12 was also tested *in vivo* in pigs (Steidler *et al.*, 2003). Lyophilized *L. lactis* MG1363 (parental strain) and Thy12 were put into enteric-coated capsules to protect the bacteria against lysis by bile. Because pigs chew their food and would destroy the enteric coat on the capsules, a fistula was made to access the proximal duodenum. A second fistula was created at the terminal ileum, where the enteric coat should dissolve and release the bacteria. Viability was determined in an ileum sample and the faeces. From the pig that received MG1363, we detected 3.64×10^8 cfu in the ileum and 2.94×10^8 cfu in the faeces. This corresponds to a reduction in viability of 19.2%. From the pig that was given Thy12, we obtained an ileal count of 2.69×10^8 and 0.125×10^8 cfu in the faeces. This corresponded to a drop in viability of 95.3% (see *Figure 12.1c*). This proves that, in the 'physically contained' environment, the viability of Thy12 drops much faster than the wild-type strain, and with a rate similar to that measured *in vitro*.

A concern of the containment system based on the deletion of *thyA* is that the GM strain would pick up a new *thyA* gene and become independent of thymidine or thymine for its growth. We tried to mimic this situation by adding traceable Thy12 to a high number of donor bacteria, ranging from Gram-negative, such as *Escherichia coli* and *Salmonella choleraesuis*, to closely related Gram-positive species, such as *L. lactis* MG1363 (Steidler *et al.*, 2003). We were unable to pick up Thy12 bacteria that had reacquired *thyA* so growth had become thymidine indepentent. Alpert and co-workers showed that, even with chromosomally located mobile elements such as transposons, it is very unlikely to obtain gene transfer *in vivo* (Alpert *et al.*, 2003). Although we cannot assure *ad infinitum* that Thy12 will not pick up a foreign *thyA* gene, the event is likely to be extremely infrequent, so is unlikely to tip the balance against its use for the treatment of such severe diseases as Crohn's disease and ulcerative colitis.

Ecological containment, i.e. all boundaries, such as environmental stress, availability of nutrients, that originally confined the presence of a bacterial species to its habitat, also form an inherent and substantial blockade on the dissemination of any GM derivative. *L. lactis* was originally isolated from raw milk (Hirsch, 1952). Although the species had ample opportunity, no colonization of any other ecological niche has been reported. Uninhibited propagation of GM lactococci in, for example, soil is therefore unlikely to occur.

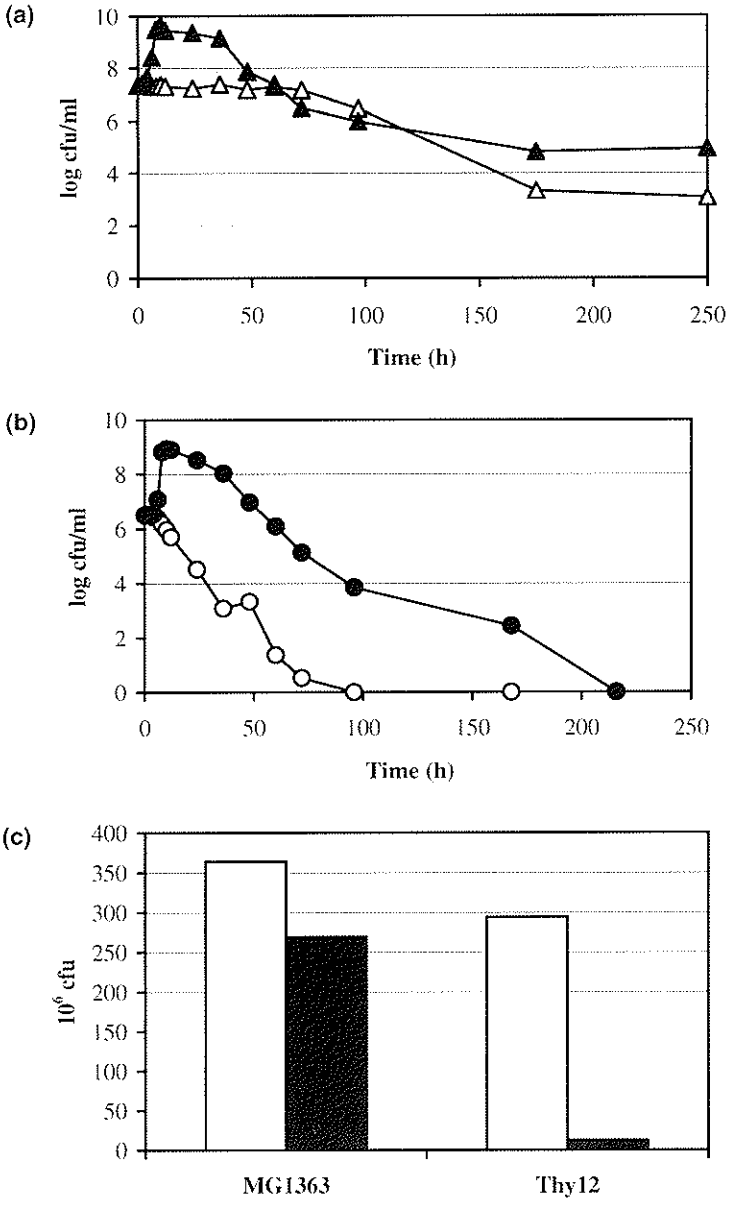

Figure 12.1. Viability of MG1363 and Thy12 *in vitro* and *in vivo*. a) Survival of parental strain MG1363 in glucose-free M17 medium (open triangles) versus 0.5% added glucose (closed triangles). Under both conditions viability decreases but never reaches zero. b) Survival over time of the *thyA* knockout strain, Thy12, grown in thymidine-free medium supplemented with 0.5% glucose. The absence of thymidine (open circles) was compared to the addition of 10 µM thymidine (closed circles). Without thymidine supplemented in the growth medium, bacteria start to die off very quickly. If thymidine is present, the bacteria grow until this is exhausted and start to die shortly afterwards. c) Viability of MG1363 and Thy12 after *in vivo* passage in pigs. The bacterial count in the ileum (white bars) is compared to the faecal count (black bars). Thy12 shows a faster drop in viability than MG1363.

Conclusions

The strong economic impact of cheese production has inspired industrial and academic researchers to scrutinize in detail the physiology, metabolism, molecular biology, and genetics of LAB. With the concomitant advent of suitable genetic engineering tools, imaginative ideas for new applications appeared. It had long been felt that consuming fermented foods is a booster for health. Why not, therefore, genetically engineer LAB for the construction of, perhaps, live vaccines and therapeutics? Such GM strains may form an attractive option to circumvent some of the more prominent difficulties of conventional medicine; high production cost, major side effects due to systemic release, complexity of localized delivery. Apart from the technical feasibility, this concept also looked attainable for a number of other reasons, the major argument in favour probably being that extensive consumption of LAB processed foods has shown that they are very safe to be used. Unlike many other potential bacterial carriers, we definitely know that eating cheese or yoghurt does not harm the strong but neither does it hurt pregnant women, infants, children, elderly people, and people with disease.

More than a decade has gone by since the original seeding of these appealing ideas, so it appears to be the right time to ask ourselves how far we have advanced along this particular avenue. Many in the field will be more than happy to acknowledge that it has not quite been a speedway. Views and visions have not always come across as clear and obvious to policy makers, and the debate on the acceptance of GM foods and their use – a completely different discussion from the medical applications envisaged here – has clearly had a negative impact on the eagerness of both the public sector and industry to invest in this field. Nevertheless, proof of concept has been given in a satisfactory number of applications. Antigens can readily be expressed in a variety of cellular compartments. Functional cytokines, mucosal healing peptides, single-chain antibodies and antigens can be secreted in culture supernatant and have been shown to perform as expected in various animal models. Nowadays, synthesis of a considerable number of immune tools belongs to the state of the art, with a neat collection of 'off-the-shelf' elements available, when designing new applications.

Moving from the experimental setting to 'real world' human or veterinary medicine is obviously a giant leap but vital to avoid drowning in the 'semantic swamp'. Practical issues, such as rebuilding strains in accordance with the human system, sometimes reveal major difficulties. Legitimate concerns exist on the use of live GMO in medicine. For this reason, robust and simple biological containment systems have been designed, some of which have been approved by national governments. A definite highlight is that the first human clinical trial using GM LAB to treat Crohn's disease patients is now finalized and is only awaiting publication. So, fortunately, the answer to the question whether we have, then, already been able to cure a disease is not a mere 'no' but rather 'not yet, but just you wait and see'.

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