



Bioengineered Microbes: The Dawn of Living Therapeutics

Sanmeet Kour^{1*}, Deepti Narang², Ram Saran Sethi¹, Amreena Hassan³ and Shruthi Reddy⁴

¹College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA

²Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA

³Department of Animal Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Shuhama Ganderbal, Srinagar, Jammu and Kashmir, INDIA

⁴Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA

*Corresponding author: S Kour; E-mail: sanmeetkour131@gmail.com

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ABSTRACT

Microbes are no longer viewed solely as vicious pathogens; they are recognized as pivotal players in human and animal health. The innate ability of microbes to interact with host systems provides a unique platform for the delivery of therapeutics via genetic engineering. “Designer” or bioengineered microbes represent a breakthrough for treating challenging chronic diseases such as cancer and addressing the alarming rise in antibiotic-resistant pathogens. These living therapeutics can be programmed to produce hormones, enzymes, antigens, and vitamins at the site of disease. As safety concerns regarding recombinant DNA persist, the development of sophisticated biocontainment devices offers a path forward. This review explores the architecture, applications, and challenges of bioengineered microbes as a judicious alternative to traditional antibiotics.

HIGHLIGHTS

- Bioengineered microbes have therapeutic potential.
- There are challenges of bioengineered microbes as a judicious alternative to traditional antibiotics

Keywords: Bioengineered microbes, Biocontainment, Therapeutics, Genetic Engineering

Microorganisms have historically been categorized primarily as “vicious pathogens”; however, modern biological research has revealed their indispensable roles in maintaining both human and animal physiological homeostasis. The emergence of synthetic biology has allowed for the transition from viewing microbes as mere residents to viewing them as programmable platforms (Bober *et al.*, 2018). By redesigning biological systems for increased efficiency, scientists can develop bioengineered microbes—often referred to as “designer microbes”—specifically tailored to treat and prevent complex illnesses.

Microbial systems are the preferred substrate for synthetic biology because of their relative simplicity, well-characterized genomes, and ease of genetic manipulation compared to multi-cellular eukaryotes (Riglar and Silver,

2018). The inherent ability of commensal microbes to colonize specific niches, such as the gastrointestinal tract, skin, or vaginal mucosa, allows them to serve as localized delivery vehicles for therapeutic molecules. These “living factories” are capable of synthesizing and secreting biologically active substances, including antigens for mucosal vaccines, enzymes to correct metabolic deficiencies, vitamins and hormones for endocrine support, and antimicrobial peptides to target specific pathogens (Cubillos-Ruiz *et al.*, 2021).

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While traditional antibiotics have historically been the cornerstone of infection control, the rapid dissemination of antibiotic-resistant genes (ARG) among global pathogens has severely compromised the current clinical repertoire (O'Neill, 2016). This “post-antibiotic era” necessitates the development of alternative strategies. Bioengineered microbes offer a transformative solution by providing dynamic, adaptive therapies that can actively sense and respond to pathogens, reducing the selective pressure that leads to widespread resistance (Charbonneau *et al.*, 2020).

Challenges of Synthetic Biology in Eukaryotes

The application of synthetic biology techniques in higher eukaryotes presents a level of complexity not typically encountered in microbial engineering. While bacteria possess a relatively straightforward genomic structure—usually consisting of a single, circular chromosome—eukaryotes are defined by sophisticated compartmentalization and regulatory mechanisms that hinder rapid manipulation (Khalil and Collins, 2010).

Several critical factors make direct synthetic biology applications in plants and animals particularly challenging:

- ❑ *Precision and Targeted Integration:* Unlike the highly efficient homologous recombination seen in many microbes, higher eukaryotes often lack methods for the precise and accurate integration of large DNA fragments into specific genomic loci.
- ❑ *Cellular Complexity and Differentiation:* Eukaryotes consist of multiple tissue types, each characterized by distinct gene expression patterns and epigenetic landscapes. Ensuring a genetic circuit functions consistently across different cell types is a significant hurdle (Voigt, 2012).
- ❑ *Post-Transcriptional Modifications:* The presence of introns, RNA splicing mechanisms, and complex post-translational modifications (such as glycosylation) adds layers of regulation that bacteria do not possess, making it difficult to predict the final function of a synthetic protein.
- ❑ *Nuclear Membrane Barriers:* The physical separation of the genome within the nucleus requires specialized transport mechanisms for any synthetic machinery to access the target DNA.

However, engineering the microbiome offers a viable strategic “workaround” to these challenges. By augmenting the existing naturally occurring microorganisms within a host, researchers can introduce new functions into a complex organism without needing to modify the host’s own genome (Charbonneau *et al.*, 2020). Modern efforts are focused on using engineered probiotic strains as either biosensors or mobile therapeutic delivery systems to combat systemic diseases (Mee and Wang, 2012).

The Rationale for Bioengineered Microbes

Bioengineered or designer microbes function as autonomous, “smart” living factories. Unlike traditional pharmacological agents, these organisms can replicate independently, sense pathological environments, and execute a pre-programmed therapeutic response, including self-destruction (lysis) upon completion of the task (Din *et al.*, 2016). The necessity for such microbes stems from the following distinct advantages over traditional systemic treatment modalities.

- ❑ *Economic and Industrial Efficiency:* A primary driver for the development of microbial therapeutics is the significant reduction in production costs. Traditional biopharmaceuticals require extensive “downstream” processing, including complex purification, stabilization, and cold-chain logistics. Designer microbes bypass these requirements by producing the therapeutic agent in situ, directly within the host’s body eliminating the overhead associated with industrial-scale protein purification (Landry and Tabor, 2017).
- ❑ *Enhanced Pharmacokinetics and Targeted Delivery:* Traditional systemic drug administration often suffers from “off-target” effects, where healthy tissues are exposed to toxic compounds. Bioengineered microbes provide a non-invasive administration route (e.g., oral or topical) and localized delivery which in turn reduces the dosage because the production unit is located in immediate proximity to the target tissue (e.g., a tumour or inflamed gut mucosa), the required therapeutic dose is significantly lower than systemic alternatives and also it has minimum side effects because its localized secretion ensures

that high concentrations of the drug reach the site of disease while systemic exposure remains negligible (Riglar and Silver, 2018).

- *Versatility in Chronic Disease Management:* Living therapeutics offer a dynamic approach to chronic and recalcitrant diseases like cancer, where they can be programmed to respond only to the unique micro-environmental cues of a tumour (e.g., hypoxia or low pH). Furthermore, these microbes serve as a critical defence against the global rise of multidrug-resistant (MDR) pathogens by delivering narrow-spectrum antimicrobial peptides that do not disturb the wider commensal microbiome (Cubillos-Ruiz *et al.*, 2021).

Designing of bioengineering microbes

The construction of a successful “designer microbe” is an iterative process that relies on the principles of the Design-Build-Test-Learn (DBTL) cycle. For a microbe to function reliably within a host, its genetic architecture must be precisely tuned to balance therapeutic output with the host’s metabolic capacity (Khalil and Collins, 2010).

- *Host Selection and Chassis Optimization:* The first step in design is selecting an appropriate chassis—the host organism. Selection criteria include Genetic Tractability i.e. the ease of culturing and availability of high-quality genome sequences, Niche Specificity i.e. the ability to colonize the target environment (e.g., *L. lactis* for the gut or *S. epidermidis* for the skin), Safety Profile preference for “Generally Recognized as Safe” (GRAS) organisms or probiotic strains like *E. Coli* Nissle 1917 (Riglar and Silver, 2018).
- *Molecular Assembly:* Traditional plasmid-based expression often suffers from genetic instability and metabolic drain. Modern design favours chromosomal integration to ensure the synthetic genes are inherited stably. It is achieved by a specialized technique known as “Clonetegration” that combines one-step cloning and site-specific integration into the bacterial chromosome, drastically reducing the time required to build complex pathways (St-Pierre *et al.*, 2013).

- *Control Circuits:* To ensure the microbe only acts when necessary, gene expression is mediated by promoters that function as biological “on/off” or “dimmer” switches. There are Inducible Promoters that are activated by specific external stimuli (e.g., an inflammatory biomarker or a specific sugar), ensuring that the metabolic burden on the host is minimized until the therapy is required (Voigt, 2012) and the Boolean Logic Gates which are created by combining multiple promoters and repressors. Using these gates designers can create “AND,” “OR,” and “NOT” logic gates. For example, a microbe might only release a drug if it detects *both* a tumor marker AND a hypoxic environment (AND gate), significantly increasing therapeutic precision (Voigt, 2012).

Requirements for constructing bioengineered microbes

It starts with a basic requirement of a strong carrier organism with a big toolbox for manipulation of gene expression which is altogether important for constructing smart living therapeutics. A living therapeutic in the form of bioengineered microbe should be constructed in such a way that it should contain input sensors to detect any signal, control devices to integrate those input signals and actuators for generation of response to them. It also must have biocontainment devices in it to address safety issues. Such devices are necessary to prevent contamination and spread of the pathogenic bacteria or recombinant DNA into the environment. Drug delivery system for constructing designer microbes includes eukaryotic cells, bacteriophages and bacteria. The concept of synthetic biology in higher eukaryotes is quite challenging, mainly because of its complexity and lack of methods for the accurate and targeted integration of DNA fragments. All these together make application of synthetic biology difficult in animals and plants. However among bacteria, most commonly used and preferred gut chassis is *E. coli* which is used as a model organism because of its widely studied & known genome and its high prevalence in human gut especially enterotoxigenic *Escherichia coli* (ETEC) which is the most common form of traveler’s diarrhoea affecting thousands of people every year. Scientists have engineered non-pathogenic *E. coli* to sense enterotoxigenic *Escherichia coli* which can respond to its presence and packs the non-pathogenic *E. coli* in cellulose



matrix to detect ETEC from the environment (Bête *et al.*, 2018). *L. lactis* on the other hand is used as probiotic bacteria from longer periods. Bacteria like *Lactobacillus jensenii* & *Staphylococcus epidermidis* have been used for engineering therapeutics for skin, vaginal tract and latter for tumors respectively (Yoon *et al.*, 2011).

Components of engineered living therapeutics

An efficient engineered microbial therapeutic is characterized by a sophisticated integration of a memory device, sensors, a delivery system, and a biocontainment module. The memory module often utilizes bi-stable gene regulatory networks, such as toggle switches, which incorporate “transcriptors”—biological transistors that regulate the flow of RNA polymerase along the DNA. By controlling the transcription unit through unidirectional terminators, these transcriptors allow the microbe to “remember” past environmental exposures, ensuring a consistent therapeutic response even after the initial trigger has dissipated (Siuti *et al.*, 2013). This memory is coupled with high-precision sensors, primarily composed of specialized proteins that bind to specific target molecules to regulate promoter activity. These sensors act as the primary interface between the microbe and the host environment, effectively turning gene expression “on” or “off” in response to real-time physiological changes (Riglar and Silver, 2018).

Once a signal is detected and processed by the memory and sensor modules, the delivery system ensures that the therapeutic cargo reaches its destination. Microbes can be programmed to produce a variety of molecules, including proteins, peptides, and small metabolites. The release of these substances into the target environment is often mediated by membrane transporters that recognize specific signal peptides linked to the therapeutic protein. Alternatively, the system may be programmed for controlled lysis, where internal or external signals trigger the rupture of the cell membrane, releasing the cytoplasmic contents directly into the surroundings.

To mitigate the ecological risks associated with the release of recombinant DNA, biocontainment devices are integrated into the architecture. These safety modules often function through toxin/antitoxin counteraction; in the default state, the microbe produces an antitoxin to neutralize a lethal toxin. If the microbe escapes its

intended niche or completes its medical protocol, the antitoxin production is suppressed, leading to automated cell death (Ozdemir *et al.*, 2018). This multi-layered approach ensures that the bioengineered microbe remains a localized, safe, and highly efficient medical tool.

Use of engineered bacteria

- *As quorum sensing molecules and memory devices:* Many bacterial species utilize quorum sensing, a sophisticated communication mechanism defined by the ability to detect and respond to cell population density through gene regulation. By coordinating gene expression across a population, bacteria can synchronize behaviours that would be inefficient for a single cell. Researchers have successfully engineered bacteria to function as inter-species quorum sensing molecules, allowing for the detection and inhibition of specific pathogens. For instance, strains of *E. coli*, *L. lactis*, and *Lactobacillus reuteri* have been reprogrammed with quorum sensing systems to actively combat infections caused by *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Staphylococcus aureus* (Lubkowitz *et al.*, 2018; Mao *et al.*, 2018). Beyond immediate therapeutic action, these bacteria can be engineered to record external inputs within a living system and display diagnostic outputs upon excretion. These biological memory devices can report the presence of disease markers, potentially turning on the production of a localized therapy only when specific diagnostic thresholds are met.
- *Transcriptional Manipulation and Promoter Engineering:* The manipulation of gene expression in designer microbes is predominantly performed at the transcriptional level. The promoter serves as the central regulatory component in the genetic toolbox, acting as the primary switch for sensing and control. By combining various regulated promoters, scientists can construct internal logic circuits capable of integrating multiple external inputs. These promoters can be specifically tuned to respond to disease-specific environmental conditions. Notable examples include tetrathionate-sensing devices in *E. coli*

and the nitrite-sensitive promoter, which has been utilized in both *E. coli* and *Bacillus subtilis* to achieve inflammation-dependent gene expression (Courbet *et al.*, 2015).

- **Oncology and Anti-Cancerous Applications:** When constructed with precise genetic components, designer microbes serve as potent agents for cancer diagnosis and treatment. Techniques such as multivariate modular pathway engineering have optimized the production of anti-cancerous precursors within *E. coli*. Historically, various anaerobic bacteria have shown promise in treating solid tumors because they naturally home to the hypoxic, poorly vascularized regions of large tumor masses—environments that are typically resistant to traditional chemotherapy. Bacteria such as *Clostridium* can germinate and proliferate within these hypoxic zones, exerting natural oncolytic effects (Heppner *et al.*, 1978). To enhance this natural activity, attenuated strains of *Listeria*, *Salmonella*, and *E. Coli* Nissle 1917 have been upgraded to deliver tumor-associated antigens or express cytokines like IL-2 and TNF-alpha to elicit anti-tumor immunity (Liu *et al.*, 2002; Stritzker *et al.*, 2007). Furthermore, microbes can be engineered to deliver pro-drug-converting enzymes, such as cytosine deaminase, which converts the non-toxic 5-fluorocytosine into the potent chemotherapeutic 5-fluorouracil directly at the tumour site, thereby minimizing systemic toxicity.
- **Combating Multidrug Resistance and Allergy Immunotherapy:** Beyond oncology, bioengineered microbes provide a defense against multidrug-resistant (MDR) organisms. For example, reprogrammed *E. coli* has been designed to produce antimicrobial and anti-biofilm proteins specifically targeting *P. aeruginosa*, while bioengineered *L. lactis* has demonstrated the ability to inhibit the growth of MDR *Enterococcus faecalis* (Xiang *et al.*, 2006). In the field of allergy immunotherapy, lactic acid bacteria (LAB) are engineered to deliver allergens or allergen-derived peptides to mucosal surfaces to induce immune tolerance. The development of “actobiotics”—orally delivered biopharmaceuticals produced *in*

situ by *L. lactis*—has shown efficacy in treating chemically induced colitis via the production of IL-10. This approach has also been linked to a reduction in food-induced anaphylaxis and the prevention of tumor formation in various animal models.

- **Surface Display and Mucosal Vaccines:** Another innovative approach involves displaying therapeutic proteins on the microbial surface to promote adhesion or entry into target cells. By using tags such as the ice nuclease protein (INP) from *E. coli*, researchers can export polypeptides to the cell membrane. This technique allows for the targeted delivery of compounds or the creation of mucosal vaccines. Such vaccines can elicit protective immune responses at aero-digestive, intestinal, and urogenital membranes. Examples include engineered probiotic vaccines against *Helicobacter pylori* and vaginal *Lactobacillus* strains engineered to produce neutralizing antibody fragments against the Human Immunodeficiency Virus (HIV) (Marcobal *et al.*, 2016). Finally, microbes have been optimized to detect and report inflammation biomarkers like nitrogen oxide, tetrathionate, and thiosulfate, providing a colored reaction or reporter gene expression as a diagnostic output (Daeffler *et al.*, 2017).

Applications in animal biotechnology

The utility of designer microbes extends beyond human medicine into the field of veterinary science. The animal microbiome—comprising the collective genomes of bacteria, fungi, and viruses—directly influences physiological functions, metabolic health, and even behaviour. By engineering the microbiomes of livestock, researchers can significantly impact food production and environmental health. For instance, manipulating rumen microbes offers a potential pathway to reduce enteric methane emissions, thereby mitigating the environmental footprint of cattle farming. Furthermore, engineering insect microbiomes presents opportunities to alter disease transmission patterns and improve food security through enhanced pollination and pest resilience.



- ❑ **Fecal Microbiota Transplantation (FMT) and Stool Banks:** A significant advancement in animal health is the standardization of Fecal Microbiota Transplantation (FMT), particularly in porcine models (Hu *et al.*, 2018). FMT is utilized to treat intestinal disorders in ruminants, pigs, and equines by restoring a healthy microbial balance. To ensure safety and efficacy, the procedure begins with the selection of optimal donors who undergo rigorous serological and phenotypic testing to prevent disease transmission. Fresh feces from these healthy donors are homogenized with sterile saline and can be preserved at -80°C in a 10% sterile glycerol solution. The establishment of “stool banks” allows for the ready transfer of these microbial populations to restore gut health in animals, serving as a promising therapeutic alternative to the prophylactic use of antibiotics in agriculture.
- ❑ **Programming Animal Physiology and Behaviour:** Synthetic biology also allows for the programming of animal behaviour and physiology through bacterial genetic circuits. By utilizing logic gates and gene-editing tools like CRISPR, scientists can control RNA expression to modulate host responses. A notable proof-of-concept involved the use of engineered *E. coli* to program the physiology of *Caenorhabditis elegans*. By placing RNA expression under the control of bacterial “AND” and “OR” logic gates, researchers successfully modified the GFP expression profiles and behavioural responses of the nematodes (Gao and Shun, 2020). This highlights the potential for using microbes to exert precise control over the biological functions of higher organisms.

Other Applications: Biofuels and Bioremediation

- ❑ **Production of Sustainable Biofuels:** Beyond clinical targets, engineered microbes are serving as the foundation for the next generation of energy. Microbes are reprogrammed to overexpress central carbon metabolism elements, such as CsrB, to optimize the production of biofuels like n-butanol and amorphadiene.

Recent breakthroughs in *E. coli* engineering have also enabled the creation of novel biosynthetic pathways for the production of pentanol, a high-energy second-generation biofuel.

- ❑ **Bioremediation of Pollutants:** To address the global challenge of non-biodegradable and radioactive waste, bioengineered microbes are employed in bioremediation—the process of breaking down hazardous substances into less toxic forms. “Mycoremediation,” which utilizes fungal mycelia, leverages the natural ability of fungi to decompose lignin and cellulose to clean contaminated environments. Construction of designer microbes with enhanced degradative capabilities offers a vital tool for restoring ecosystems damaged by industrial pollutants.
- ❑ **Combating Obesity:** Obesity represents a critical global health concern that increases the risk of numerous metabolic disorders. Engineering probiotic *E. coli* to produce appetite-suppressing compounds—typically secreted by the intestines in response to food intake—offers a novel therapeutic strategy. While research in this area is still in its nascent stages, the ability to modify the natural microbiome to satisfy appetite autonomously could revolutionize metabolic health management.

Limitations of Bioengineered Microbes

Despite the transformative potential of designer microbes, several technical and biological constraints remain. One of the primary challenges is the metabolic burden imposed by heterologous gene expression. When a microbe is reprogrammed to prioritize the synthesis of foreign therapeutic proteins, its internal resources (such as ATP and amino acids) are diverted away from essential growth and replication. This often results in reduced fitness, making the engineered strain less competitive against the natural, non-engineered members of the host microbiome.

Genetic stability is another significant hurdle. Most early-stage engineered microbes rely on recombinant DNA carried on plasmids, which are prone to being lost during rapid cell division. To ensure long-term therapeutic efficacy, researchers are increasingly moving

toward chromosomal integration of synthetic circuits. Furthermore, the risk of horizontal gene transfer—where recombinant DNA might be transferred to wild-type environmental bacteria—necessitates the use of high-fidelity biocontainment. While devices like the iDNA system (which utilizes CRISPR-Cas9 to degrade the host genome and recombinant genes upon an environmental trigger) are promising, they require further optimization to ensure they do not fail due to spontaneous mutations.

Further Research and Future Perspectives

The transition of bioengineered microbes from the laboratory to the clinic requires addressing a complex web of societal, ethical, and technical issues. A critical priority for future research is the education of both the public and healthcare providers. Because these therapies involve “living GMOs,” transparent communication regarding safety protocols and biocontainment is essential to foster societal acceptance. Furthermore, health care workers must be specifically trained to handle, administer, and monitor these novel biological agents.

As antibiotic resistance continues to escalate, the need for new “carrier microbes” beyond the standard *E. coli* and *S. cerevisiae* models is urgent. Future efforts should focus on characterizing non-model organisms, such as various species of *Bifidobacteria* and *Lactic acid bacteria*, which may offer superior colonization and safety profiles for specific human tissues. Key areas for development include:

- ❑ *Advanced Biosensors:* Developing sensors capable of detecting a broader array of toxic compounds, volatile organic compounds (VOCs), and disease-specific protein markers.
- ❑ *Precision Actuators:* Creating more efficient delivery mechanisms that allow for a reduction in the initial bacterial dose while maintaining therapeutic efficacy at the target site.
- ❑ *Smart Biocontainment:* Researching “nutritional safeguards” and synthetic auxotrophy that link microbial survival to a rare, synthetic nutrient provided only during the treatment regimen.

CONCLUSION

Bioengineered microbes represent a shift toward a more judicious and precise form of medicine. While the biological concerns regarding metabolic burden and ecological escape are significant, the continued refinement of synthetic genetic tools promises a future where living therapeutics work alongside traditional medicine to solve some of the most challenging health crises of the 21st century.

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