

Designer probiotics: Development and applications in gastrointestinal health

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Abstract

Given the increasing commercial and clinical relevance of probiotics, improving their stress tolerance profile and ability to overcome the physiochemical defences of the host is an important biological goal. Herein, I review the current state of the art in the design of engineered probiotic cultures, with a specific focus on their utility as therapeutics for the developing world; from the treatment of chronic and acute enteric infections, and

their associated diarrhoeal complexes, to targeting HIV and application as novel mucosal vaccine delivery vehicles.

Key words: Gastrointestinal tract; Probiotics; BetL; *Listeria monocytogenes*; Bifidobacterium; Lactobacillus

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Core tip: Genetically engineered probiotic bacteria, with improved *in vivo* stress survival and persistence, have the potential to enhance, and in some instances replace, conventional prophylactic and therapeutic measures. This is particularly relevant in the developing world, where chronic and acute infections, and their associated sequelae impose a significant clinical and economic burden.

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INTRODUCTION

Probiotics are commensal organisms that can be harnessed for therapeutic benefit^[1]. In acute infections probiotics may enhance protection mediated by commensal flora through direct antagonism, *i.e.*, competition for niches and nutrients, or *via* the production of antimicrobials, such as bacteriocins^[2]. In chronic clinical conditions, such as immuno-suppression, microbe-host signalling is likely more relevant to effective probiotic function. This bacterial-host dialogue within the gut lumen, functions to maintain an effective mucosal barrier while also priming the host for further

responses to injury^[3]. These health promoting benefits of probiotics, coupled with fact that they are cheap to produce, transport and store, makes them an attractive alternative to traditional therapies, especially in underdeveloped and war-torn territories.

Herein, I review key milestones in the development of probiotic based therapies, focusing particularly on problems encountered in developing countries such as acute and chronic enteric infections, as well HIV (AIDS) which continues to devastate sub-Saharan Africa^[4]. Little or no access to appropriate medical care, often coupled with compromised immunity means that the malnourished are significantly more predisposed to infections by enteric pathogens, leading to incapacitating and dehydrating diarrhoea which in turn leads to a dramatic worsening of an already severely compromised nutritional status. Superimposing the HIV (AIDS) pandemic on an already distressed situation has created a state of affairs which needs to be urgently addressed^[5].

Probiotic therapy, specifically the use of engineered probiotic strains, is a viable alternative to traditional approaches to alleviate suffering, to fight existing diseases and to protect against future infections^[6]. Herein, I review the current state of the art in the design and application of probiotic cultures as therapeutics for the developing world.

Probiotics as therapeutics for the developing world

Almost a third of the world's population are malnourished^[7], a quarter of which are children in the developing world. Malnutrition significantly reduces cell-mediated immunity, immunoglobulin A (IgA) concentrations and cytokine production^[7]. This in turn leads to an increased risk of infection, accompanied by bouts of acute and recurrent diarrhoea – which further exasperates an already depressed nutritional status. Indeed, almost half of all diarrhoea-related mortalities are linked to malnutrition^[8], while morbidity is also disturbingly high (approximately 4 times more episodes per child per annum in developing countries than in the developed world). Diarrhoeal disorders exists as either acute diarrhoea; associated with sudden onset infections, characterized by recovery within two weeks, and chronic diarrhoea which lasts more than two weeks and usually arises as a symptom of malnutrition or immunodeficiency^[9].

Probiotic bacteria have been shown to considerably limit the incidence and duration of diarrhoea associated with both acute infectious illness and chronic episodes linked to malnutrition^[10]. Shornikova *et al*^[11] showed that *Lactobacillus reuteri* can reduce the duration of acute diarrhoea in infants by one full day. Guandalini^[12] observed similar effects with *Lactobacillus rhamnosus*, which also decreased the duration of hospital stays. Furthermore, in addition to alleviating the symptoms of malnutrition and diarrhoea, probiotics have also been used to specifically target bacterial and viral pathogens^[13-16]. *Lb. casei* Shirota, for example was

shown by Ogawa *et al*^[17] to reduce *Escherichia coli* O157:H7 colonization, while Pascual *et al*^[18] observed complete exclusion of *Salmonella enteritidis* by *Lb. salivarius*. Furthermore, even more impressive effects have been observed with mixed probiotic cocktails. Casey *et al*^[19] reported significant amelioration of clinical symptoms of *Salmonella* Typhimurium infection in pigs using LIVE5; a cocktail of two *Lactobacillus murinus* strains with one strain each of *Lb. salivarius* subsp. *salivarius*, *Lb. pentosus* and *Pediococcus pentosaceus*. Pigs administered this mixture exhibited significantly lower levels of *Salmonella* infection, reduced frequency, severity and duration of diarrhoea, and enhanced weight gain relative to animals fed on a skim milk placebo. Nisbet *et al*^[20] observed similar decreases in *Salmonella gallinarum* mediated mortality using a commercial probiotic mixture, while Johnson-Henry *et al*^[21] showed that a *Lactobacillus* mixture reduced inflammation in *Helicobacter pylori*-infected animals. Furthermore, clinical trials in colonized humans revealed significantly lower levels of *H. pylori*, and decreased adverse side effects^[22]. Probiotics are also effective against rotavirus, an enteric virus which accounts for approximately 60% of all diarrhoeal episodes in developing countries^[23,24]. Specifically, *Lactobacillus casei* subsp. *casei* strain GG (LGG) has been shown to stimulate a rotavirus-specific IgA antibody response, which may confer immunity against future rotavirus infections^[25].

However, one of the most significant limitations in the clinical application of probiotics is that the most clinically relevant probiotics are often the most physiologically fragile. Improving probiotic stress tolerance is thus a biological imperative. Below we describe the application of the "Patho-biotechnology" concept for the development of improved probiotic cultures^[26-29] (Figure 1).

DEVELOPMENT AND APPLICATION OF PROBIOTIC THERAPEUTICS

Fluctuations in temperature and water availability (a_w) are the most common stresses associated with food production^[30,31]. The ability to overcome these stresses is thus an important criterion in the selection of commercially viable and clinically effective probiotics^[32]. A common strategy to overcome temperature and osmotic stress is the accumulation of compatible solutes, such as the plant derived trimethyl ammonium compound glycine betaine^[33], which serves to stabilise cellular function under stressful conditions^[34].

Improving compatible solutes accumulation is thus an important first step in the development of more physiologically robust probiotic strains^[35]. Several sophisticated mechanisms for compatible solute accumulation have evolved over time^[34]. Indeed, the intracellular pathogen *L. monocytogenes*, which serves as a useful model for Gram positive osmotolerance^[36], has three compatible solute uptake systems (BetL, Gbu and OpuC^[37]), the first to be identified being the secondary betaine transporter BetL^[38-40]. By cloning

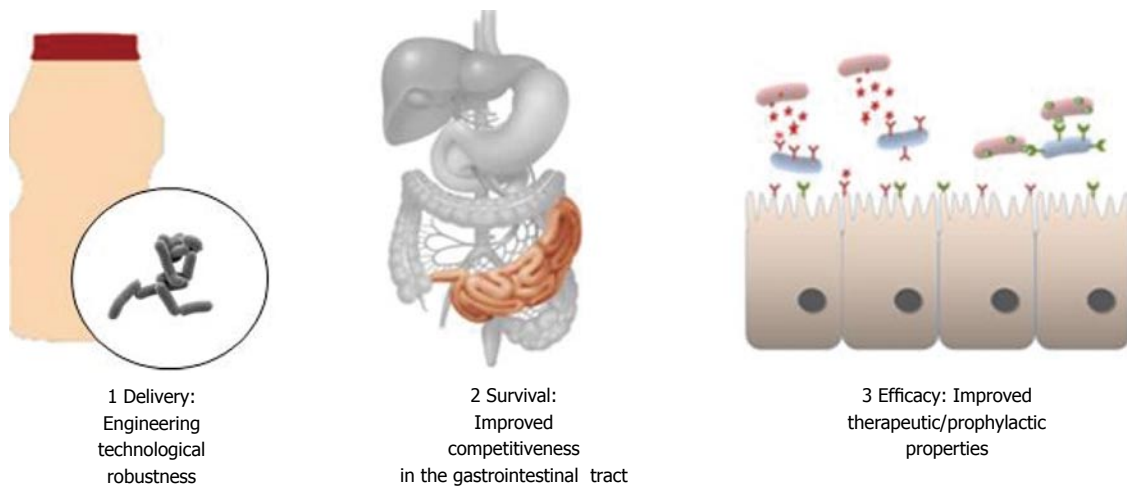


Figure 1 Overview the patho-biotechnology concept; enhancing probiotic delivery in the food matrix, gastrointestinal persistence and clinical efficacy.

betL downstream of the nisin inducible promoter *PnisA*, we were able to assess the ability of BetL to contribute to probiotic survival under a variety of stresses^[41,42]. As expected, the *Lb. salivarius* strain heterologously expressing BetL exhibited a significant increase in betaine uptake compared to the wild type, untransformed control. Indeed, the increased betaine uptake was sufficient to confer improved resistance to chill and cryotolerance, freeze-drying, spray-drying and barotolerance^[41,43-46].

In addition to *ex vivo* stresses, probiotic bacteria must also overcome the *in vivo* defences of the host^[30,31,47,48]. We demonstrated heterologous expression of BetL in *Bifidobacterium breve* UCC2003, significantly improved survival of the probiotic in gastric juice^[49]. In support of this, Termont *et al*^[50] also reported similar effects in a *L. lactis* strain expressing the *E. coli* trehalose synthesis genes, suggesting a novel protective role for compatible solutes in the gastric environment. Furthermore, we have reported roles for carnitine and proline in contributing to bacterial gastrointestinal survival^[51-54]. Upon exiting the stomach, bacteria enter the upper small intestine where they are exposed to elevated osmolarity (equivalent to 0.3 mol/L NaCl). As was observed with *L. salivarius*^[41], a significant osmoprotective effect was evident following BetL expression in *B. breve*, facilitating growth of the probiotic in conditions similar to those encountered *in vivo*. Furthermore, whilst stable colonisation of the murine intestine was achieved by oral administration of *B. breve* UCC2003, strains expressing BetL were recovered at significantly higher levels than the parent in the faeces, intestines and caecum of inoculated animals. Additionally, BetL significantly improved the clinical efficacy of the probiotic; resulting in significantly reduced levels of systemic infection following oral inoculation with *L. monocytogenes*, compared to the control.

In addition to improving physiological stress tolerance, “designer probiotics” have been designed to specifically inhibit enteric infections by blocking ligand-

receptor interactions between the pathogen and/or secreted toxins and the host^[55]. Blocking receptor binding prevents infection, while toxin neutralization dampens clinical symptoms. Engineered to express receptor-mimic structures on their surface^[56], orally administered probiotics neutralize toxins and inhibit pathogen adherence to the intestinal epithelium. Examples of such constructs include an *E. coli* strain expressing a chimeric lipopolysaccharide terminating in a shiga toxin (Stx) receptor, which binds to and neutralises Stx1 and Stx2^[56], as well those with receptor blocking potential against cholera toxin (Ctx) and Enterotoxigenic *E. coli* (ETEC) toxin LT^[57,58].

As well as treating enteric infections, “designer probiotics” have also been enlisted to target HIV. Every day approximately 14000 people become infected with HIV, a majority of which are in developing countries. Rao *et al*^[59] recently described the construction of an *E. coli* strain engineered to secrete HIV-gp41-haemolysin A hybrid peptides, which block HIV fusion and entry into target cells. This “live microbicide”, administered either orally or *via* rectal suppository, colonizes the gut mucosa creating a protective barricade against HIV infection for four weeks^[60]. Other anti-HIV probiotics include a human vaginal isolate of *Lactobacillus jensenii* engineered to secrete two-domain CD4 which inhibits HIV entry into target cells and *Streptococcus gordonii* modified to produce cyanovirin-N, a HIV-inactivating protein originally isolated from cyanobacterium^[61].

PROBIOTICS BASED PROPHYLAXIS

The most effective prophylaxis for infectious diseases is vaccination; resulting in the mobilisation of an immune response capable of specifically targeting invading pathogens^[29]. In addition to the classical approach to vaccination, involving induction of acquired immunity to specific antigens, there is a growing awareness of the importance of innate immunity, associated primarily with our commensal microflora^[2,62-64]. Indeed, optimal development and functioning of the mucosal immune

response is dependent on microbial exposure early in post-natal life^[65]. In the absence of such stimulation, development of the intestinal-associated lymphoid tissue is stunted and immune responses are suboptimal^[66].

The development of efficient vaccine delivery vehicles remains challenging since traditional vaccines are typically based on either recombinant proteins or killed whole pathogens which, although safe, typically induce only weak immunity^[67-69]. The alternative is to use viable or attenuated pathogens^[15]. However, while this approach improves targeted delivery, it carries with it the possibility of reversion to virulence^[70]. Using a patho-biotechnology based approach, probiotics are being engineered to function as novel vaccine delivery vehicles which, lack the possibility of reversion to virulence, and effectively stimulate both the innate and acquired arms of the immune response^[68,69]. In line with this approach, Guimarães *et al*^[71] engineered a *Lactococcus lactis* strain capable of delivering either DNA or protein into the epithelial cells of the small intestine. Heterologous expression of *inlA*, encoding a listerial eukaryotic cell adhesion factor, converted the otherwise non-invasive *L. lactis* strain into a safe and effective vaccine delivery platform. Furthermore, the addition of *hlyA* (encoding listeriolysin) to *L. lactis inlA*⁺ enables phagosomal escape within the macrophage allowing MHC I and II stimulation^[72].

Mucosal vaccine delivery, achieved using probiotic delivery platforms, has significant medical and methodical advantages, particularly for use in the developing world, including: reduced pain and the possibility of cross contamination associated with intramuscular injection, no requirement for expensive medically trained personnel and no cold chain requirement - a significant advantage in the tropical climates most often associated with the developing world.

CONCLUSION

While conventional medical research continues to provide effective prophylactics and therapeutics, these often remain beyond the reach of the developing world. In this context, probiotics provide a viable and cost effective alternative to fighting infection, modulating the immune response and alleviating the symptoms of malnutrition and its associated sequelae, all of which will ultimately contribute to health and social gain, particularly in the developing world.

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