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The Holy Grail of Designer Probiotics: The Probiotics with Multiple Health Benefits

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Abstract

Alteration in gut microbiome is a major underlying causative factor of diverse inflammatory and metabolic diseases. Role of probiotics to promote perinatal health, prevention of obesity, irritable bowel syndrome are the current hot topics of research. The bioengineered probiotics with multiple immunogenic and antagonistic properties can deliver drugs, therapeutic proteins into host cells. Already the probiotics have been engineered for their stress tolerance profile, overcoming the physiochemical stresses of the host, and their applications in human nutrition and health. The aim of this article is to provide interest on mining the host microbiota for novel therapeutics and using designer probiotics as therapeutics and nutraceuticals in clinical practice.

At the same time, novel high-throughput technologies such as NGS (New Generation Sequencing) allow a much more detailed and stable analysis of the microbiota than the one done by PCR so to direct industries in designing more selected mixtures and physicians to tentatively apply a more tailor-made gut eco-system interventions and rely on a reliable follow up tool.

Keywords: Designer probiotics; Gut; Health applications; NGS

Introduction

The healthy human gut microbiota is unique in that it is a naturally evolved home to trillions of microorganisms including prokaryotes, eukaryotic microorganism, archaea and phages that potentially inhabit every tissue. Genome sequencing, molecular techniques and bioinformatics have unravelled a lot about complex and essential relationship between gut, genitourinary tract, documentation of patterns of microbial colonization associated with disease states, neurodevelopment and behaviour of the host [1,2]. The societal changes including overly use of antibiotics, disinfectants and food additives, not only for medical applications, but also their use in farm animal and crops, have provoked the gut microbial aggression that may be a contributing factor to increase obesity epidemic, cardiovascular diseases (CVD), dyslipidemia, and patho-physiological conditions such as allergy, intestinal inflammatory diseases and cancers [3-6].

The probiotics and their metabolites viz., lactic acid, antimicrobial proteins, peptides, short chain fatty acids (SCFAs), and H2O2 are favourably viewed as alternative novel strategies for controlling some pathogens, metabolic diseases and intestinal diseases like inflammatory bowel disease (IBD) and cancer through multiple mechanisms [7-10]. Bacteria that are normally found in high number, such as bifidobacteria and lactobacilli (LAB) are involved in maintaining a healthy genitourinary and GI tract by providing nutrients, vitamins, immunomodulation through various pathways, prevention against pathogens, production of short chain fatty acids (SCFAs) for providing energy to intestinal enterocytes as well as inhibition of carcinogenesis, and protection of intestinal barrier defence system [11,12]. Bioengineered probiotics expressing exogenous biomolecules such as antimicrobial proteins (AMPs) may have a dual role in preventing microbial infections [13,14]. A potential microbiome-modulating strategy could be the incorporation of bioengineered bacteria that colonise the gut and express therapeutic factors therein [15-17]. Key issues, such as quality control, dose optimization, threat of lateral gene transfer from designer probiotics to pathogens and clinically germane studies have to be embarked upon.

Why the designer probiotics?

Microbial infectious diseases are emerging as serious health problems, especially in patients with compromised illness or those receiving immune-suppressant therapies. In addition, the indiscriminate use of antibiotics in food chain has led to emergence of resistance to multiple antibiotics among microorganisms [18]. In view of the ban of antibiotics as in poultry, the suitable alternatives to sub-therapeutic growth promoters must be developed. The probiotics can be genetically modified to express AMPs with high affinity to bind viruses and reduce their ability to invade the host. Besides cytokines and growth factors, the genetically modified LAB can be evolved to deliver antibodies against gut and genitourinary infectious agents. Development of a recombinant Lactococcus lactis strains aimed to treat IBD in murine models is regarded as the revolutionary development and application of designer probiotics for health applications. Intragastric administration of L.lactis expressing recombinant interleukin-10 (IL-10), a cytokine used in clinical trials for treating inflammatory bowel disease (IBD), could successfully prevent colitis in IL-10-/- murine models. As the treatment prevented

the onset of colitis in IL-10 (-/-) mice, it was regarded as an improved method for cost-effective and long term management of IBD in humans [19]. LAB, Saccharomyces, E. coli Nissle and some Bacillus sp. are the prospective microbial species whose efficiency and utility can be enhanced by genetic engineering.

Designer probiotics in nutrition and health

The drug delivery systems targeting specific mucosal sites is an important strategy to improve topical bioavailability of therapeutics and avoid the side effects associated with systemic administration of therapeutics. Orally formulated LAB and other species (actobiotics) engineered to produce therapeutic AMPs in the GI tract are envisioned as potential candidates. Ever since the pioneering report of development of recombinant probiotic strain [20], the field has rapidly advanced. The probiotic strains such as Lactococcus lactis due to being non-pathogenic, non-colonizing species is reported to secrete correctly processed, bioactive molecules [21].

The efficacy of protein antigens administered through oral route is impeded during its passage through alimentary canal. Hence, tolerogenic bacterial delivery technology or bioengineered microbes may provide topical feasibility of in situ delivery of immunomodulatory agents at the sites of inflammation that are otherwise difficult to reach, such as colon [22-25].

Oral tolerance is an important alternative for inducing tolerance to a particular antigen. Active delivery of recombinant autoantigens or allergens at the intestinal mucosa by recombinant probiotic strains could be a novel therapeutic approach to develop effective therapeutics for systemic and intestinal immune-mediated inflammatory diseases. Intragastric administration of ovalbumin (OVA)-secreting L. lactis was found to deliver OVA at the mucosa and suppressed the local and systemic OVA-specific T-cell responses in murine models [26].

The human interleukin -10 (IL-10) is an important immunoregulator in the intestinal tract, and plays a critical role in regulation of mucosal immune system as it affects immunoregulation and inflammation, enhances B cell survival, proliferation, and production of antibodies. The patients treated with recombinant Lactococcus lactis (LL-Thy 12) expressing human IL-10 experienced reduced disease activity during a placebo-uncontrolled trial. It was inferred that use of designer bacteria for mucosal delivery of therapeutics could be a safer and novel strategy for treating chronic intestinal diseases [27]. Subsequently, while studying the modulating effect of L. lactis secreting human IL-10 (L. lactis (IL-10)) on dendritic cells (DCs), the proliferation of Th-cells was found to be suppressed. It was inferred that spatially restricted delivery of IL-10 by food-grade bacteria could be a promising strategy to induce suppressor T- cells in vivo and treat the inflammatory disease [28].

Bioengineered L. lactis producing native (and pilin-deleted) surface piliation appendage (SpaCBA) with immunomodulating capacity that were assembled in a functional form and anchored to cell surface had mucus-binding functionality. The lactococcal constructs showed that SpaCBA could activate Toll-like recpror 2-dependent signalling in human embryonic kidney (HEK) cells and modulated pro- and anti-inflammatory cytokine (TNF-alpha, IL-6, Il-10 and IL-12) production in human monocyte-derived DCs [29]. Functionality of stress-inducible controlled expression system (SICE) in L. lactis for the production and delivery of proteins of health interest at mucosal surface, was validated in vivo by using two different routes of administration in different murine models of pathologies [30].

Mucosal vaccine based on probiotic bacteria is a novel concept for preventing and treating allergic diseases, and construing the mechanisms involved. Oral pre-treatment of L. lactis allergen Der-p2 as a mucosal vaccine induced immune tolerance against house dust mite allergy in murine model [31].

Bioengineered probiotics as anticancer therapeutics

The ability of certain bacteria to colonise solid tumors opens the ways to develop novel technologies in both the tumor diagnosis and therapy [32]. These bacteria can colonize solid tumors and may be directed to deliver therapeutic biomolecules to cure tumors. Human Trefoil Factor 1 (hTFF1), a stable secretory peptide expressed in intestinal mucosa is required for repairing epithelial damage occurring during chemotherapy or radio-therapy induced oral mucositis (OM) in cancer patients. Administration of a recombinant L. lactis strain sAGX0085 carrying an htfff1 cassette in its genome encoding expression of hTFFF1, in mouth rinse formulation in a hamster model was found to reduce the radiation-induced OM [33]. Non-pathogenic strains such as E. coli Nissle can target tumours and replicate near tumor and necrotic tissues [34].

The natural tropism of certain bacteria for cancers make them ideal tools for delivery of conventional or novel therapeutic modalities, and potentially spares the patients from adverse effects associated with toxicity to healthy cells [35]. The strategy may benefit by the use of tumor-colonizing obligate or facultative anaerobes such as Salmonella, Shigella, certain strains of E. coli or clostridia, Bifidobacteria or E. coli Nissle and certain oncolytic viruses [36-40], and may be effective for treating primary as well as metastatic melanomas [41]. As the mechanisms by which specific bacteria affect the body are established, it could be easier to pinpoint the combination of microorganisms that could be deployed to treat different conditions.

Designer probiotics with antimicrobial peptides

The probiotics are already known to exhibit a variable range of antagonism against pathogenic microorganisms including food borne-pathogens. Probiotics producing antagonistic biomolecules produced by commensal bacteria could be an alternative strategy to prevent human and veterinary drug-resistant pathogens. AMPs which mainly act via membrane active mechanisms have emerged as an exciting class of antimicrobial agents with potential to overcome the global epidemic of antibiotic-resistance infections [42]. The alternative strategy would be the use of probiotic strains that express various AMPs, yielding a combination strategy that brings the benefits of AMPs and probiotics simultaneously [43].

Higher titres recombinant AMPs can be obtained from the engineered probiotics by cloning and expression of genes encoding antimicrobial peptides in them.

Designer probiotics in metabolic diseases

It is now evident that obesity has a microbial component, which might have therapeutic implications [44]. Large body of evidence suggest that probiotics reduce inflammatory responses and oxidative stress, and increase the expansion of adhesion proteins with the intestinal epithelium, reducing intestinal permeability that increases insulin sensitivity and reduces autoimmune responses [45]. Food grade LAB are engineered for use as live vaccines for treating T1DM. The recombinant Lactobacillus casei/pSW501 could induce SP (Usp45)-INS-specific antibodies and raise levels of IL-4 in the sera of NOD

mice and protect them from pancreas injury. Though further studies are warranted, this approach might be a new way to the treatment of T1DM [46]. Equally, type 2 diabetes is another worldwide public health crisis that threatens the economies of all nations, particularly the developing countries. Fuelled by fast paced urbanization, nutrition transition, and gradually more sedentary lifestyles, the epidemic has grown parallel with the global rise in obesity [47].

The suitable alteration of genome of gut microbiota might provide long term protection against obesity. Genetically engineered bacteria expressing therapeutic factors that increase the satiety and sensitivity to adipose-derived negative feedback signals, such as leptin, could be a potential strategy [48]. Orally administered engineered-acylphosphatidylethanolamines (NAPE)-expressing E. coli bacteria in drinking water for 8 weeks reduced the levels of obesity in mice fed a high-fat diet [49].

Designer probiotics vis-a-vis reproductive health

Recurrent urinary tract infections (UTIs) are the common infections that can lead to significant morbidity including stricture, abscess formation, bacterimia, sepsis, pyelonephritis and kidney dysfunction. The incidences are defined as ≥2 episodes in the last six months or ≥3 episodes in the last 12 months, indicating that management and prevention of UTIs is of utmost significance [50,51]. Mortality rates are as high as 1% in men and 3% in women due to development of pyelonephritis [52]. Probiotics have a great deal of applications in managing women health that are at higher risk of heterosexually transmitted viral infection [53]. Epidemiological, experimental and clinical evidences convincingly reveal that normal vaginal microbiota dominated by LAB could offer protection bacterial vaginosis (BV) and sexually transmitted infections (STIs) such as human papilloma virus (HPV) and HIV. Although studies are needed, the use of probiotics should be a useful and safer alternative to antibiotics to circumvent the incidences of BV and STIs.

Although the vaccination is the most effective strategy for controlling HIV/AIDS, the microbiocides capable of preventing HIV-1 transmission at mucosal levels are envisioned as safer and effective candidates. Protein-based microbiocides may offer a target specificity ensuring prolonged protection against HIV. The LAB represents ideal expression systems as they not only produce antagonistics of choice at mucosal surface, but also easily colonise the gut or genitourinary tract and confer valuable homeostatic effects. Developing effective designer microbiocides for preventing HIV-1 sexual transmission represents a primary goal for controlling global AIDS epidemic.

Safety aspects

The microbial therapy has the potential to significantly impact the global morbidity and mortality if they are successfully translated from the basic laboratory triumph into human or veterinary clinics. As recombinant probiotics contain additional genetic elements for inducing antigenecity, immunomodulation, and affects on normal metabolic pathways, hence safety of bioengineerd probiotics is an important issue. Large well designed randomized controlled clinical trials along with culture-independent metagenomic analyses should be carried out to elucidate the role and safety of bioengineered probiotic formulations. One of the major concerns is that probiotic-mediated induction of immune response in patients could lead to acute inflammatory responses, unhealthy metabolic activities and overstimulation of immune system. Besides, the overproduction of antagonistic substances by bioengineered probiotics may inhibit the growth of gut bacteria that are important for a healthy gut.

Future prospective and challenges

Probiotics are under intensive research, because the concept holds potential for human and animal health and consistent commercial prospects. Though the regulation of introduction of probiotics in human food vary by geographical regions, and regulatory authorities, the surge in the use of functional food market is likely to help growing probiotic market. The concept is gaining popularity in view of increasing link between health, diet and nutrition. The use of potential designer microbes may circumvent side effects of antibiotics and allow long term protection against chronic diseases.

Consumer protection and the requirement for health prerogatives to be confirmed with authentic scientific evidences, several studies have proved the efficacy and pro-health effects of bioengineered probiotics. Further, the approach of synthetic biology viz., introduction of synthetic devices that allow design and construction of sophisticated and reliable genetic circuits, precise fine-tuning of transgene expression may open up new frontiers and will greatly contribute towards advancing the development and applications of designer probiotics. The bioengineering of microorganisms could be an important toolbox in curing cancers, development of therapeutics and nutraceuticals. However, compared with the speed with which genome sequencing and de novo DNA synthesis has advanced and offered us a wealth of data to study and manipulate biological functions, it is a slow process.

Treating humans with bioengineered probiotics raises critical questions about safety of human subjects per se and the biological containments of the transgenes introduced into bacteria. The key issues concerning research and use designer products include making publically available the genomic sequences, profiling of antibiotic resistance, use of appropriate gene transfer vectors and in vivo models for validating the safety of probiotics, toxicological aspects of the probiotics and definition of target populations. Relevant means of monitoring vector trafficking and levels over times, and development of bacterial-specific real-time imaging modalities are the key requirements for successful development of clinical bacterial gene delivery. Pre-clinical and clinical investigations involving bacterial vectors require relevant means of monitoring vector trafficking and levels over time. A thorough understanding of the factors responsible for composition of gut microbiome and consequences of exogenously introduced recombinant microorganisms should be the prioritised area of research.

Novel diagnostics avenues applicable in clinical practice

Till recently, a main hindrance in bacterial stool culture is represented by the major bias that only a few gut bacteria can be properly detected and cultivated in the laboratory. On the other hand, capillary sequencing or PCR-based approaches need culture medium with its inner complexities of multiple separate analysis. In this scenario, a rising star is represented by the next-generation sequencing (NGS) which, by combining multiple samples in a sequencing run, is able to analyse the entire microbial community within a sample. In particular, Illumina NGS technology uses clonal amplification and sequencing by synthesis chemistry and this allows a simultaneous identification of DNA bases while incorporating them into a nucleic acid chain. By this process, each base is triggered to emit a unique

fluorescent signal which is used to determine the order of the DNA sequence i.e., each fragment being classified independently from millions of individual DNA molecules. Thus this unique ability enables to catalogue resident organisms within the very complex gut polymicrobial bacterial communities, whether they are commensal, symbiotic and/or pathogenic microorganisms. This new analysis has raised a great deal of interest in view of an amenable application in clinical practice although facing considerable laboratory investments and a team work to make a DNA-stable sampling and producing a report intelligible to physicians and ideally endowed with a nutritionist and a gastroenterologist commented interplay so to make it a valid diagnostic, a treatment-guided result and a follow up tool as well. This has been quite recently achieved by a spin off of dedicated genetists and biologist (Next Genomics, Prato, Italy) which avail itself of a kit allowing small sampling and remaining stable for 14 days at room temperature and with a detection accuracy of 0.0001% of the original sample. The report gathers all the above mentioned expertise so to make it also ready applicable to practice (MMC Milano, Milan, Italy). This test is currently used in research and clinical center and help us also identifying the enterotype and the presence of bacterial species correlated with inflammatory bowel disease, cardiovascular disease, coeliac disease, obesity, diabetes, autoimmune arthropaties, metabolic syndrome etc. However, the flexibility of this technology allows also to customize it by choosing the bacterial species of interest.

Concluding remarks

Using recombinant probiotics as carriers of heterogenetic antigens for oral vaccines is promising. Engineered probiotics can be tailored to deliver drugs, therapeutic proteins and gene therapy vectors with great efficiency, with higher degree of site specificity. Techniques and tools are available for genome engineering and gene expression control to achieve desired phenotypes of bacteria and use them for particular functions. Genetic engineering of probiotics combined with progression of metagenomic studies should be used for enhancing efficacy of candidate probiotic strains as well as the future of recombinant probiotic therapy. The merging therapies require further evaluation before they are recommended for human applications.

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