REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

Future MICROBIOLOGY

Bioengineered probiotics as a new hope for health and diseases: an overview of potential and prospects

Manoj Kumar[1], Ashok Kumar Yadav[1], Vinod Verma[2], Birbal Singh[3], Gorakh Mal[3], Ravinder Nagpal[4] & Rajkumar Hemalatha*[,1]

Despite the use of microorganisms as therapeutics for over a century, the scientific and clinical admiration of their potential is a recent phenomenon. Genome sequencing and genetic engineering has enabled researchers to develop novel strategies, such as bioengineered probiotics or pharmabiotics, which may become a therapeutic strategy. Bioengineered probiotics with multiple immunogenic or antagonistic properties could be a viable option to improve human health. The bacteria are tailored to deliver drugs, therapeutic proteins or gene therapy vectors with precision and a higher degree of site specificity than conventional drug administration regimes. This article provides an overview of methodological concepts, thereby encouraging research and interest in this topic, with the ultimate goal of using designer probiotics as therapeutics in clinical practice.

First draft submitted: 28 July 2015; Accepted for publication: 13 January 2016; Published online: 12 April 2016

KEYWORDS
• bioengineering
• biotherapeutics
• gut microbiota
• probiotics • recombinant

The human body serves as a niche for multitudinous micro-organisms, viruses and eukaryotic microbes [1]. The acquisition and colonization of the microbiota starts soon after a baby leaves the uterus [2]. Research advances, primarily the technologies in genome sequencing, molecular biology and bioinformatics, have enabled us to discover remarkable complex and indispensable relationships between the microbiota of the GI tract, skin, urogenital tract, patterns of microbial colonization associated with disease states, and their association with health and disease of the host [3]. While the mechanisms and host's responses to invading infectious microbes are being vigorously explicated, emphasis is also given to discover novel and safer therapies using beneficial microbes. The probiotic metabolites, namely, lactic acid, antimicrobial proteins, peptides, short-chain fatty acids and H_2O_2 are conceived as alternative strategies for controlling pathogens and preventing intestinal diseases such as inflammatory bowel disease (IBD) and GI cancer [4,5]. Given that the GI microbiota can be manipulated by prebiotics, probiotics, antibiotics, fecal transplants and dietary interventions, altering the gut microbiota could be a tractable approach to otherwise intractable problems of obesity and unhealthy eating [6,7].

The traditional non-GI vaccines are less effective against mucosal (oral, intranasal or vaginal) infections due to their inability to induce sufficient immune response at the mucosa [8]. Owing to their generally recognized as safe (GRAS) status, lactic acid bacteria (LAB) serve as safer and alternative vehicles for developing vaccines. Expression of specific immunogens on cell surface of probiotic lactobacilli may augment the specific binding of these probiotics in the gut. The specific

[1]Department of Clinical Microbiology & Immunology, National Institute of Nutrition, ICMR Hyderabad, India
[2]Centre of Biotechnology, Nehru Science Complex, University of Allahabad, Allahabad, India
[3]ICAR-Indian Veterinary Research Institute, Regional Station, Palampur, India
[4]Probiotics Research Laboratory, Graduate School of Medicine, Juntendo University, Tokyo
*Author for correspondence: rhemalathanin@gmail.com

Future Medicine part of fsg

proteins made to express in bioengineered probiotics will not only act as immunogen, but will also inhibit the binding of pathogens to the enterocytes in the host. Bioengineered probiotics expressing exogenous antimicrobial proteins and peptides (AMPs) or other therapeutic biomolecules may have a supplementary role in preventing microbial infectious diseases [9–11]. Therapeutic options of modulating the normal microbiota through administration of bioengineered probiotics are underscored in several experimental animal models as well as in human trials [12–14].

Taking these issues into account, the incorporation of bioengineered probiotics that colonize the gut and express therapeutic factors could be a promising strategy. Herein we present an overview of designer probiotics and their clinical applications, and address some of the future directions. The outcomes from clinical trials using designer probiotic interventions, and the challenges associated with their use are also highlighted. As the majority of the studies are conducted in cell lines or experimental animal models, relevant examples are cited.

What is a healthy microbiome?

Humans have co-evolved to exist with microorganisms in a mutualistic manner, where we as hosts rely on microbes for vital functions including nutrition, immunity, metabolism and protection against pathogens and xenobiotics [15–18]. The composition of the human gut microbiome depends primarily on various pre- and postnatal factors, such as type of delivery, gestational age, mode of feeding during infancy, environmental hygiene and medications.

Rapidly emerging research on the human microbiome is transforming our understanding of human physiology, drug targets and development of microbes as probiotics or even drugs. From involvement in the complexities of reproduction and fetal or neonatal development, to delaying the onset of diseases and overcoming various maladies, the healthy microbiome offers hopes for the general wellbeing of humans [19]. The technological advances made from human microbiome studies may decipher the highly complex and intriguing host–microbe interactions, the key factors that influence these interactions, and also the appropriate intervention time needed to stop aberrant microbial invasions or other disease-causing activities due to dysbiosis.

The human gut ecosystem in genomics & metagenomic era

The human microbiota is highly complex but the least understood microbial ecosystem. While conventional culturing techniques are unable to draft the blueprint of the GI microbiome [20], current sequencing technologies and bioinformatics algorithms have revolutionized our understanding of the human microbial consortia [21]. Distinctive populations of anaerobes belonging to each of the three domains of life, archaea, bacteria and eukaryota, inhabit and impact our body, including the placenta [22]. Intriguingly, a strong correlation has been revealed between the intestinal microbiome composition and pathophysiological conditions such as allergy, intestinal inflammatory diseases, cancers, obesity, diabetes, cardiovascular diseases (CVD) and dyslipidemia.

On average, the human GI tract contains approximately ten [14] micro-organisms and includes over 5000 bacterial species, although this varies between individuals. Approximately 90% of the bacterial species belong to the phyla Bacteroidetes and Firmicutes. Other species are members of the phyla Actinobacteria, Proteobacteria, Verrumicrobiota, Fusobacteria and Cyanobacteria [21,23]. Given that the metabolic capacity of the gut microbiota is comparable to that of the liver, the intestinal microbiota is tagged as an additional organ. The gut microbiome contains more than 5 million genes, many of which are the biosynthetic enzymes, proteases and glycosidases that, along with other enzymes produced by the host, increase the overall metabolic capacity of host [21,23]. The normal GI microbiota is beneficial in a number of ways. The bacteria that are normally found in a greater number 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 for providing energy to intestinal enterocytes as well as inhibiting carcinogenesis, and protection of intestinal barrier defense system **(Figure 1)** [24–26].

The concept of designer probiotics

Although antibiotic therapy is the first choice against microbial infection, haphazard use of antibiotics has led to the rapid emergence of microbial resistance to conventional antibiotics and disinfectants [27]. This has prompted

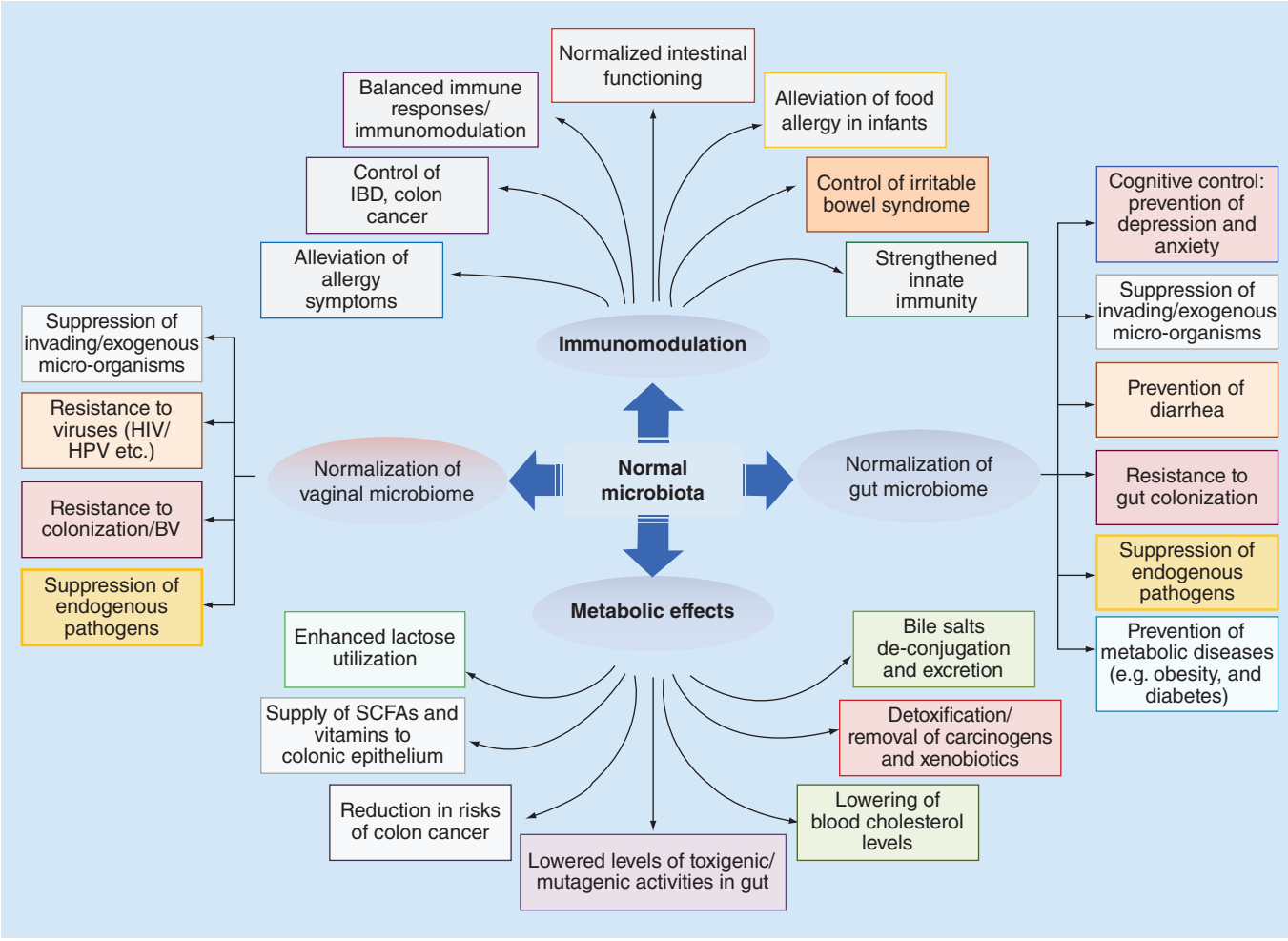

Figure 1. A generalized view of functions of the normal microbiota. The gut microbiota affects the immune system, regulates metabolic diseases, such as diabetes and obesity, and controls brain activities. The microbial metabolites are indispensable for the majority of biological effects of microbiota. Besides, exogenously provided probiotics are important in maintaining feminine and infant health. Designer probiotics may provide additional beneficial effects when used as probiotics.
BV: Bacterial vaginosis; IBD: Inflammatory bowel disease; SCFA: Short-chain fatty acid.

the search for novel antimicrobials that are safer, biodegradable and effective against multiple drug-resistant microorganisms. Microorganisms (generally LAB) can be genetically modified to express AMPs with a high affinity to bind bacteria and viruses, and then reduce their ability to invade the host **(Figure 2)**. Besides cytokines and growth factors, bioengineered LAB can be developed to deliver antibodies against infectious agents. In one such example, a recombinant *Lactococcus lactis* strain was developed to treat IBD in murine models [28]. In this study, the intragastric administration of *L. lactis* expressing recombinant IL-10, a cytokine used in clinical trials for treatment of IBD, could successfully prevent colitis in IL-10 [-/-] murine models with 50% reduction in artificially induced colitis. Given that the treatment prevented the onset of colitis in IL-10 (-/-) mice, it was suggested as an improved method for cost-effective and long-term management of IBD in humans [28]. The LAB, *Saccharomyces* spp., *Escherichia coli* Nissle 1917 and some *Bacillus* species are the prospective species whose efficiency and utility should be improved for use as probiotics. The combination of long-established standard protocols of synthesizing genes and oligonucleotides, their modifications, high efficiency vectors for transferring genes into a wide range of living cells and hosts, and incorporation of exogenous genes into bacterial host genomes have provided the way to more effective, large scale and robust methods of microbial genome modifications [29–31].

Advances in developing designer probiotics

Probiotic effects are highly specific to certain strains and variable traits; however, with genetic engineering, it is possible to develop strains that can exert a variety of beneficial attributes. During the past two decades, the field of probiotic genetic engineering has advanced as a leading area of research for applications in humans and animals [11,32–35]. *L. lactis,* a nonpathogenic and noncolonizing species, has been studied in detail and has increasingly been used as a production host for heterologous proteins. Furthermore, *L. lactis* is a food-grade bacterium that is able to secrete correctly processed, bioactive molecules, originally derived from eukaryotic systems [36]. In addition, several orally formulated LAB and other species (actobiotics), engineered to produce therapeutic biomolecules in GI tract are already in advanced stages of preclinical and clinical trials.

Owing to the gut microbiota's effect on numerous metabolic functions, gut dysbiosis leads to the onset of various metabolic diseases. Metagenomic studies have shown that

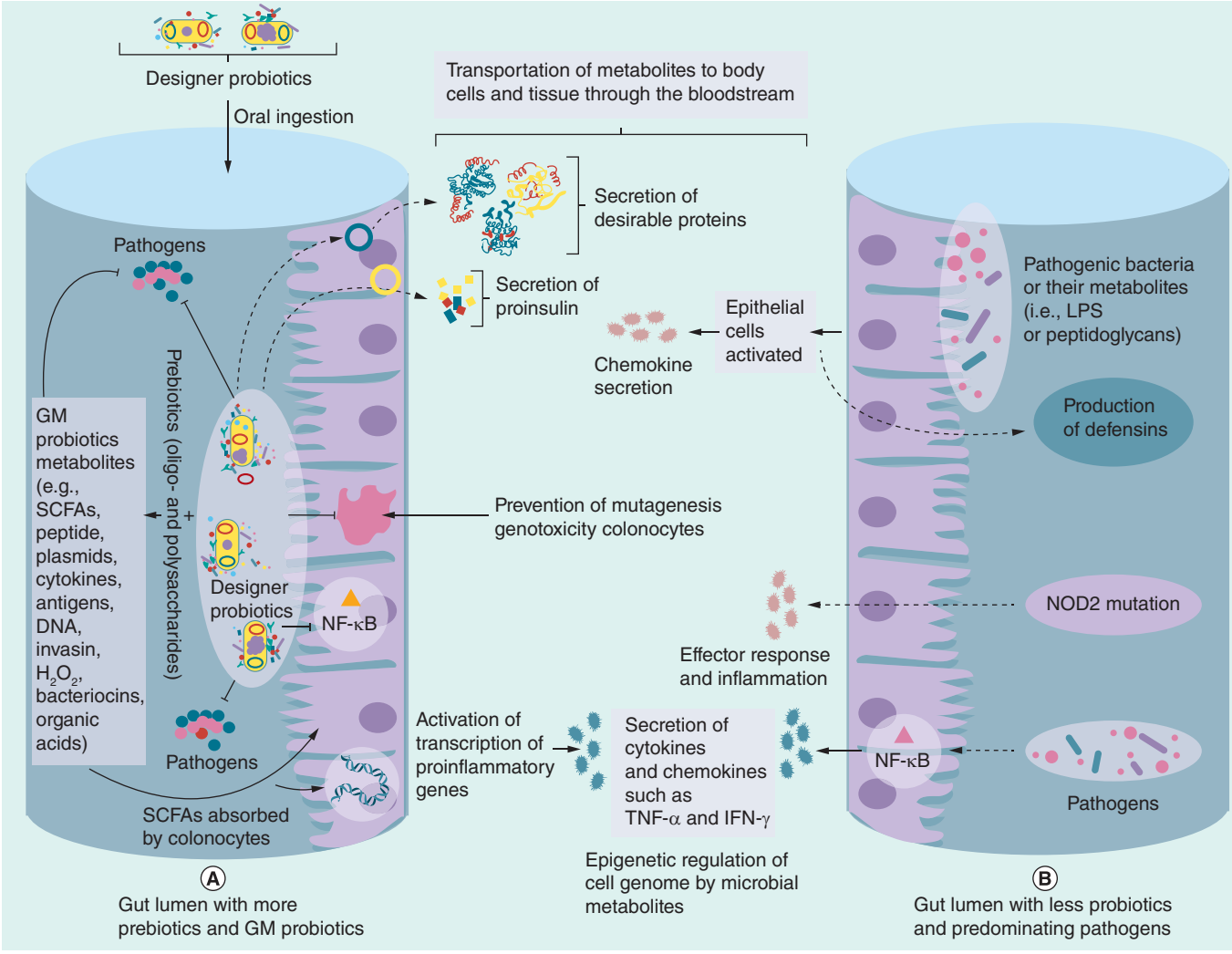

Figure 2. The effects of bioengineered probiotics. The metabolites, such as SCFAs, exert beneficial effects on intestinal epithelium and the gut immune system. The recombinant probiotics can deliver diverse biomolecules into intestinal lumen. **(A)** Examples of ingested designer probiotics, e.g., expressing Elatin preventing inflammation in murine models of colitis by reducing elastolytic activity and restoring gut homeostasis. SOD synthesized by bioengineered probiotics decreases colonic inflammation, and leads to anti-inflammatory impact. The probiotics have beneficial effects through binding and detoxification of mutagens and carcinogens, while metabolites (e.g., butyrate) mediate epigenetic regulation of gut epithelial cells. **(B)** Gut lumen without probiotics or containing less number of beneficial microbiota. Details are described in the text.
SCFA: Short-chain fatty acid; SOD: Superoxide dismutase.

obesity and Type 2 diabetes are associated with a profound dysbiosis, altered bacterial genes and respective metabolic pathways [37]. The inferences that butyrate-producing intestinal bacteria (e.g. *Roseburia intestinalis* and *Faecalibacterium prausnitzii*) are reduced in Type 2 diabetes patients and that potent antidiabetic drugs, for example, metformin, interferes with *Akkermansia muciniphilia* lining the intestinal mucus, pave the way for developing novel microbial therapeutic principles [37].

Designer probiotics in nutrition & health

Drug delivery systems targeting specific mucosal sites may be an important strategy to improve topical bioavailability of therapeutics and avoiding the side effects associated with systemic administration of therapeutics. Further, since the efficacy of protein antigens administered orally is often impeded during their passage through the alimentary canal, mainly due to the digestive nature of the GI tract, the tolerogenic bacterial delivery technology or bioengineered microbes may provide topical feasibility of *in situ* delivery of immunomodulatory agents at the sites of inflammation [38–40]. The IL-10, encoded by the *IL-10* gene [41], which signals through a receptor complex of four IL-10 receptor molecules, plays a critical role in regulation of mucosal immunity. It affects immunoregulation and inflammation, and enhances B-cell survival, proliferation and antibody production, and hence is considered as an important immunoregulator in the intestinal tract. Patients treated with recombinant *L. lactis* expressing mature human IL-10 (LL-Thy12) were observed to experience reduced disease activity during an open trial, comprising ten patients; it was inferred that the use of designer probiotics for mucosal delivery of proteins could be a safer and novel strategy against chronic intestinal diseases [38]. Subsequently, while elucidating the molecular mechanisms of action, it was observed that the modulating effect of recombinant *L. lactis* on dendritic cells (DCs) suppressed the proliferation of Th-cells. In this context, the spatially restricted delivery of IL-10 by *L. lactis* across intestinal epithelium and physical contact of the recombinant probiotic with DCs in lamina propria and/or mesenteric lymph node was proposed to be a promising strategy to induce suppressor T cells *in vivo* and treating the inflammatory diseases [42]. *L. lactis* bioengineered to produce native (and pilin-deleted) surface pili (SpaCBA) with immunomodulating capacity was assembled in a functional form and anchored to the cell surface was found to retain mucus-binding properties [43]. Here, the *L. lactis* showed that SpaCBA could activate Toll-like receptor 2-dependent signaling in HEK cells and modulate pro- and anti-inflammatory cytokine (TNF-α, IL-6, Il-10 and IL-12) production in human monocyte-derived DCs [43]. The functionality of stress-inducible controlled expression system in recombinant *L. lactis* producing and delivering the proteins of health interest at mucosal surface was validated *in vivo* by using different routes of administration in different murine models of pathologies [44]. Oral pretreatment of recombinant *L. lactis* expressing dust mite allergen Der-p2 as a mucosal vaccine is found to induce immune tolerance against house dust mite allergy in murine model [45]. This is because the system is episomal in nature and is composed of a vector carrying an expression cassette under the transcription control of a stress-inducible promoter. Nevertheless, development of mucosal vaccines based on bioengineered probiotics is a new concept, not only for prevention and treatment of allergic diseases, but also in context of the underlying mechanisms. Additional information on bioengineered probiotics with health benefits are summarized in **Table 1**.

Designer probiotics as therapeutics

The development and production of nutraceuticals have attracted a great deal of attention. For instance, omega-3 long chain polyunsaturated fatty acids (LC-PUFA) have received attention due to their role in human nutrition, visual and neurodevelopment of infants, and reduction of CVD (cardiovascular disease) in adults [59–64]. Since the natural sources of LC-PUFA, namely marine fish and certain sea foods, cannot meet the ever-increasing global demand, the metabolic engineering of oleaginous micro-organisms could be a promising alternative for bulk production of omega-3 LC-PUFA including eicospentenoic acid and docosohexenoic acid. Similarly, due to the potential roles of antioxidants to protect against harmful oxidation, there is a rise in the production and consumption of various antioxidants, and hence, metabolic engineering, in other words, the successful reconstitution of heterologous pathways in reliable bacteria as host [65].

Bioengineered probiotics as anticancer therapeutics

Identification of therapies that are selective for tumors, but have minimal toxicity to normal

Table 1. Bioengineered microorganisms for use as designer probiotics in humans.

Microbial species (origin)	Modification induced	Model	Inferences/remarks	Ref.
Bacillus subtilis	*B. subtilis* expressing human IL-1 receptor antagonist (IL-1RA)	Rat and rabbit	Expression of intact and active IL-10 IL-1RA protein, mucosal administration of recombinant *B. subtilis* released cytoplasmic recombinant protein with biological activity *in vivo* that prevented endotoxin-induced shock and death	[46]
Bacillus subtilis subtilis	Expression of *Helicobacter pylori* urease B protein on *B. subtilis* spore coat protein CotC as fusion reporter	Mice	Prolonged colonization of recombinant *B. subtilis* in GI tract of mice, significant (84%) reduction in *H. pylori* load in the stomach, indicating that orally administered urease B-producing spores being immunogenic could provide protection against *H. pylori* infection	[47]
Escherichia coli Nissle 1917	Expression of CAI-1 in *E. coli* Nissle 1917, termed as (Nissle-cqsA)	Infant mice	Pretreatment for 8 h with Nissle-cqsA increased survival of mice against *Vibrio cholerae*. The strategy was suggested to be an inexpensive approach to use bioengineered commensal bacteria to prevent humans from invading bacterial pathogens	[48]
Lactobacillus jejuni 1153 (Human vaginal strain)	Surface-anchored two domain CD4 (2D, CD4) linked to a peptidoglycan in the cell wall of *L. jejuni* 1153	*In vitro* modeling	Uniform expression of recombinant protein on *Lactobacillus* cell surface. The recombinant protein adopted a native functional conformation	[49]
Lactobacillus jensenii (Human vaginal strain)	Secretion of 2D, CD4 proteins, anti-CD4 recognizing onformation-dependent antibody, and bound HIV-1 gp120	HeLa cells	Inhibition of HIV-1 entry into target cells in a dose-dependent manner.The study represents an important step toward development of engineered commensal bacteria within vaginal microbiota to inhibit heterosexual transmission of HIV	[50]
Lactobacillus jensenii (Human vaginal strain)	Expression of anti-HIV chemokine RANTES and C1C5 RANTES	CD(+) T cells and macrophages	Inhibition of HIV in $CD4^+$ cells and macrophages by both the variants	[51]
Lactobacillus jensenii 1153	Expression of potent HIV-inhibitor cyanivirin-N (CV-N), inhibition of CCR5-HIV (BaL), infectivity *in vitro* with 50% inhibitory concentration of 0.3 nM	Mice	Successful colonization of vaginal epithelium by the engineered strains administered to mice in estrus phase. The study was reported to be an expensive and durable approach to prevent HIV infection in women	[52]
Lactobacillus jensenii (Human vaginal strain)	Expression of HIV1-entry inhibitor, modified cyanovirin-N (mCV-N) in *L. jensenii* (LB-mCV-N)	Rhesus macaque model SHIVSF162P3	Detection of higher IL-1RA, lower load of Simian HIV, indicating the potential of engineered LB-mCV-N as a safer microbiocide	[53]
Lactobacillus jensenii 1153 (Human vaginal strain)	Expression of HIV-entry inhibitor modified cyanovirin N (mCV-N)	Human cervical	Expression of mCV-N with anti-HIV activity conserved in epithelial cell lines, expression of higher immunomodulatory potential by recombinant *L. jensenii* activity compared with control strains of *L. jensenii* 1153. Recombinant *L. jensii* 1153 were recommended for clinical trials in humans	[54]
Lactococcus lactis	Expression of Der p2 in *L. lactis* in different cell components (extracellular, intracellular and cell wall)	Mouse model	Oral pretreatment of mice with live recombinant *L. lactis* prevented the development of allergen-induced airway inflammation by induction of specific mucosal immune tolerance	[45]
L. lactis (food grade strain)	Expression of cytokine IL-27 in *L. lactis* (LL-IL-27)	Mouse model	LL-IL-27-mediated protection of mice from T-cell transfer-induced enterocolitis and death, mucosal delivery of LL-IL-27 was proposed to be an effective and safer therapy for IBD	[55]

Most of the trials are conducted either in vitro using cell lines or model animals.
DC: Dendritic cell; IBD: Inflammatory bowel disease; IL-1RA: IL-1 receptor antagonist; $IL\text{-}2^{-/-}$ mice: Knock-out for the *IL-2* gene; SICE: Stress-inducible controlled expression; SOD: Superoxide dismutase.

Table 1. Bioengineered microorganisms for use as designer probiotics in humans.

Microbial species (origin)	Modification induced	Model	Inferences/remarks	Ref.
L. lactis NZ9000 (food grade strain)	Expression of IGF-1 (rtmIGF-1)	–	Functional and stable expression of rtmIGF-1 in *L. lactis*, recombinant *L. lactis* could act as host for producing rtmIGF-1, and delivery system for IGF-1	[56]
L. lactis	A thymidine-dependent recombinant strain expressing mature human IL-10	Monocyte-derived DCs	Reduced symptoms of Crohn's disease, ease of biological containment [38]; anti-inflammatory effects mediated by programming DCs to induce suppression of Th cells	[42]
L. lactis sAGX0085	Expression of human Trefoil factor (hTFF-1)	Hamster model	Improved repair of gut epithelial damage likely to occur during chemotherapy or radiotherapy-induced mucositis in cancer patients	[12]
L. lactis	Development of SICE system in *L. lactis* delivering protein of therapeutic value at mucosal surface	Mouse models	Validation of functionality of SICE *in vivo* in murine models of human pathologies against IBD and vaccination against HPV-16	[44]
L. lactis	Secretion of pro-insulin autoantigen and immunomodulatory cytokine IL-10	NOD mice	The combination therapy with low dose systemic anti-CD3 stably reverted diabetes in NOD mice, increased frequency of local 'Tregs' that suppressed immune response in an auto-antigen-specific way. The strategy is thought to be effective for treating T1DM in humans	[9]
L. lactis	Development of recombinant strains for secreting T1D autoantigen GAD65370-575 and IL-10 in the gut	NOD mice	The orally treated murine models exhibited stabilized insulitis, preserved functional β-cell mass and restored normoglycemia in recent-onset NOD mice. The recombinant bacterial strains were thought to have therapeutic potential in Type 1 diabetes	[40]
Salmonella enteritica sv *typhimurium*	Eukaryotic expression of plasmids encoding Cu-Zn SOD and MCP-1 to intestinal cells	Male Balb/c mice	Bactofection-mediated improved total antioxidant capacity, reduced histological colitis score compared with untreated controls	[57]
Streptococcus gordonii (oral origin)	Recombinant *S. gordonii human* IL-10 composed of amino acid residues RVFP of transporterat N-terminus	Mice model	Display of full biological activity by RFVP/IL-RA *in vitro*, recombinant strain was proposed to be useful as delivery system for selective targeting of mucosal surface	[58]
Saccharomyces boulardii (yeast)	Secretion of IL-10 in yeast		*S. boulardii* proved to be suitable for secretory expression of biologically active IL-10	

Most of the trials are conducted either in vitro using cell lines or model animals.

DC: Dendritic cell; IBD: Inflammatory bowel disease; IL-1RA: IL-1 receptor antagonist; IL-2$^{-/-}$ mice: Knock-out for the *IL-2* gene; SICE: Stress-inducible controlled expression; SOD: Superoxide dismutase.

tissue in patients is one of the biggest challenges for oncology researchers and clinicians. The side effects of conventional anticancer therapies such as chemotherapy, and resistance to conventional anticancer treatments in patients with advanced stage of disease have prompted the need to explore alternative therapeutic strategies. In this context, bioengineered probiotics may overcome the limitations of traditional therapy by specifically targeting tumors. Certain bacteria, such as clostridia, are capable of homing to tumors when injected systematically, thereby resulting in higher level of replication locally, either external to (noninvasive species) or within tumor cells [66]. *Salmonella typhimurium* A1-R, an engineered tumor-targeting variant, may be used to replace earlier therapies like clostridial delivery system for treating metastatic cancer [67,68].

As a result, bacteria being invaluable sources of delivering tumor-specific anticancer genes, toxins, polysaccharides for synthesis of nanodrugs and gene delivery vectors have emerged as important candidates for preventing cancer. The ease of genetic manipulation and ability of certain bacteria to colonize solid tumors offers novel avenues to develop novel technologies, both in tumor diagnosis and therapy [69–71]. Such bacteria could be directed to deliver therapeutic biomolecules to cure tumors. The human trefoil factor 1 (hTFF1), a stable secretory peptide expressed in intestinal

mucosa, is required for repairing epithelial damage occurring during chemotherapy or radiotherapy-induced oral mucositis (OM) in cancer patients. A recombinant *L. lactis* strain sAGX0085 carrying an *htff1* cassette in its genome and producing hTFF1 is developed where its administration through a mouth rinse formulation in a clinically relevant hamster model was found to significantly reduce the severity and the course of radiation-induced OM [12]. These results demonstrated that *in situ* secretion of hTFF1 by topically administrated *L. lactis* could provide a safe and efficacious therapeutic tool for preventing OM [12]. Nonpathogenic strains such as *E. coli* Nissle 1917 can also target tumors, and replicate near tumor and necrotic tissues [53]. Rather, the entire colonization and intratumoral migration process serves as a passive mechanism that could be influenced by the tumor microenvironment and bacterial metabolome [72]. This natural tropism for cancers makes certain microbes ideal for the delivery of conventional or novel therapeutic modalities, and could save patients from the adverse effects of current drugs associated with toxicity to healthy cells [79]. The strategy could also benefit by the use of tumor-colonizing obligate or facultative anerobes, such as *Salmonella, Shigella*, certain strains of *E. coli* or clostridia, bifidobacteria and certain oncolytic viruses [73,74], and thus might be effective for treating primary and metastatic melanomas [75]. Since the mechanisms by which specific bacteria affect the body are not yet fully established, it might be easier and more effective to exploit a combination of microbes that could be deployed to treat different conditions. Nevertheless, studies have shown that chemotaxis and motility do not play a significant role in tumor colonization and bacterial distribution within the tumor. Evidence of dysbiosis in humans suggests that cancers and disorders of the GI and genitourinary tract may be worth exploring further for bacterial therapeutic manipulation [25–27].

Designer probiotics with antimicrobial peptides

Another remarkable and inquisitive challenge for modern medicine is to develop alternative therapies to overcome rapidly developing microbial resistance to conventional antibiotics. Probiotics and bacteriocins or AMPs could prove to be novel strategies to control drug-resistant pathogens. The cationic AMPs could be used to develop efficient drugs for human and veterinary applications. AMPs, which mainly act via membrane-active mechanisms, are emerging as an exciting class of antimicrobial agents [76]. Since the production, purification and delivery of AMPs may have certain limitations, novel mathematical models, simulations and computational programs for identifying and evaluating antimicrobial peptides from genomic databases could be a strategy to design peptides with biotechnological potential from natural sources [77–79]. Nevertheless, an alternative approach might be the use of probiotic strains that express various AMPs, thereby yielding a combination strategy that brings the benefits of AMPs and probiotics simultaneously, especially since probiotics are already known to exhibit a variable range of antagonism against pathogens. In this context, high-titer recombinant AMPs could be obtained from the engineered probiotics by cloning and expression of genes encoding AMPs in them. **Table 2** presents a summarized view of examples of designer probiotics with multiple heath benefits.

Designer probiotics in management of metabolic diseases

Obesity and diabetes are strongly associated with excess calorie intake and reduced energy expenditure, resulting in negative energy balance. Obesity increases the risk of CVD, diabetes and other diseases, whose global frequency has substantially increased during the past decades [86–88]. Current medical and lifestyle treatments have largely failed to offer long-term protection against the malady. The major challenge is the need for lifelong dietary adherence and/or exercise to maintain modest effects in view of evolutionary driven compensatory responses to weight loss induced by voluntary caloric restriction [13]. Evidence suggests that probiotics can reduce inflammatory responses and oxidative stress, and increase the expansion of adhesion proteins within the intestinal epithelium, thereby reducing intestinal permeability and eventually results in increased insulin sensitivity and reduced autoimmune responses [89].

The success of probiotics in the delivery of health benefits depends on their ability to withstand the technological and gastrointestinal conditions, hence, development of robust cultures is critical to the probiotic industry. Combinations of probiotic microbes are more effective than the use of a single culture for treating heterogeneous diseases. A number of food-grade probiotics have been developed as live biotherapeutics for treating metabolic diseases. The recombinant *Lactobacillus casei* pSW501 has been shown to

Table 2. Summary of antimicrobial proteins synthesized by genetically modified LAB and other species bioengineered for use as probiotics.

Expression host (expression vector)	Name of AMP	Nature of AMPs/mode of action/recommendation (Refs)	Ref.
Bacillus cereus and *Lactobacillus plantarum* (pNZ8048, PNZ44)	ABP-118 (heat-stable bacteriocin)	ABP-118 is a class IIb-two peptide bacteriocin, originally isolated from *Lactobacillus salivarius* from human	[80]
Lactobacillus sake LB790 (pMG36e)	Pediocin PA-1, Sakacin P, Curvacin A, Enterocin A, Leucocin A (hybrid bacteriocins)	The hybrid bacteriocins were found to be active and highly potent. The C-terminal domain was found to be important in determining target cell specificity that is different for different bacteriocins	[81]
Lactobacillus salivarius (pNZ44)	Bactofencin A (new type of bacteriocin)	Mass of bactofencin is 2782 Da, shares similarity with eukaryotic cationic AMPs, inhibitory against medically significant pathogens (*Staphylococcus aureus* and *Listeria monocytogenes*)	[82]
Lactococcus lactis (pMK-RQ)	Alyteserin and A3APO	Alyteserin and A3APO-inhibited *Escherichia coli* and *Samonella* by up to 20-fold, found to be nontoxic to the producing host. The system was recommended to deliver AMPs against Gram-negative bacteria	[83]
L. lactis (pINT29)	Enterocin P (Sec-dependent bacteriocin)	The study revealed that synthesis, processing and secretion progresses efficiently in recombinant *L. lactis* as host	[84]
L. lactis and *Pichia pastoris* (pNZ8048)	Hiracin JM79 (Sec-dependent bacteriocin)	Synthesis, processing and secretion of HirJM79 was efficient in recombinant LAB and *Pichia pastoris*	[85]

Safety and bio-containment of recombinant probiotics are the important criteria before recommending them for human applications.
AMPs: Antimicrobial proteins and peptides; LAB: Lactic acid bacteria.

induce SP(Usp45)-INS-specific antibodies and raise the levels of IL-4 in the sera of NOD mice and protect them from pancreas injury, thereby suggesting that this approach might be a new way to treat Type 1 diabetes. However, further studies are warranted to clinically validate this [90].

Type 2 diabetes is another worldwide public health crisis that equally threatens the economies of all nations, particularly developing countries. As a result of fast-paced urbanization, nutritional transition and gradually increasing sedentary lifestyles, the epidemic has grown in parallel with the global rise in obesity [83]. Intriguingly, it is indicated that obesity has a microbial component, which might have therapeutic implications [91,92]. Studies based on gut microbiota interventions in humans, or transfer of disease-associated microbiota into gnotobiotic mice have underscored that a normal gut microbiota is a key regulator of host metabolism and plays a vital role in metabolic diseases such as obesity, insulin resistance, Type 2 diabetes and CVD [93]. Thus, the potential of microbiome-based therapeutics to treat epidemic human diseases could be of significance. Therapeutic paradigms such as second-generation personalized probiotics, prebiotics, narrow-spectrum antibiotic treatment and fecal microbiome transplantation may provide safer alternatives to traditional clinical interventions of chronic metabolic diseases [88,94]. The suitable manipulation of the gut microbiota might provide long-term protection against obesity. Also, genetically engineered bacteria expressing therapeutic factors that increase sensitivity to adipose-derived negative feedback signals such as leptin, could be a potential strategy [13]. Orally administered engineered acylphosphatidylethanolamines (NAPE)-expressing *E. coli* Nissle 1917 in drinking water for 8 weeks had reduced the levels of obesity in mice fed a high-fat diet [13].

Probiotics & cognitive health

A newly coined class of probiotics, called psychobiotics, is capable of producing and delivering neuroactive substances such as gamma-aminobutyric acid and serotonin, which act on the gut–brain axis and possess an antidepressant or anxiolytic effect on the host [95]. There is now ample evidence to suggest that gut bacteria have an effect on brain chemistry and behavior of the host, and this intriguing field is now gradually transitioning from purely descriptive studies to the understanding of the neural circuitry and the elucidation of mechanism underlying the influence of the microbiota on the CNS and human general behavior [18,96,97]. In the context of the gut–brain axis, the vagus nerve has emerged as an important mean of communicating signals from gut microbes to the CNS and mediating the effects of certain microbes on the brain and,

subsequently, on the host's behavior. The vagus nerve connects approximately 100 million nerve cells from the digestive tract to the base of the brain, by which signals released by gut microbes can influence various physiological and behavioral responses. From *in vitro* and model animal studies, it is envisioned that gut microbes and their metabolites can affect neural circuitry, development of CNS and the stress responses [98]. A recent study in a murine model has provided mechanistic explanations of ingestion of a commensal bacterium *Lactobacillus rhamnosus* JB-1 and its mode of yielding immunoregulatory effects, by influencing nerve-dependent colon migrating motor complexes, enteric nerve function and behavioral aspects [18]. There is evidence to suggest that the intestinal bacteria affects mental health, and there is a strong link between gut microbiota and autism, behavior and depression in humans [80,81]. As a result, neuroscientists are now taking notice of this dynamic phenomenon, not because of its clinical implications, but also for what the link could mean for future experimental designs [80]. Indeed, the studies of gut microbiota–brain communications have provided us with a deeper understanding of the relationship between microbes and the host, while also suggesting the potential for microbial-based therapeutic strategies that may aid in the treatment of mood disorders [82]. For instance, LAB can lower the levels of potentially neurotoxic compounds such as ammonia, amines and indoles [84]. Thus, it may only be a matter of time before such designer microbes could also be developed for this area of clinical research.

Designer probiotics in feminine health

Recurrent urinary tract infections (UTIs) are common in women and are defined as ≥2 episodes in the last 6 months or ≥3 episodes in the last 12 months, indicating that management and prevention of UTIs is of utmost significance [85]. Several probiotic strains have shown efficacy in managing the health of women who are at higher risk of heterosexually transmitted viral infection, with the mucosal epithelium of cervix and vagina serving as a major portal of entry [19,27,99]. Epidemiological, experimental and clinical evidence has revealed that a normal vaginal microbiota primarily dominated by LAB plays a protective role against acquisition of bacterial vaginosis and other sexually transmitted infections, including viral infections such as human papillomavirus and HIV [27,100,101]. Even though several studies have reported contradictory results, most studies have supported the application of probiotics in the prevention of UTIs, bacterial vaginosis and sexually transmitted infections [5,19,102].

While the development of a protective vaccine could be one of the most effective strategies for the control of HIV/AIDS, microbiocides capable of preventing HIV-1 transmission at mucosal levels are envisioned as safer and effective candidates. Proteins or protein-based microbiocides might offer an effective target specificity ensuring prolonged protection against HIV/AIDS [51]. The genitourinary and gut LAB may represent ideal expression systems as they not only produce antagonistics of choice at the mucosal surface, but can also easily colonize the GI or urinary tract and confer valuable homeostatic effects [27,103]. In this context, development of effective designer microbiocides preventing HIV-1 sexual transmission could represent a key target for controlling the global AIDS epidemic [103], and the use of bacteria originating from the vaginal microbiota as live microbiocides for the topical production of HIV inhibitors could represent a safe and promising approach. It was proposed that commensal bacteria producing antiviral peptides may protect the host against HIV. In an attempt to block HIV infection, probiotic strain *E. coli* Nissle 1917 (EcN) was engineered to secrete HIV-gp41-hemolysisn A hybrid peptides that block HIV fusion and its entry into target cells. The bioengineered EcN could colonize mice for periods of weeks to months, predominantly in the colon and cecum, with their lower populations in rectum, small intestine and vaginal epithelia. Bacterial growth and antiviral secretion of hybrid peptide throughout the luminal mucosa indicated that bioengineered live probiotics could serve as anti-HIV microbiocides [104].

Safety aspects & guidelines

Safety is one of most important and critical issues in the therapeutic use of microbes to ensure that the possibility of any adverse effect(s) under defined conditions is completely excluded.

Probiotics are ubiquitously appreciated for their prohealth attributes. However, some side effects, such as unhealthy metabolic activities, overstimulation of the immune system and mutagenesis, have been noted, including theoretical risks described in some case reports, clinical trial results, hyperstimulation of immune system in susceptible patients and some gastrointestinal side effects [109], the data are inconclusive on the use and efficacy of probiotics [105]. Furthermore, reports are sparse on the pathogenesis of lactobacilli, correlation among

consumption of probiotics and their side effects. The risk of probiotic lactobacillus infection is estimated at approximately one case per 10 million people over a century of probiotic consumption in various trials [106]. Moreover, the widespread use of probiotics in conjunction with the use of antibiotics can establish a reservoir of antibiotic resistance genes in probiotics. While the intrinsic antibiotic resistance is the desirable attribute of probiotics for restoring gut microbial balance during the course of antibiotics, the transfer of antibiotic resistance genetic determinants from probiotics to gut pathogens could pose serious clinical threats [107,108]. Importantly, the frequency of drug resistance gene transfer and its persistence are high among lactobacilli, and from lactobacilli to pathogens and vice versa [108]. The curative strategies such as the removal of genetic elements harboring antibiotic resistance may be applied to improve safety of probiotics. The strategy was applied to a probiotic *Lactobacillus reuteri* DSM 17938 where plasmids carrying tet(W) tetracycline and lnu(A) lincosamide resistance were successfully knocked out from parent *L. reuteri* (ATCC55730), while not affecting the probiotic potential of the strain [109].

Bioengineered microorganisms are utilized in industry for the production of valuable metabolites in closed industrial systems [13,14,36,110,111]. However, applications of bioengineered bacteria in open or clinical or the environment require stringent safety and security measures [108]. Since designer probiotics contain additional genetic elements for inducing antigenicity and immunomodulation, and can also affect normal metabolic pathways, the safety of bioengineered probiotics is of utmost importance. Therefore, large, well-designed, randomized controlled clinical trials along with the culture-independent metagenomic analyses should be meticulously carried out.

The effects from one species or strain of probiotics vary from others, and different preparations of the same species or strain may also be required. Thus, it is recommended that healthcare providers must be consulted before bioengineered probiotic formulations are consumed. One of the major concerns is that probiotic-mediated induction of immune response in patients could also lead to acute inflammatory responses, unhealthy metabolic activities and overstimulation of the immune system. Furthermore, the overproduction of antagonistic substances by bioengineered probiotic supplements may also inhibit the growth of other gut bacteria that are important in sustaining normal health and function of the intestine. In this context, some of the newly developed biocontainment strategies that include overlapping 'safeguards' – engineered riboregulators that tightly regulate the expression of essential genes, and the engineered addiction module based on nucleases – should be extended to clinically or industrially relevant genetically engineered bugs [1,12]. Construction of genomically recoded micro-organisms whose growth is restricted by the expression of multiple essential genes that depend on exogenously supplemented synthetic amino acids may represent another approach toward the biocontainment of bioengineered microbes [107].

Future perspective

Gut microbes may eventually impact all major aspects of health management including drug identification, evolving predictive models of disease, toxicology and drug metabolism, and better patient subtyping for clinical trials as well as developing novel therapeutic agents. One of the major concerns is that, with a few exceptions such as the use of fecal transplants for treating critical *Clostridium difficile* infections, research on the human microbiome has not generated novel and efficacious therapies [80]. Hence, further research is warranted to expedite the developments in this important area of health management. A combined therapy with combined benefits of direct antagonistic and anticancer effects of recombinant bacteria and their immunomodulatory potential could be used as an effective weapon against pathogenic bacteria, cancers and metabolic diseases.

Although regulation of the introduction of probiotics in human food varies by geographical regions and regulatory authorities, the surge in the functional food market is likely to help a growing probiotic market. This is because the concept of the therapeutic micro-organism is gaining popularity owing to the increasing links between health, diet and nutrition. Thus, the use of bioengineered microbes may circumvent side effects of antibiotics and could allow long-term protection against various chronic diseases.

Nevertheless, consumer protection and the requirement for health prerogatives need to be confirmed with scientific evidence. The application of synthetic biology techniques, namely, introduction of synthetic genes that allow design and construction of reliable genetic circuits, precise fine tuning of transgene expression may offer new frontiers toward advancing the development of designer probiotics.

Treating humans with bioengineered probiotics raises critical questions about the safety of human subjects *per se* and also the biological containments of the transgenes introduced into bacteria. Tumor-detecting bioengineered bacteria are envisioned to provide a sensitive and minimally invasive strategy

EXECUTIVE SUMMARY

Background

- The human body serves as a niche for multitudinous micro-organisms, viruses and eukaryotic microbes.

What is a healthy microbiome?

- The composition of the human gut microbiome depends primarily on various pre- and postnatal factors.
- Factors affecting these complex interactions can result in dysbiosis.

The human gut ecosystem in genomics & metagenomic era

- Current sequencing technologies and bioinformatics algorithms have revolutionized our understanding of the human microbial consortia.

The concept of designer probiotics

- Emerging antibiotic resistance in bacteria means there is a need for novel antimicrobials.
- Micro-organisms can be genetically modified to express antimicrobial proteins and peptides with a high affinity to bind bacteria and viruses, and then reduce their ability to invade the host.

Bioengineered probiotics asanticancer therapeutics

- The side effects of conventional anticancer therapies such as chemotherapy, and resistance to conventional anticancer treatments in patients with an advanced stage of disease, have prompted the need to explore alternative therapeutic strategies.
- Bioengineered probiotics may overcome the limitations of traditional therapy by specifically targeting tumors.

Designer probiotics with antimicrobial peptides

- Probiotics and bacteriocins or antimicrobial proteins and peptides could prove to be novel strategies to control drug-resistant pathogens.

Designer probiotics in management of metabolic diseases

- The global frequency of obesity and diabetes has substantially increased in recent years.
- The suitable manipulation of the gut microbiota might provide long-term protection against obesity.

Probiotics & cognitive health

- This section highlights the recent advances in understanding the role of probiotics and normal microbiota in maintaining cognitive health.

Designer probiotics in feminine health

- Recurrent urinary tract infections are common in women.
- Some studies have supported the application of probiotics in the prevention of urinary tract infections.

Safety aspects & guidelines

- The widespread use of probiotics in conjunction with the use of antibiotics can establish a reservoir of antibiotic-resistance genes in probiotics.
- Further research into the risks associated with the prolonged use of bioengineered probiotics is needed.

Future perspective

- Gut microbes may eventually impact all major aspects of health management.
- However, important key issues such as microbial characterization, quality control, dose optimization, threat of lateral gene transfer from designer probiotics to pathogens, and clinical safety are needed.

to detect tumor recurrence, monitoring tumor-treatment efficacy and identification of the onset of metastatsis.

However, important key issues such as microbial characterization, quality control, dose optimization, threat of lateral gene transfer from designer probiotics to pathogens, and clinical safety should be meticulously addressed. More information is needed on the safety of bioengineered probiotics in young children, the elderly and individuals with compromised immune systems. The key issues concerning research and the use of designer products include making the genomic sequences publically available, profiling of antibiotic resistance, use of appropriate gene transfer vectors and *in vivo* models for validating the safety of designer probiotics, toxicological aspects of the designer probiotics and definition of target populations. In these contexts, relevant means of monitoring vector trafficking and bacterial levels over time and the development of bacteria-specific, real-time imaging modalities appear to be the key requirements for the successful development of clinical bacterial gene delivery. The bioengineering of microorganisms could be important in treating cancers and developing potential therapeutics against various other diseases, even though it is still a slow process. Therefore, a thorough understanding of the factors responsible for modulating the composition and function of the gut microbiome and consequences of exogenously introduced recombinant micro-organisms should be the prioritized area of research.

Conclusion

Given that the concept of probiotics could hold the potential for human and animal health, several potential probiotic strains are being intensively researched. Indeed, the future of using LAB as carriers of heterogenetic antigens for oral vaccines or production of other biotherapeutic molecules appears to be promising. Engineered probiotics could be tailored to deliver drugs, therapeutic proteins and gene therapy vectors with great efficiency, with a higher degree of site specificity. In recent years, a number of techniques are now available for genome engineering and controlling gene expression to achieve desired phenotypes of bacteria. Hence, genetic engineering of probiotics and the high-throughput sequencing technologies may be used for enhancing the efficacy of candidate probiotic strains to enhance the impact of recombinant probiotic therapy. Nevertheless, these emerging therapies warrant further evaluation before they are recommended for human application.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest.

1 Eloe-Fadrosh EA, Rasko DA. The human microbiome: from symbiosis to pathogenesis. *Annu. Rev. Med.* 64, 145–163 (2013).

2 Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat. Rev. Microbiol.* 9, 279–290 (2011).

3 Goodrich JK, Waters JL, Poole AC *et al.* Human genetics shape the gut microbiome. *Cell* 159, 789–799 (2014).

4 Olle B. Medicines from microbiota. *Nat. Biotechnol.* 31, 309–315 (2013).

• **Highlights the need to explore micro-organisms for medicine.**

5 Homayouni A, Bastani P, Ziyadi S *et al.* Effects of probiotics on the recurrence of bacterial vaginosis: a review. *J. Low. Genit. Tract Dis.* 18, 79–86 (2014).

6 Smits LP, Bouter KE, de Vos WM *et al.* Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* 145, 946–953 (2013).

7 Alcock J, Maley CC, Aktipis CA. Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *Bioessays* 36, 940–949 (2014).

8 Bahey-El-Din M, Gahan CG. *Lactococcuslactis*-based vaccines: current status and future perspectives. *Hum. Vaccin.* 7, 106–109 (2011).

9 Takiishi T, Korf H, Van Belle TL *et al.* Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified *Lactococcus lactis* in mice. *J. Clin. Invest.* 122, 1717–1725 (2012).

10 Gardlik R, Palffy R, Celec P. Recombinant probiotic therapy in experimental colitis in mice. *Folia Biol. (Praha).* 58, 238–245 (2012).

11 Pöhlmann C, Thomas M, Förster S *et al.* Improving health from the inside: use of engineered intestinal microorganisms as *in situ* cytokine delivery system. *Bioengineered* 4, 172–179 (2013).

12 Caluwaerts S, Vandenbroucke K, Steidler L *et al.* AG013, a mouth rinse formulation of *Lactococcus lactis* secreting human Trefoil Factor 1, provides a safe and efficacious therapeutic tool for treating oral mucositis. *Oral Oncol.* 46, 564–570 (2010).

13 Chen Z, Guo L, Zhang Y *et al.* Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. *J. Clin. Invest.* 124, 3391–3406 (2014).

14 Del Carmen S, de Moreno de LeBlanc A, Martin R *et al.* Genetically engineered

immunomodulatory *Streptococcus thermophilus* strains producing antioxidant enzymes exhibit enhanced anti-inflammatory activities. *Appl. Environ. Microbiol.* 80, 869–877 (2014).

15 Turnbaugh PJ, Ley RE, Mahowald MA *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027–1031 (2006).

16 Kumar M, Nagpal R, Kumar R *et al.* Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Exp. Diabetes Res.* Article ID 902917(2012), 1-14 (2012).

17 Nagpal R, Kumar A, Kumar M *et al.* Probiotics, their health benefits and applications for developing healthier foods: a review. *FEMS Microbiol. Lett.* 334, 1–15 (2012).

18 Al-Nedawi K, Mian MF, Hossain N *et al.* Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *FASEB J.* 29, 684–695 (2014).

19 Reid G, Brigidi P, Burton JP *et al.* Microbes central to human reproduction. *Am. J. Reprod. Immunol.* 73, 1–11 (2015).

20 Singh B, Gautam SK, Verma V *et al.* Metagenomics in animal gastrointestinal ecosystem: potential biotechnological prospects. *Anaerobe* 14, 138–144 (2008).

21 Human Microbiome Project Consortium (HMPC). Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–214 (2012).

22 Aagaard K, Ma J, Antony KM *et al.* The placenta harbors a unique microbiome. *Sci. Transl. Med.* 6, 237ra65 (2014).

23 Qin J, Li R, Raes J *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65 (2010).

24 Louis P, Flint HJ. How our gut microbes influence our behaviour. *J. Neuroendocrinol.* 25, 517–518 (2013).

25 Kumar M, Nagpal R, Verma V *et al.* Probiotic metabolites as epigenetic targets in the prevention of colon cancer. *Nutr. Rev.* 71, 23–34 (2013a).

•• **An important review highlighting the epigenetic role of probiotic metabolites and certain phytometabolites in preventing human colon cancer.**

26 Kumar M, Hemalatha R, Kumar R *et al.* Epigenetics, probiotic metabolites and colon cancer prevention: an overview of progress, opportunities and challenges. *Med. Epigenet.* 1, 60–69 (2013).

27 Singh B, Mal G, Bharti D *et al.* Probiotics in female reproductive health: paradigms, prospects and challenges. *Curr. Womens Health Rev.* 9, 236–248 (2013).

28 Steidler L, Hans W, Schotte L *et al.* Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 289, 1352–1355. (2000).

29 Cambray G, Mutalik VK, Arkin AP. Toward rational design of bacterial genomes. *Curr. Opin. Microbiol.* 14, 624–630 (2011).

30 Fehér T, Burland V, Pósfai G. In the fast lane: large-scale bacterial genome engineering. *J. Biotechnol.* 160, 72–79 (2012).

31 Qin J, Wang X, Kong J *et al.* Construction of a food-grade cell surface display system for *Lactobacillus casei*. *Microbiol Res.* 169, 733–740 (2014).

32 LeBlanc JG, Aubry C, Cortes-Perez NG *et al.* Mucosal targeting of therapeutic molecules using genetically modified lactic acid bacteria: an update. *FEMS Microbiol. Lett.* 344, 1–9 (2013).

33 Amalaradjou MA, Bhunia AK. Bioengineered probiotics, a strategic approach to control enteric infections. *Bioengineered* 4, 379–387 (2013).

34 Bermúdez-Humarán LG, Aubry C, Motta JP *et al.* Engineering lactococci and lactobacilli for human health. *Curr. Opin. Microbiol.* 16, 278–283 (2013).

35 DeGrandis S, Law H, Brunton J *et al.* Globotetraosyl ceramide is recognized by the pig edema disease toxin. *J. Biol. Chem.* 264, 12520–12525 (1989).

36 Van Huynegem K, Loos M, Steidler L. Immunomodulation by genetically engineered lactic acid bacteria. *Front. Biosci. (Landmark Ed).* 14, 4825–4835 (2009).

37 Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. *Gut* 63, 1513–1521 (2014).

38 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.* 4, 754–759 (2006).

39 Paton AW, Morona R, Paton JC. Bioengineered microbes in disease therapy. *Trends Mol. Med.* 18, 417–425 (2012).

•• **Describes the role of designer probiotics in disease prevention.**

40 Robert S, Gysemans C, Takiishi T *et al.* Oral delivery of glutamic acid decarboxylase (GAD)-65 and IL10 by *Lactococcus lactis* reverses diabetes in recent-onset NOD mice. *Diabetes* 63, 2876–2887 (2014).

41 Eskdale J, Kube D, Tesch H *et al.* Mapping of the human IL10 gene and further characterization of the 5′ flanking sequence. *Immunogenetics* 46, 120–128 (1997).

42 Huibregtse IL, Zaat SA, Kapsenberg ML *et al.* Genetically modified *Lactococcus lactis* for delivery of human Interleukin-10 to dendritic cells. *Gastroenterol. Res. Pract.* 2012, 639291 (2012).

43 von Ossowski I, Pietilä TE, Rintahaka J *et al.* Using recombinant lactococci as an approach to dissect the immunomodulating capacity of surface piliation in probiotic *Lactobacillus rhamnosus* GG. *PLoS ONE* 8, e64416 (2013).

44 Benbouziane B, Ribelles P, Aubry C *et al.* Development of a stress-inducible controlled expression (SICE) system in *Lactococcus lactis* for the production and delivery of therapeutic molecules at mucosal surfaces. *J. Biotechnol.* 168, 120–129 (2013).

45 Ai C, Zhang Q, Ren C *et al.* Genetically engineered *Lactococcus lactis* protect against house dust mite allergy in a BALB/c mouse model. *PLoS ONE* 9, e109461 (2014).

46 Porzio S, Bossù P, Ruggiero P *et al.* Mucosal delivery of anti-inflammatory IL-1Ra by sporulating recombinant bacteria. *BMC Biotechnol.* 4, 27. (2004).

47 Zhou Z, Gong S, Li X *et al.* Expression of *Helicobacter pylori* urease B on the surface of *Bacillus subtilis* spores. *J. Med. Microbiol.* 64(Pt 1), 104–110 (2015).

48 Duan F, March JC. Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model. *Proc. Natl Acad. Sci. USA* 107, 11260–11264 (2010).

49 Liu X, Lagenaur LA, Lee PP *et al.* Engineering of a human vaginal *Lactobacillus* strain for surface expression of two-domain CD4 molecules. *Appl. Environ. Microbiol.* 74, 4626–4635 (2008).

50 Chang TL, Chang CH, Simpson DA *et al.* Inhibition of HIV infectivity by a natural human isolate of *Lactobacillus jensenii* engineered to express functional two-domain CD4. *Proc. Natl Acad. Sci. USA* 100, 11672–11677 (2003).

51 Dey B, Lagenaur LA, Lusso P. Protein-based HIV-1 microbicides. *Curr. HIV Res.* 11, 576–594 (2013).

52 Liu X, Lagenaur LA, Simpson DA *et al.* Engineered vaginal *Lactobacillus* strain for mucosal delivery of the human immunodeficiency virus inhibitor cyanovirin-N. *Antimicrob. Agents Chemother.* 50, 3250–3259 (2006).

53 Brichacek B, Lagenaur LA, Lee PP *et al. In vivo* evaluation of safety and toxicity of a

Lactobacillus jensenii producing modified cyanovirin-N in a rhesus macaque vaginal challenge model. *PLoS ONE* 8(11), e78817 (2013).

54 Yamamoto HS, Xu Q, Fichorova RN. Homeostatic properties of *Lactobacillus jensenii* engineered as a live vaginal anti-HIV microbicide. *BMC Microbiol.* 13, 4 (2013).

55 Hanson ML, Hixon JA, Li W *et al.* Oral delivery of IL-27 recombinant bacteria attenuates immune colitis in mice. *Gastroenterology* 146, 210–221.e13 (2014).

•• **One of the preliminary reports demonstrating the role of recombinant food-grade microbial strains in preventing immune colitis in a murine model.**

56 Gao G, Qiao JJ, Yang CH *et al.* Functional expression of mouse insulin-like growth factor-I with food-grade vector in *Lactococcus lactis* NZ9000. *Lett. Appl. Microbiol.* 54, 404–409 (2012).

57 Palffy R, Gardlik R, Behuliak M *et al.* Salmonella-mediated gene therapy in experimental colitis in mice. *Exp. Biol. Med. (Maywood)* 236, 177–183 (2011).

58 Ricci S, Macchia G, Ruggiero P *et al. In vivo* mucosal delivery of bioactive human interleukin 1 receptor antagonist produced by *Streptococcus gordonii*. *BMC Biotechnol.* 3, 15 (2003).

59 Voigt RG, Jensen CL, Fraley JK *et al.* Relationship between omega-3 long-chain polyunsaturated fatty acid status during early infancy and neurodevelopmental status at 1 year of age. *J. Hum. Nutr. Diet* 15, 111–120 (2000).

60 Das UN, Fams MD. Long-chain polyunsaturated fatty acids in the growth and development of the brain and memory. *Nutrition* 19, 62–65 (2003).

61 Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* 34, 1249–1257 (2011).

62 Demaison L, Moreau D. Dietary n-3 polyunsaturated fatty acids and coronary heart disease-related mortality: a possible mechanism of action. *Cell. Mol. Life Sci.* 59, 463–477 (2002).

63 Von Schacky C. The role of omega-3 fatty acids in cardiovascular disease. *Curr. Atheroscler. Rep.* 5, 139–145 (2003).

64 Gong Y, Wan X, Jiang M *et al.* Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids. *Prog. Lipid Res.* 56C, 19–35 (2014).

65 Lin Y, Jain R, Yan Y. Microbial production of antioxidant food ingredients via metabolic engineering. *Curr. Opin. Biotechnol.* 26, 71–78 (2014).

66 Heap JT, Theys J, Ehsaan M *et al.* Spores of *Clostridium* engineered for clinical efficacy and safety cause regression and cure of tumors *in vivo*. *Oncotarget* 5, 1761–1769 (2014).

67 Hoffman RM. Back to the future: are tumor-targeting bacteria the next-generation cancer therapy? *Methods Mol. Biol.* 1317, 239–260 (2015).

68 Hiroshima Y, Zhao M, Zhang Y *et al.* Tumor-targeting *Salmonella typhimurium* A1-R arrests a chemo-resistant patient soft-tissue sarcoma in nude mice. *PLoS ONE* 10, e0134324 (2015).

69 Panteli JT, Forkus BA, Van Dessel N *et al.* Genetically modified bacteria as a tool to detect microscopic solid tumor masses with triggered release of a recombinant biomarker. *Integr. Biol. (Camb)* 7, 42334 (2015).

70 Panteli JT, Forkus BA, Van Dessel N *et al.* Genetically modified bacteria as a tool to detect microscopic solid tumor masses with triggered release of a recombinant biomarker. *Integr. Biol. (Camb)* 7, 423–434 (2015).

71 Van Dessel N, Swofford CA, Forbes NS. Potent and tumor specific: arming bacteria with therapeutic proteins. *Ther. Deliv.* 6, 385–399 (2015).

72 Stritzker J, Weibel S, Seubert C *et al.* Enterobacterial tumor colonization in mice depends on bacterial metabolism and macrophages but is independent of chemotaxis and motility. *Int. J. Med. Microbiol.* 300, 449–456 (2010).

73 Stritzker J, Szalay AA. Single-agent combinatorial cancer therapy. *Proc. Natl Acad. Sci. USA* 110, 8325–8326 (2013).

74 Chang WW, Lee CH. Salmonella as an innovative therapeutic antitumor agent. *Int. J. Mol. Sci.* 15, 14546–14554 (2014).

75 Lee CH, Wu CL, Shiau AL. Systemic administration of attenuated *Salmonella choleraesuis* carrying thrombospondin-1 gene leads to tumor-specific transgene expression, delayed tumor growth and prolonged survival in the murine melanoma model. *Cancer Gene Ther.* 12, 175–184 (2005).

76 Ong ZY, Wiradharma N, Yang YY. Strategies employed in the design and optimization of synthetic antimicrobial peptide amphiphiles with enhanced therapeutic potentials. *Adv. Drug Deliv. Rev.* 78C, 28–45 (2014).

77 Amaral AC, Silva ON, Mundim NC *et al.* Predicting antimicrobial peptides from eukaryotic genomes: in silico strategies to develop antibiotics. *Peptides* 37, 301–308 (2012).

78 Kaznessis YN. Multiscale models of antibiotic probiotics. *Curr. Opin. Chem. Eng.* 6, 18–24 (2014).

79 Andreu D, Torrent M. Prediction of bioactive peptides using artificial neural networks. *Methods Mol. Biol.* 1260, 101–118 (2015).

80 Reardon S. Microbiome therapy gains market traction. *Nature* 509, 269–270 (2014).

81 Dash S, Clarke G, Berk M *et al.* The gut microbiome and diet in psychiatry: focus on depression. *Curr. Opin. Psychiatry* 28, 1–6 (2015).

82 Forsythe P, Kunze WA, Bienenstock J. On communication between gut microbes and the brain. *Curr. Opin. Gastroenterol.* 28, 557–562 (2012).

83 Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. *J. Am. Coll. Nutr.* 20, 5–19 (2001).

84 Kopp-Hoolihan L. Prophylactic and therapeutic uses of probiotics: a review. *J. Am. Diet. Assoc.* 101, 229–238; quiz 239–241 (2001).

85 Aydin A, Ahmed K, Zaman I *et al.* Recurrent urinary tract infections in women. *Int. Urogynecol. J.* 26, 795–804 (2014).

86 Flegal KM, Carroll MD, Ogden CL *et al.* Prevalence and trends in obesity among US adults, 1999–2008. *JAMA* 303, 235–241 (2010).

87 Lam DW, LeRoith D. The worldwide diabetes epidemic. *Curr. Opin. Endocrinol. Diabetes Obes.* 19, 93–96 (2012).

88 Yadav H, Lee JH, Lloyd J *et al.* Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. *J. Biol. Chem.* 288, 25088–25097. (2013).

89 Gomes AC, Bueno AA, de Souza RG *et al.* Gut microbiota, probiotics and diabetes. *Nutr. J.* 13, 60 (2014).

90 Chen SW, Zhong J, Huan LD. Expression of human insulin in lactic acid bacteria and its oral administration in non-obese diabetic mice. *Wei Sheng Wu Xue Bao* 47, 987–991 (2007).

91 Gérard P. Gut microbiota and obesity. *Cell Mol. Life Sci.*73, 147-162 (2016).

92 Ley RE. Microbial ecology: human gut microbes associated with obesity. *Nature* 444, 1022–1023 (2006).

93 Khan MT, Nieuwdorp M, Bäckhed F. Microbial modulation of insulin sensitivity. *Cell Metab.* 20, 753–760 (2014).

94 Peterson CT, Sharma V, Elmén L *et al.* Immune homeostasis, dysbiosis and

therapeutic modulation of the gut microbiota. *Clin. Exp. Immunol.* 179, 363–377 (2015).

95 Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. *Biol. Psychiatry* 74, 720–726 (2013).

96 Moloney RD, Desbonnet L, Clarke G *et al.* The microbiome: stress, health and disease. *Mamm. Genome* 25, 49–74 (2014).

97 Howland RH. Can a bug in the gut act like a drug in the brain? *J. Psychosoc. Nurs. Ment. Health Serv.* 53, 22–24 (2015).

98 Forsythe P, Kunze WA, Bienenstock J. On communication between gut microbes and the brain. *Curr. Opin. Gastroenterol.* 28, 557–562 (2012).

99 Abramov V, Khlebnikov V, Kosarev I *et al.* Probiotic properties of *Lactobacillus crispatus* 2,029: homeostatic interaction with cervicovaginal epithelial cells and antagonistic activity to genitourinary pathogens. *Probiotics Antimicrob. Proteins* 6, 165–176 (2014).

100 Brotman RM, Shardell MD, Gajer P *et al.* Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *J. Infect. Dis.* 210, 1723–1733 (2014).

101 Buve A, Jespers V, Crucitti T *et al.* The vaginal microbiota and susceptibility to HIV. *AIDS* 28, 2333–2344 (2014).

102 Borges S, Silva J, Teixeira P. The role of lactobacilli and probiotics in maintaining vaginal health. *Arch. Gynecol. Obstet.* 289, 479–489 (2014).

103 Vangelista L, Secchi M, Liu X *et al.* Engineering of *Lactobacillus jensenii* to secrete RANTES and a CCR5 antagonist analogue as live HIV-1 blockers. *Antimicrob. Agents Chemother.* 54, 2994–3001 (2010).

104 Rao S, Hu S, McHugh L *et al.* Toward a live microbial microbicide for HIV: commensal bacteria secreting an HIV fusion inhibitor peptide. *Proc. Natl Acad. Sci. USA.* 102, 11993–11998 (2005).

105 Stadlbauer V. Immunosuppression and probiotics: are they effective and safe? *Benef. Microbes*6, 823-828 (2015).

106 Sanders ME, Akkermans LM, Haller D *et al.* Safety assessment of probiotics for human use. *Gut Microbes* 1, 164–185 (2010).

107 Broaders E, Gahan CG, Marchesi JR. Mobile genetic elements of the human gastrointestinal tract: potential for spread of antibiotic resistance genes. *Gut Microbes* 4, 271–280. (2013).

108 Wong A, Ngu DY, Dan LA *et al.* Detection of antibiotic resistance in probiotics of dietary supplements. *Nutr. J.* 14(14), 95 (2015).

109 Rosander A, Connolly E, Roos S. Removal of antibiotic resistance gene-carrying plasmids from *Lactobacillus reuteri* ATCC 55730 and characterization of the resulting daughter strain, *L. reuteri* DSM 17938. *Appl. Environ. Microbiol.* 74, 6032–6040 (2008).

110 Piñero-Lambea C, Ruano-Gallego D, Fernández LÁ. Engineered bacteria as therapeutic agents. *Curr. Opin. Biotechnol.* 35, 94–102 (2015).

111 Rovner AJ, Haimovich AD, Katz SR *et al.* Recoded organisms engineered to depend on synthetic amino acids. *Nature* 518, 89–93 (2015).

•• **Important paper on biocontainment of the engineered micro-organism.**

112 Gallagher RR, Patel JR, Interiano AL *et al.* Multilayered genetic safeguards limit growth of microorganisms to defined environments. *Nucleic Acids Res.* 43, 1945–1954 (2015).