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Synthetic biology in probiotic lactic acid bacteria: At the frontier of living therapeutics

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The trillions of microbes hosted by humans can dictate health or illness depending on a multitude of genetic, environmental, and lifestyle factors that help define the human ecosystem. As the human microbiota is characterized, so can the interconnectivity of microbe–host-disease be realized and manipulated. Designing microbes as therapeutic agents can not only enable targeted drug delivery but also restore homeostasis within a perturbed microbial community. Used for centuries in fermentation and preservation of food, lactic acid bacteria (LAB) have a long history of safe, and occasionally health promoting, interactions with the human gut, making them ideal candidates for engineered functionality. This review outlines available genetic tools, recent developments in biomedical applications, as well as potential future applications of synthetic biology to program LAB-based therapeutic systems.

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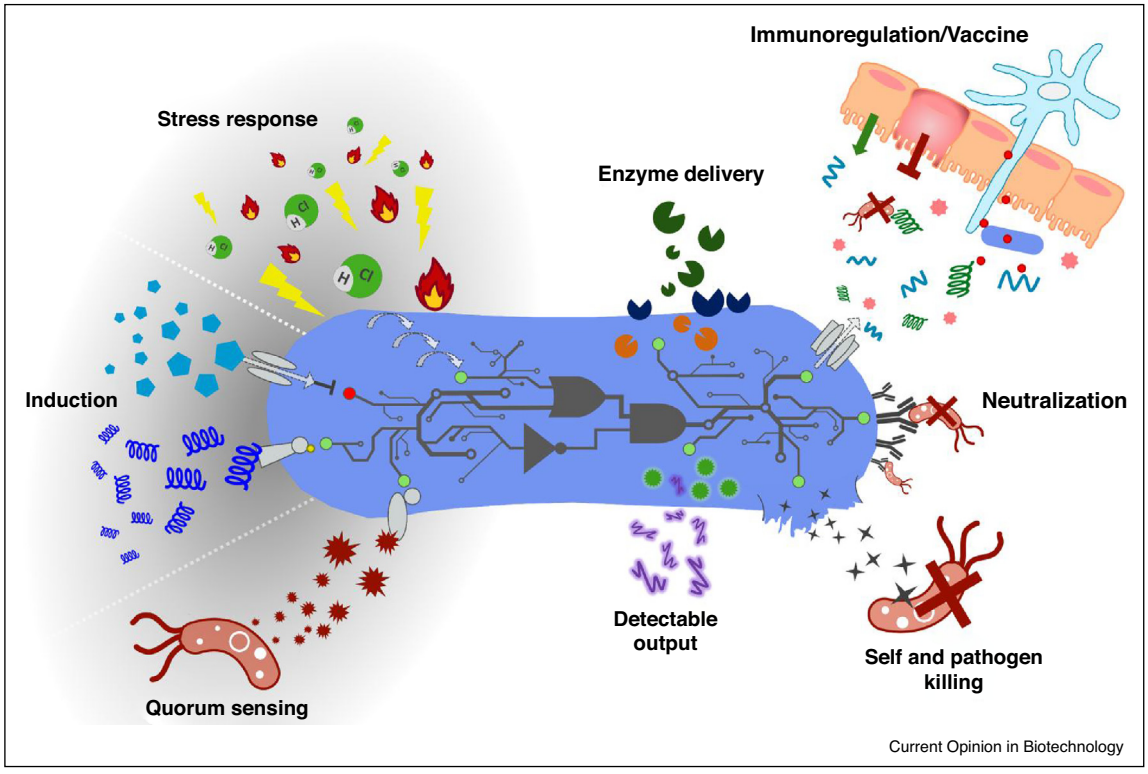
Introduction

The advent of next-generation sequencing has provided a new lens into the vast and diverse microbial taxa present in and on the human body, with their collective genomes termed the microbiome. Consequently, the continued resolution of the microbiome in the post-genomic era has modified the ontological definition of an individual, evoking a re-formation of human beings as holobionts, pluralistic ecosystems in which humans are not only hosts *to* but members *of* a community functioning as a whole being.

With this framework, microbiota health is interminably linked to human development and wellness, and in turn, microbial dysbiosis can stem from, or spur, disease. With the largest microbial community present in the gastrointestinal (GI) tract, a perturbed gut microbiota can invite opportunistic overgrowth, nosocomial infection, immunodysregulation, and metabolic imbalances, each of which have been associated with a multitude of diseases [1]. The gut microbiota has been further associated with aging, asthma and atopy, cardiovascular disease, neuropsychiatric disorders, cancer, and childhood diseases. Although historically contextualized within traditional medicine and home remedies, recent explorations into treatments of these conditions have sought to leverage the microbe–host connection by restoring homeostasis through the supplementation of *good bugs*, colloquially termed probiotics.

Lactic acid bacteria (LAB), largely from the order Lactobacillales and long used in food and agricultural processing, have become the centerpiece of probiotics research [2]. Extending beyond LAB to *Bifidobacterium*, non-pathogenic *Escherichia coli* Nissle 1917 (EcN), and some yeasts, conventional probiotics have been employed in several successful clinical trials to combat food borne enteric pathogens and impart general immunoregulatory benefits [3]. While probiotics are often sold as over-the-counter health supplements, they have an opaque connection to wellness, resulting in symptom alleviation or general health promotion without a designated mode-of-action. An adjuvant approach, conventional drug therapy (i.e. small molecules) along with prebiotic and probiotic supplements, can sometimes provide synergistic benefit. For example, prebiotic and/or probiotic supplementation was more effective at eradicating *Helicobacter pylori* or *Clostridium difficile* infections when antibiotics were often not sufficiently efficacious [4,5]. This adjuvant strategy is now in a second iteration as LAB probiotics are being directly engineered to produce these conventional drugs, acting as living therapeutics. This review highlights recent publications and outlines the toolsets being implemented in probiotic microorganisms, with particular emphasis on LAB, to localize, tune, control, and remember functional outcomes with therapeutic, preventative, and diagnostic potential (Figure 1). While this review focuses on the most recent advances in LAB engineering, a comprehensive and historical perspective of LAB, their relationship to human health, and the fundamentals of their expression systems can be found in *Lactic Acid Bacteria: Microbiological and Functional Aspects* [6].

Figure 1



Summary of current genetic tools and modes of action for probiotic therapeutics. The various synthetic biological components corresponding to input (left), computation (center), output (right) are outlined. Environmental signals like heat, low pH, and UV-irradiation can initiate a stress cascade. Concentration gradients of salts, sugars, and antimicrobial peptides can induce or repress gene expression. Quorum-sensing machinery can be co-opted to control population-wide responses. These gene circuits can be used to actuate several therapeutic outputs. Enzymes can be secreted, displayed, and cytoplasmically expressed; production of cytokines, antimicrobial peptides, hormones, antigens, and enzymes can manipulate immunoregulatory signals; pathogens can be neutralized or killed by the production of antibodies and toxins; and detectable outputs can be produced to diagnose subclinical disease states.

Developing synthetic biological parts libraries for LAB probiotics

Expression systems

A cornerstone of synthetic biology is the forward engineering of cellular behavior using well-defined parts libraries. Supported by several decades of basic and applied research due to its importance in the fermented foods industries, *Lactococcus lactis* has emerged as a model and platform LAB for synthetic biology. Early gene cloning vectors constructed using plasmids isolated from *L. lactis* strains — pWV01 and pSH71 (as well as pAMβ1 from *Enterococcus faecalis*) — are still the backbone for more advanced genetic tools for gene expression, inducible systems, chromosomal integration, and recombineering.

A considerable number of constitutive promoters and terminators of varying strength have already been isolated from *L. lactis* and have provided sufficient gene expression for initial characterization studies. Using promoter mutagenesis, synthetic constitutive promoter libraries were generated in *L. lactis* and *Lactobacillus plantarum* with wide dynamic ranges [7,8]. Alternatively, inducible promoters offer conditional expression and are often more useful for synthetic biological applications. The nisin-controlled expression (NICE) system is the most widely used and reviewed, constructed based on the autoregulatory production of the antimicrobial peptide nisin. One drawback to the NICE system is leaky basal expression, which can limit its utility to applications that require tight control or for expression of toxic proteins. The P_{Zn}-*zit*R expression system [9] and the stronger zinc-regulated expression (Zirex) system [10] can each be implemented simultaneously with the NICE system to provide tight co-expression under non-toxic Zn^{2+} levels. The agmatine-controlled expression (ACE) system, adapted from the eponymous locus in *L. lactis* [11], also offers a tightly controlled and dose-responsive alternative to the NICE system. Another widely used induction system is based on the *Lactobacillus sakei* sakacin-P regulatory machinery and