

Proceedings of Women in Academia, Research and Management for Work-life Initiatives for Sustainable Health & Empowering Safety (WARM-WISHES 2026)

## Engineered Bacteria as Living Therapeutics: Design, Applications, and Clinical Challenge

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Received: 18 Apr 2026 | Received Revised Version: 30 Apr 2026 | Accepted: 11 May 2026 | Published: 13 May 2026

Volume 08 Issue 05 2026 | DOI: 10.37547/tajas/warm-06

### Abstract

*Living therapeutics" is an innovative category of living organisms that can be engineered to prevent, detect, and treat human diseases. With the help of synthetic biology and genetic engineering, microbial cells with the ability to recognize disease-associated cues, synthesize drugs within the host body, and respond to complex biological environments have now been created. This review explores the fundamental approaches used for constructing engineered bacteria, such as the creation of synthetic gene circuits, selecting suitable hosts or chassis, and incorporating biosensing and biocontainment strategies. Finally, we discuss the primary applications of engineered bacteria with a focus on gastrointestinal disease treatment, cancer therapy, metabolic disease management, and infectious disease treatment. Despite all of this, however, there are numerous hurdles yet to be conquered, including issues related to safety, genetic stability, targeted delivery, and regulatory clearance. For bacterial systems to move from the lab bench to the bedside, certain parameters need to be taken care of. On the whole, genetically modified bacteria represent an exciting frontier within the field of precision medicine, with the capacity to revolutionize therapeutic strategies with highly selective interventions.*

**Keywords:** Genetic engineering, CRISPR-Cas, Escherichia coli, Lactobacillus, Bacteroides, Bacterial therapeutics

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**Cite This Article:** Ishika Baranwal, & Aditi Singh. (2026). Engineered Bacteria as Living Therapeutics: Design, Applications, and Clinical Challenge. The American Journal of Applied Sciences, 56–63. <https://doi.org/10.37547/tajas/warm-06>

### 1. Introduction

A novel class of biomedical therapies called "living therapeutics" employs living cells, particularly microorganisms, to recognize, inhibit, or cure diseases

using the metabolic activities of the host. Unlike conventional drugs whose formulations remain constant, living therapeutics are dynamic systems capable of detecting cues from the environment, adapting to physiological conditions, and generating therapeutic

substances, such as antibiotics or signaling molecules (Riglar & Silver, 2018). One class of living therapeutics that has attracted much attention includes genetically engineered bacteria owing to their flexibility, easy manipulation, and wide distribution in body sites.

The concept of using microbes for health purposes is not novel as it was associated with the use of probiotics to maintain gut flora. Owing to the natural positive impacts of the microbes on the body, conventional probiotics such as *Lactobacillus* and *Bifidobacterium* strains have been used traditionally. However, owing to advancements in molecular biology and genetic manipulation, the naturally existing microbes can be engineered with greater therapeutic prospects (Landry et al., 2021). One of the important aspects of precision medicine, whereby bacteria can be programmed to perform specific diagnostic and therapeutic functions, can be observed through the shift from conventional probiotics to genetically engineered microorganisms.

Use of bacteria as a source of beneficial medicines is being increasingly recognized owing to the natural adaptability and programmability of these living organisms. Some of the complex processes that can be performed using bacteria include the targeted delivery of medicines, sensing of specific disease signals, and production of medicinal substances through the regulation of certain processes in the body. Their ability to multiply and respond to external stimuli also ensures continued effect after administration in a single dose (Zalila-Kolsi, 2025). In addition, their well-understood biochemistry and very small genomes allow for genetic modifications using modern technologies such as CRISPR technology. Moreover, certain strains of

bacteria preferentially seek specific sites, such as the digestive tract and cancerous tissue (Chowdhury et al., 2019).

The purpose of this review article is to provide an elaborate summary of the topic of engineered bacteria as living drugs, with a particular focus on the multiple uses of engineered bacteria in biomedicine, methods used to engineer them, and challenges to their practical use in medicine.

## 2. Design and Engineering Strategies

### 2.1 Genetic Engineering Approaches

The development of genetically engineered microorganisms that can serve as living medicines is made possible by genetic engineering, which allows precise modifications to be performed on microorganism genomes. Table 1 illustrates the various strategies employed for the purpose. To increase the efficiency and safety of the process, traditional strategies like genetic knockout and gene introduction have become commonplace to introduce desired features or delete unwanted attributes, like virulence genes (Riglar & Silver, 2018).

CRISPR-Cas technology, which can precisely edit genes and DNA sequences, has been highly instrumental in transforming bacterial genome modification. The technology allows successful knockout, addition, and regulation of gene expression using CRISPR interference (CRISPRi) and activation (CRISPRa), without any negative impacts on other genome parts and without posing any genomic instability (Pickar-Oliver & Gersbach, 2019).

**Table 1: Genetic Engineering Strategies in Bacterial Therapeutics**

Approach	Description	Example Application	Reference
Gene insertion	Introduction of therapeutic genes	Enzyme production for metabolic disorders	Smanski, M. J., et al. (2020)
Gene deletion	Removal of virulence/metabolic genes	Attenuated bacterial strains	Liu, Y., et al. (2022)
CRISPR-Cas editing	Precise genome modification	Targeted gene knockouts	Pickar-Oliver, A., & Gersbach, C. A. (2019)
Synthetic gene circuits	Programmable regulatory networks	Disease-responsive drug delivery	Cubillos-Ruiz, A., et al. (2021)

In addition, bacteria can also be engineered to detect specific cues in the environment and respond by generating regulated therapies through the use of synthetic gene circuits. In relation to their therapeutic applications, these gene circuits will make the modified bacteria both selective and safe (Din et al., 2016).

**2.2 Host Strain Selection**

For developing therapeutic bacteria, selecting the appropriate bacterial strain is crucial because it has an impact on the bacteria’s safety, functionality, and efficacy. Among the commonly used bacterial strains in developing modified bacteria include *Escherichia coli*, *Lactobacillus* bacteria, and *Bacteroides* bacteria (table 2).

**2.2.1 *Escherichia coli***, Due to its well-understood genetic makeup, ease of modification, and fast growth rate, non-pathogenic strains, such as *E. coli* (Nissle 1917), are some of the most frequently used chassis. In applications ranging from drug delivery to

metabolic disorder treatments, significant engineering has been conducted on it (Isabella et al., 2018).

**2.2.2 *Lactobacillus***, The human GI tract also contains Gram-positive lactobacilli that are considered GRAS organisms. These can serve as viable delivery agents for drug molecules due to their probiotic properties and proven safety records, especially in diseases related to the gastrointestinal tract (Steidler & Rottiers, 2006).

**2.2.3 *Bacteroides species*** Owing to the fact that they are established and stable colonizers within the gastrointestinal tract, along with their important role in the microbiome of the gut, Bacteroides strains provide notable advantages as therapeutic chassis. In view of the ability of these microorganisms to thrive in anaerobic conditions and interact metabolically with the host, they represent interesting options for engineered therapies (Wexler & Goodman, 2017;Whitaker et al., 2017).

**Table 2: Common Bacterial Chassis for Living Therapeutics**

Chassis	Key Features	Advantages	Limitations	Reference
<i>E. coli</i> (Nissle 1917)	Well-characterized, easy to engineer	Rapid growth, versatile	Potential safety concerns if not controlled	Isabella et al., 2018
<i>Lactobacillus</i> spp.	Probiotic, GRAS status	Safe, gut-adapted	Limited genetic tools compared to <i>E. coli</i>	Steidler & Rottiers, 2006
<i>Bacteroides</i> spp.	Anaerobic gut commensals	Stable colonization	Difficult to culture and engineer	Wexler & Goodman, 2017;Whitaker et al., 2017

Colonisation ensures that the modified bacteria have the potential to stay on the required site to deliver treatment. Stability is essential for the engineered features to stay stable over time. To ensure success in the clinic, optimization of these aspects becomes mandatory (Landry et al., 2021).

**2.3 Control Systems**

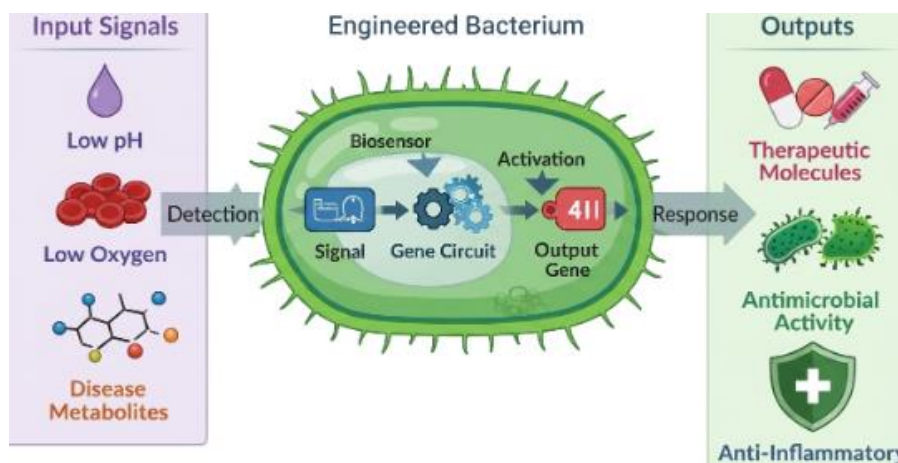
Regulation is essential for genetically modified bacteria in order for them to function specifically in the host depending on circumstances. This technology increases the safety and effectiveness of these organisms by

enabling them to respond to certain environmental cues and changing their gene expression.

In order to manage bacteria behavior in reaction to certain physiological indicators such as pH, oxygen concentration, and metabolites, inducible systems are commonly employed (figure 1). Such systems enhance the specificity of therapy by ensuring that the genetically altered bacteria produce genes useful for treatment under certain conditions. For example, when the bacteria are subjected to an acidic environment often observed during inflammatory processes or tumors, pH-responsive promoters are utilized to promote gene expression. In

addition, in response to low oxygen environments, which are usually characteristic of solid tumors, oxygen-responsive systems enable selective activation. By linking gene expression with specific metabolic

indicators, metabolite-inducible systems, which respond to sugars, amino acids, or compounds associated with the disease state, can further enhance therapeutic specificity (Kotula et al., 2014).



**Figure 1:** Schematic representation of control systems in engineered bacteria. Environmental or disease-specific inputs (e.g., pH, oxygen, metabolites) are detected by biosensors, which activate synthetic gene circuits to produce therapeutic outputs in a controlled manner. (Created in <https://BioRender.com>)

For designing bacterial therapies to detect disease-related biomarkers and trigger customized treatment along with inducible systems, biosensors become an important component of the process. Regulatory proteins or engineered gene networks that respond to environmental stimuli such as inflammation molecules, quorum sensing chemicals, or metabolites associated with pathogens are often employed in the construction of these devices. Therapeutic agents like antimicrobial peptides and anti-inflammatory agents can be produced by genetically altered microorganisms in response to these stimuli, resulting in localized and instant treatment. This targeted action helps ensure effective treatment while reducing unwanted side effects, thus increasing the safety and effectiveness of microorganism-mediated therapies (Pedrolli et al., 2019).

#### 2.4 Biocontainment Strategies

By avoiding unchecked growth and unintentional dissemination, biocontainment techniques are crucial to ensuring the safe use of modified microorganisms.

##### 2.4.1 Genetic Kill Switches

Genetic circuits that are manipulated to ensure the death of the bacteria depending on certain conditions

The bacteria are stimulated once they leave their intended surroundings or fail to receive the required signals.

They can respond to environmental conditions like temperature, nutrients, or chemicals.

Help reduce the viability of artificial bacteria (Din et al., 2016).

##### 2.4.2 Auxotrophy

Bacterial modification that makes bacteria dependent on specific nutrients or metabolic compounds

The absence of such nutrients outside their natural environment means that their life is restricted.

Prevents uncontrolled growth in foreign environments

Artificial amino acids or co-factors is one of the popular approaches (Mimee et al., 2016).

##### 2.4.3 Prevention of Horizontal Gene Transfer (HGT)

Below are some strategies to prevent the transfer of genetically modified genes into wild-type microorganisms:

Transposon deletion, plasmid removal, and elimination of other mobile genetic materials.

Minimizes the major conduits for gene transfer.

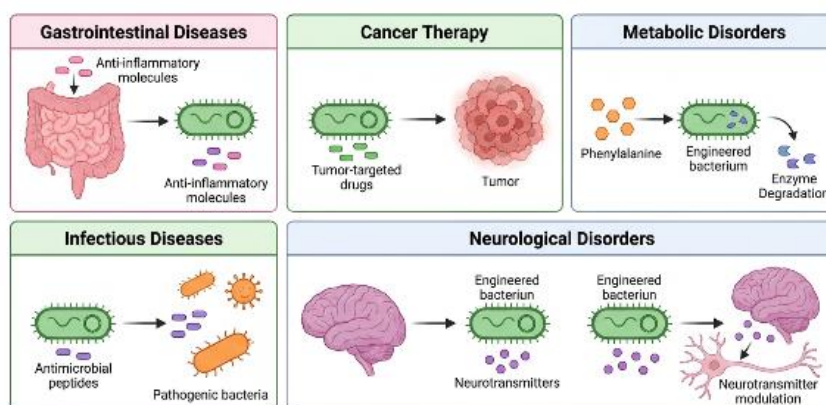
Modification of the genetic code to limit compatibility with wild-type organisms

Incorporation of systems for inactivation or rendering of transferred genetic material inactive

Prevents unintended gene dissemination and ecological consequences (Schmidt & de Lorenzo, 2016).

### 3. Applications of Engineered Bacteria

The application of microbial cells as a platform for therapy has been adopted in treating various diseases (figure 2). They have proven highly beneficial in precision medicine due to their ability to sense, respond, and produce drugs locally.



**Figure 2:** Application of engineered bacteria in treating gastrointestinal, cancer, metabolic, infectious, and neurological diseases through targeted molecule production.

(Created in <https://BioRender.com>)

#### 3.1 Gastrointestinal Diseases

Engineered microorganisms for the treatment of gastrointestinal disorders, such as Crohn's disease and inflammatory bowel disease (IBD), have extensively been studied. It is possible to restore the intestinal balance, manipulate the gut microflora composition, and deliver anti-inflammatory substances through engineered probiotic bacteria. As an example, IL-10 has been produced by the genetically engineered bacterium *Lactococcus lactis*, showing positive anti-inflammatory effects (Steidler & Rottiers, 2006).

#### 3.2 Cancer Therapy

Due to their natural ability to selectively settle into oxygen-depleted and dead zones in cancerous tumors, bacteria offer immense potential for cancer therapy. The killing of tumor cells, regulation of immune system response, and provision of

anticancer agents using specially designed bacterial species is feasible. Recent advancements in synthetic biology have allowed scientists to design customizable bacterial strains that will deliver their payload in a controlled fashion within the tumor microenvironment (Cubillos-Ruiz et al., 2021).

#### 3.3 Metabolic Disorders

With the help of bioengineering, bacteria are being developed to compensate for insufficient metabolic pathways for the cure of metabolic disorders. The main one among these includes the development of *E. coli* bacteria that can be used to degrade phenylalanine in phenylketonuria patients (Isabella et al., 2018).

#### 3.4 Neurological Disorders (Emerging Area)

Based on recent discoveries, it is possible that the gut-brain axis, which is a two-way pathway between the

digestive system and the central nervous system, could enable genetically altered microorganisms to impact the functioning of the brain. The genetic modification of microorganisms through their ability to manipulate immunological, inflammatory, and neurochemical pathways makes it feasible for the brain to be affected by such a system. This is likely to benefit the management of neurodegenerative conditions and psychological disorders, such as depression, despite the novelty of this technology (Cryan et al., 2019; Morais et al., 2021).

#### 4. Future Perspectives

The field of genetically engineered bacteria as medicinal agents is advancing rapidly, and it is expected that future applications in a clinical setting will largely be influenced by various possible approaches. One of the most promising developments is the concept of personalized bacterial therapies, where engineered bacteria are tailored according to the unique characteristics of an individual's microbiota, genome, and disease conditions. Consistent with the general aims of precision medicine, these methods have the potential for enhanced efficacy and reduced inter-patient variability (Charbonneau et al., 2020).

The integration of artificial intelligence (AI) and synthetic biology is expected to expedite the process of designing and optimizing bacteria-based systems (Cubillos-Ruiz et al., 2021). AI technology can facilitate faster experimentation and enhance system reliability by making it easier to predict gene circuitry dynamics, optimize metabolic processes, and choose the right chassis organism (Wang & Qi, 2019). This integration could potentially make more complex and versatile therapies possible.

Clinical trials and regulatory frameworks are of utmost importance for the incorporation of genetically modified bacteria in conventional clinical practices (Gulig et al., 2024). Although several genetically modified microbe-based therapeutic modalities have been introduced to early-stage clinical trials, many challenges need to be addressed, including challenges related to standardization, safety concerns, and regulatory issues (Lerner et al., 2026). The establishment of strict validation procedures, more control over the behavior of microbes, and formulation of regulatory guidelines to ensure proper utilization of these therapies are vital for addressing these challenges (Brewer et al., 2026).

#### 5. Conclusion

Through the combination of microbiology, genetic engineering, and synthetic biology to create treatment platforms that are programmable and dynamic, engineered bacteria represent an emerging field in the current treatment approaches. The major strategies for designing the platforms have been highlighted in this paper. The strategies include genetic engineering approaches, choice of chassis, control strategies, and biocontainment strategies, which make the platforms more practical and safer. Moreover, the broad range of applications for the platforms in various diseases such as cancers, infectious diseases, gastrointestinal diseases, and metabolic disorders underscores the vast potential of the modified microbial platforms.

In spite of all these promising developments, several hindrances stand in the way of their clinical application. Biosafety, genetic stability, controlled behavior of the bacteria, and regulatory approval remain considerable hurdles. The potential host immune responses and environmental implications require addressing to ensure proper and effective utilization. Nevertheless, all these challenges are increasingly being addressed due to further progress in the areas of synthetic biology, computational design, and the study of microbiomes. It is expected that engineered bacteria will shift from laboratory studies to clinical applications and become highly specialized means for treating disease conditions.

In conclusion, bacterial engineering is expected to revolutionize medicine in the future by substituting conventional treatment with smart medicine, which is alive and has the ability to respond to complicated scenarios caused by diseases in ways never seen before.

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Both authors confirm that:

Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. – **Yes / Not Applicable**√

All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged. – **Yes / Not Applicable**√

Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable. – **Yes / Not Applicable**√

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