Review Article

Probiotics as therapeutics for the developing world

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Abstract
Although described for over a century, scientists and clinicians alike are only now beginning to realize the significant medical applications of probiotics. Probiotic research in the developed world has received significant attention in recent years; however, the potential for probiotics in the developing world has remained relatively unexplored. This review focuses on the most recent advances in the application of probiotics as potential therapeutics for the developing world, from the treatment of chronic and acute enteric infections and their associated diarrhoeal complexes, to the development of designer probiotics for controlling HIV and as novel mucosal vaccine delivery vehicles.

Key Words: probiotics, patho-biotechnology, infection control, vaccination.


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Introduction
Probiotics are commensal organisms that can be harnessed for therapeutic benefit [1], usually exerting their effects by positively influencing normal microbe-microbe and host–microbe interactions. In acute infections probiotics may augment the protection afforded by commensal flora through competitive interactions, direct antagonism of pathogens, and/or production of antimicrobial factors [2]. Alternatively, in other clinical conditions involving chronic infections and immuno-suppression (associated, for example, with human immunodeficiency virus [HIV]), microbe-host signaling is probably more relevant to effective probiotic action. Gut homeostasis (the maintenance of a ‘balanced’ and beneficial flora) requires continual signaling from bacteria within the gut lumen, maintaining the mucosal barrier while at the same time priming the gut for responses to injury [3]. Given the potential health promoting benefits of probiotics, coupled with the fact that they are relatively simple and inexpensive to produce, transport and store, these microbes may herald a new era in health care, particularly for the developing world.

Herein, we review some of the major advances in the probiotic arena, dealing specifically with some of the problems encountered in the developing world such as poverty, malnutrition, acute and chronic enteric infections leading to complex diarrhoeal disorders, as well as the unshakeable scourge of HIV which continues to decimate sub-Saharan Africa. The World Health Organization estimates that by the year 2015, over 110 million children under five years of age will suffer from malnutrition, the overwhelming majority of whom will live in developing countries [4]. With weakened immunity and little or no access to proper medical support, the malnourished in developing countries are increasingly susceptible to infections by enteric pathogens, leading to bouts of debilitating and dehydrating diarrhoea which in turn worsens nutritional status. This is compounded by HIV (AIDS) which has created a critical situation which desperately needs to be addressed. We suggest that probiotic therapy, in combination with conventional therapies, may help to alleviate some of the suffering, help to fight existing diseases, and protect against future infections.

Malnutrition and Diarrhoea
Almost 30% of the world’s population suffers from malnutrition [5], including a quarter of all children in developing countries. This leads to significant impairment of cell-mediated immunity,
immunoglobulin A (IgA) concentrations and cytokine production [5]. This in turn leads to an increased risk of infection and episodes of acute and recurrent diarrhoea - further aggravating the nutritional status. Indeed, up to 40% of diarrhoea-related mortalities are linked to malnutrition [6], while morbidity rates are also worryingly high (6-7 episodes/child/year in developing countries as opposed to 1-2 in the developed world [7]). Diarrhoeal disorders can be divided into two broad categories: acute diarrhoea, primarily associated with infections characterized by an abrupt onset and resolution within 14 days, and chronic diarrhoea (lasting > 14 days) which usually arises secondary to malnutrition and/or immunodeficiency [8].

Probiotic bacteria have been shown to significantly reduce both the frequency and duration of diarrhoea associated both with acute infectious illness and chronic cases linked to malnutrition [9]. Shornikova et al. [10] demonstrated that consumption of Lactobacillus reuteri can shorten the course of acute diarrhoea in infants from 2.5 to 1.5 days. Guandalini et al. [11] observed similar effects with Lactobacillus rhamnosus which can also significantly reduce the duration of hospitalization. When combined with conventional oral rehydration therapy (ORT), probiotics may help to significantly reduce the burden of diarrhoeal disorders in developing countries.

Infection Control

In addition to alleviating the symptoms of multifactorial conditions such as malnutrition and diarrhoea, the potential of probiotics to specifically target bacterial and viral pathogens is currently the focus of intense scientific scrutiny [12-14]. Perhaps the best evidence for probiotic efficacy is the treatment and prevention of enteric infections [2], a considerable burden in developing countries. To date the beneficial effect of probiotics has been observed in several models of gastrointestinal infection. Ogawa et al. [15] reported that L. casei Shirota reduces Escherichia coli O157:H7 colonization while Pascual et al. [16] noted complete exclusion of Salmonella enteritidis by L. salivarius. In addition to the application of individual probiotic strains, even more impressive results have been obtained using probiotic cocktails (combinations of two or more strains with potentially different mechanisms of antimicrobial action). Casey et al. [17] recently reported significant reductions in both clinical and microbiological signs of Salmonella typhimurium infection in a porcine model using a mixture of two strains of L. murinus and one strain each of L. salivarius subsp. salivarius, L. pentosus and Pediococcus pentosaceus (LIVE5). Animals treated with this cocktail showed lower levels of Salmonella infection, reduced incidence, severity and duration of diarrhoea, and improved weight gain relative to controls administered skim milk. Similarly, Nisbet et al. [18] using a commercial probiotic mixture observed significant decreases in mortality due to Salmonella gallinarum infection, while Johnson-Henry et al. [19] noted that a mixture of Lactobacillus strains reduces gastric inflammation and bacterial colonization in Helicobacter pylori-infected animals. Furthermore, clinical trials in colonized human adults and children show that while probiotics do not completely eradicate pathogens such as H. pylori, they maintain significantly lower levels of the bacterium and thus, in combination with antibiotics, increase eradication rates and/or decrease adverse effects [20].

Significantly, probiotics have also been demonstrated to be effective against enteric viruses, particularly rotavirus which accounts for up to 60% of all diarrhoeal episodes in developing countries. Lactobacillus casei subsp. casei strain GG (LGG) stimulates a rotavirus antibody response which may confer immunity against future rotavirus infections [21]. Duration of rotaviral shedding is reduced, as is gut permeability, reducing the incidence and severity of the illness.

Patho-biotechnology

One of the limitations of probiotics in clinical application is that the most effective probiotic strains often prove to be fragile during industrial processing such as drying or heating [22]. Improving the stress tolerance of probiotic strains is thus an important biological and clinical goal. ‘Patho-biotechnology’ describes the exploitation of highly adapted pathogenic stress survival and host evasion strategies for the development of improved probiotic cultures [12-14]. Cloning the listerial betaine uptake system, BetL, into a probiotic strain Lactobacillus salivarius UCC118...
significantly increases its resistance to several stresses, including elevated osmo-, cryo-, baro- and chill tolerance, as well as increased resistance to spray- and freeze-drying [22] – important stresses encountered during product/tablet formulation. Similarly, expressing BetL in Bifidobacterium breve UCC2003 improved gastric transit and intestinal persistence, and resulted in significantly higher levels of the probiotic in the faeces, intestines and caecum of inoculated animals. This approach significantly improved the clinical efficacy of B. breve UCC2003, resulting in lower levels of systemic infection compared to the control strain following oral inoculation with Listeria monocytogenes [23]. Similar results were observed by Termont et al. [24] in which a Lactococcus lactis strain heterologously expressing the E. coli trehalose synthesis genes exhibits increased resistance to freeze drying, bile and gastric acid.

**Designer Probiotics**

In addition to improving their physiological stress tolerance, recent studies have led to the development of ‘designer probiotics’ which specifically target enteric infections by blocking crucial ligand-receptor interactions between the pathogen and host cell [25]. Many of the pathogens responsible for the major enteric infections exploit oligosaccharides displayed on the surface of host cells as receptors for toxins and/or adhesions, enabling colonization of mucosae and entry of the pathogen or secreted toxins into the host cell. Blocking this adherence prevents infection, while toxin neutralization ameliorates symptoms until the pathogen is eventually overcome by the immune system. ‘Designer probiotics’ have been engineered to express receptor-mimic structures on their surface [26]. When administered orally these probiotics bind to and neutralize toxins in the gut lumen and interfere with pathogen adherence to the intestinal epithelium. One such construct consists of an E. coli strain expressing a chimeric lipopolysaccharide (LPS) terminating in a shiga toxin (Stx) receptor. 1 mg dry weight of this recombinant strain has been shown to neutralize >100 μg of Stx1 and Stx2 [26]. Paton et al. [27, 28] have also constructed probiotics with receptor blocking potential against Enterotoxigenic E. coli (ETEC) toxin LT and cholera toxin (Ctx).

**HIV Control**

As well as treating enteric infections, ‘designer probiotics’ have also been recruited to combat HIV. Approximately 14,000 people, most of whom inhabit the developing world, contract HIV every day. Rao et al. [29] recently described the construction of a probiotic strain of E. coli, engineered to secrete HIV-gp41-haemolysin A hybrid peptides, which block HIV fusion and entry into target cells. When administered orally or as a rectal suppository, this ‘live microbicide’ [30] colonizes the gut mucosa and secretes the peptide in situ, thereby providing protection in advance of HIV exposure for up to a month. Other anti-HIV probiotics currently in development include a genetically engineered Streptococcus gordonii which produces cyanovirin-N, a potent HIV-inactivating protein originally isolated from cyanobacterium, and a natural human vaginal isolate of Lactobacillus jensenii modified to secrete two-domain CD4 which inhibits HIV entry into target cells [31].

Although conventional molecular medical research continues to provide large collections of effective therapeutic compounds, their application is often complicated by in vivo sensitivity and spiraling production costs. Engineered probiotics provide an effective means of circumventing the short half-life and fragility of conventional therapeutics, providing a cost effective alternative which will ultimately contribute to health and social gain in the developing world [14].

**Probiotics as Vaccine Delivery Vehicles**

Vaccination, which requires the mobilisation of an appropriate immune response capable of specifically targeting and neutralising invading pathogens, remains the most effective prophylaxis for infectious diseases [12]. Its success is dependent on the acquired immune response, which is both antigen specific and possesses memory. Compartmentalised into systemic and mucosal responses, the primary mediators of acquired immunity are lymphocytes. Extracellular pathogens are dealt with primarily by B lymphocytes, which produce antibodies, while intracellular pathogens are targeted by T lymphocytes, which recognise antigens in the context of the major histocompatibility complex (MHC). 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 populations or probiotics [32, 33]. Indeed, optimal development and functioning of the mucosal immune response is dependent on microbial exposure early in post-natal life [34]. Without this stimulation, development of the gut-associated lymphoid tissue is rudimentary and generation of immune responses suboptimal [35].

Obtaining efficient vaccine delivery vehicles remains a major challenge for immunologists since traditional vaccines are usually based on either recombinant proteins or killed whole pathogens which, although relatively safe, typically induce only weak cellular immunity. The alternative is to use viable or attenuated pathogens. However, while this approach improves targeted delivery, it carries with it the possibility of the weakened pathogen reverting to its more virulent phenotype [36]. Using a patho-biotechnology based approach, probiotics are being engineered to function as novel vaccine delivery vehicles which, unlike the attenuated pathogenic platforms currently in development, lack the possibility of reversion to virulence while stimulating both innate and acquired immunity. Guimarães et al. [37] recently described the construction of a Lactococcus lactis strain expressing inlA, encoding internalin A, a eukaryotic cell adhesion factor in L. monocytogenes. In this instance the otherwise non-invasive L. lactis strain is now capable of invading the small intestine and delivering molecules (DNA or protein) into mammalian epithelial cells, making it a safer and more attractive alternative to attenuated L. monocytogenes as an antigen delivery vehicle. Furthermore, the addition of hlyA (encoding listeriolysin) to L. lactis inlA+ may promote phagosomal escape within the macrophage and induction of an immune response comparable to that of the intracellular pathogen.

Probiotic vaccine carriers administered by the mucosal route mimic the immune response elicited by natural infection and can lead to long lasting protective mucosal and systemic responses [38]. Mucosal vaccine delivery (those administered orally or by nasal spray) has significant technological and commercial advantages for use in developing countries. These include:

- Reduced pain
- Decreased possibility of cross-contamination associated with intramuscular injection
- Removal of the requirement and cost of medically trained personnel

Furthermore, probiotic vaccine delivery vehicles can be dried and stored at ambient temperatures without the need to maintain a cold chain – a significant advantage in tropical climes with little or no access to refrigeration.

Conclusions and Future Prospects

Probiotics have the potential to alleviate the symptoms of malnutrition and its associated sequelae, to fight infection, and to modulate the immune system. Furthermore, in comparison to conventional therapeutics, probiotic strains are relatively cheap to produce, deliver and store, important considerations in the context of the developing world. Notwithstanding these impressive health and economic benefits, probiotic research has really only gained scientific credence in the last decade [39], this despite the fact that Yakult launched the first probiotic fermented food drink in Japan in 1935, years before the appearance of the first commercially available antibiotics.

The major challenges facing probiotic research and application in developing countries thus centre on consumer confidence and acceptance. These may ultimately be attained, as outlined by Reid et al. [40] by the establishment of education and outreach programs involving support studies examining local customs and the feasibility of adding probiotics as alternative starter cultures to traditional fermented foods. Acceptance of genetically engineered ‘designer probiotics’, however, will likely be less easily achieved. Nevertheless, this reluctance (both in the developed and developing world) should eventually be overcome by rigorous scientific controls such as adequate biological containment, as outlined previously by Steidler et al. [41] and proper risk-benefit analysis of the potential advantages of such a strategy.

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