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Engineering bacterial warriors: harnessing microbes to modulate animal physiology

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A central goal of synthetic biology is the reprogramming of living systems for predetermined biological functions. While many engineering efforts have been made in living systems, these innovations have been mainly employed with microorganisms or cell lines. The engineering of multicellular organisms including animals remains challenging owing to the complexity of these systems. In this context, microbes, with their intricate impact on animals, have opened new opportunities. Through the utilization of the symbiotic relationships between microbes and animals, researchers have effectively manipulated animals in various ways using engineered microbes. This focused approach has demonstrated its significance in scientific exploration and engineering with model animals, coral preservation and restoration, and advancements in human health.

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Introduction

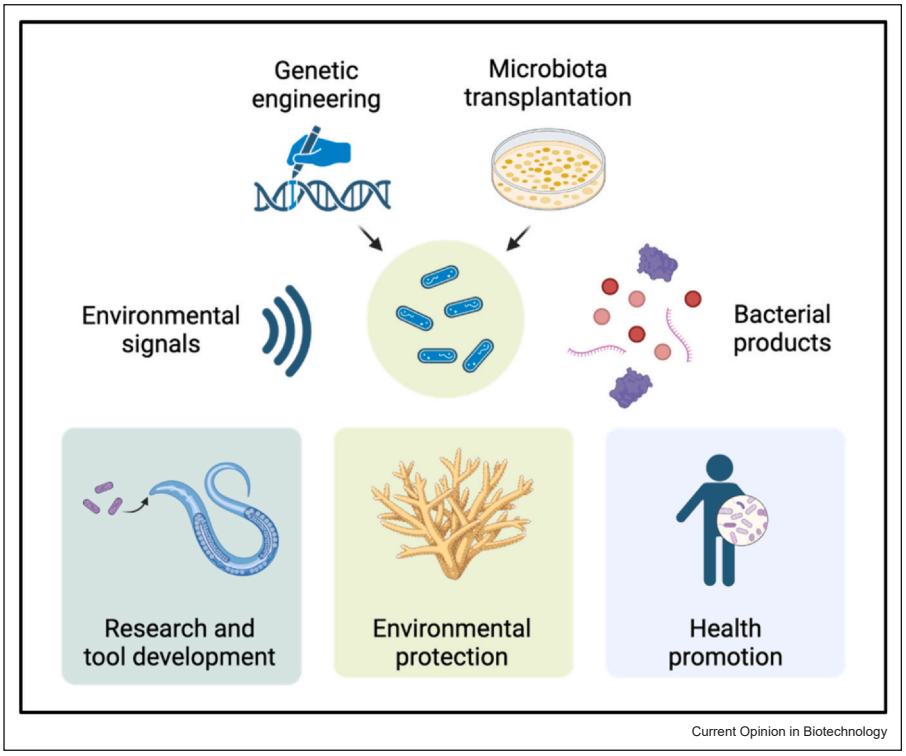
Recent advances in synthetic biology have enabled the creation of highly programmable biology for specific functions. Researchers integrated and automated the design of synthetic gene circuits in cells to respond to environmental signals [1–3]. Applying synthetic biology to multicellular organisms, such as animals, faces challenges such as coordinating gene delivery and expression across cell types, managing intercellular signaling complexity, contending with developmental and evolutionary constraints, and addressing ethical and safety concerns associated with genetic modifications.

In contrast to the complexities of modifying animals directly, microbes present a tractable platform for leveraging synthetic biology advances to alter animal physiology. Animals share intimate, lifelong relationships with microbes that impact animal immunity, behavior, development, metabolism, and more. Microbes interact with animals through a variety of mechanisms, including the exchange of metabolites, proteins, and RNA, all of which can be precisely tailored using synthetic biology methods [Figure 1]. This review will explore recent advances in using microbes for [1] reprogramming animal physiology using *Caenorhabditis elegans* as a model, [2] advancing environmental conservation with a focus on coral preservation, and [3] enhancing human health outcomes through disease diagnosis and therapeutics delivery. Using microbes to manipulate animal physiology offers new and potentially transformative ways to understand and control biological processes and to drive advancements in health, environmental sustainability, and therapeutic development.

Programming animal physiology using *Caenorhabditis elegans* as a model animal

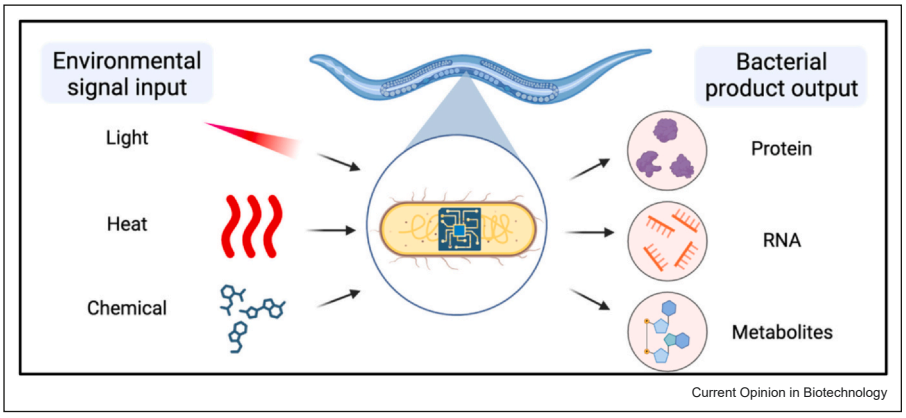
An area of particular significance in the realm of microbe–animal interactions is the potential of engineered microbes to revolutionize animal health and physiology [4–7]. Recent breakthroughs in engineered bacterial modulation of animal physiology highlight the use of *Caenorhabditis elegans*, a 1-mm-long soil nematode, as a key model organism. Chosen for its simplicity, quick life cycle, and easy maintenance, *C. elegans* is an ideal model to study how engineered bacteria influence animal health and development. WormBase offers a comprehensive repository of gene structures, mutants, RNAi phenotypes, gene expressions, and protein interactions on nematode biology [8]. Furthermore, *C. elegans* is particularly useful for studying bacteria–animal interactions as bacteria serve as its primary food source [9].

Figure 1



Engineered microbes can interact with and modulate animal physiology and behavior through multiple modalities.

Figure 2



Engineered microbes can sense environmental signals and modulate model animal *C. elegans* physiology and behavior.

Utilizing this relationship, researchers have engineered bacteria to serve as environmental sensors, impact host nutrient processing, and influence host physiology [Figure 2].

Engineered bacteria as biosensors mark a major advance in synthetic biology, providing new perspectives into environmental monitoring and biological interactions. This innovation has been applied to studies involving *C. elegans*, where researchers have utilized genetically modified bacteria to detect and respond to changes in the nematode's internal and external environments. For sensing *C. elegans* intestinal environment, a bacterium has been engineered to produce increasing levels of green fluorescent protein (GFP) in response to changing concentrations of isopropyl-β-d-1-thiogalactopyranoside

(IPTG) using a pLac promoter [10]. Although the bacterium only detected IPTG, an artificial signal, this approach could be used to detect other environmental changes. For example, using a heat switch *cI857*, Li et al. engineered a heat-sensitive *E. coli* that produced GFP in *C. elegans* gut to indicate temperature changes [11].

The symbiotic relationship between animals and their gut microbiota is crucial for nutrition, as bacterial metabolism and enzymes help unlock nutrients not readily accessible to the animal alone. As a demonstration of how an engineered gut microbiome can expand nutritional opportunities for animals, Sun et al. colonized *C. elegans* gut with cellulose-degrading microbe *Pseudomonas cellulosa*, enabling the nematodes to metabolize cellulose, a substance that was initially inaccessible to this species [3]. This showcases how changing the localized gut bacteria can extend the animal's nutritional capabilities. Furthermore, when *P. cellulosa* and *Lactobacillus plantarum* were combined in the presence of cellulose, they jointly reduced the proliferation of pathogen *Salmonella* by 10-fold compared with either of the strains. This demonstrates that engineered bacterial communities can change the host's nutrition and help fight pathogens [3].

Bacterially produced metabolites and RNA can also impact animal physiology. In *C. elegans*, ingestion of bacterially produced RNA can trigger specific and potent RNA interference that has been used previously to study gene functions [12,13]. Programmed to produce RNA under the control of synthetic gene circuits, engineered bacteria successfully silenced *C. elegans* genes to modulate its GFP expression, twitching behavior, and fat storage through feeding [14]. Importantly, the transfer of genetic circuit output from *E. coli* to *C. elegans* allows for the manipulation of *C. elegans* physiology through genetic logic gates, including 'AND' and 'OR' gates. Bacterial metabolites also influence *C. elegans* hosts in various ways, including development, lifespan, and behavior [4,15,16]. Using engineered *E. coli*, researchers were able to optogenetically control the production of colonic acid directly inside *C. elegans* gut [17]. Optogenetic production enabled researchers to study colonic acid's local effect on protecting intestinal mitochondria, extending the lifespan by preventing stress-induced hyperfragmentation [17].

Using *C. elegans* has shown engineered bacteria's capacity to sense environmental signals and alter host physiology. Beyond model organisms, the application of engineered bacteria holds promise for influencing host organisms, presenting opportunities for environmental sustainability and therapeutic innovations.

Improving coral health through microbiome engineering

Just as engineered bacteria have been used to expand the nutritional repertoire of *C. elegans* by enabling them to metabolize previously indigestible substances and mitigate environmental stresses, similar strategies are being explored to fortify coral health. The manipulation of the coral microbiome through microbial community transplantation and the introduction of beneficial bacteria aim to bolster coral resilience in the face of climate-induced stressors [Figure 3].

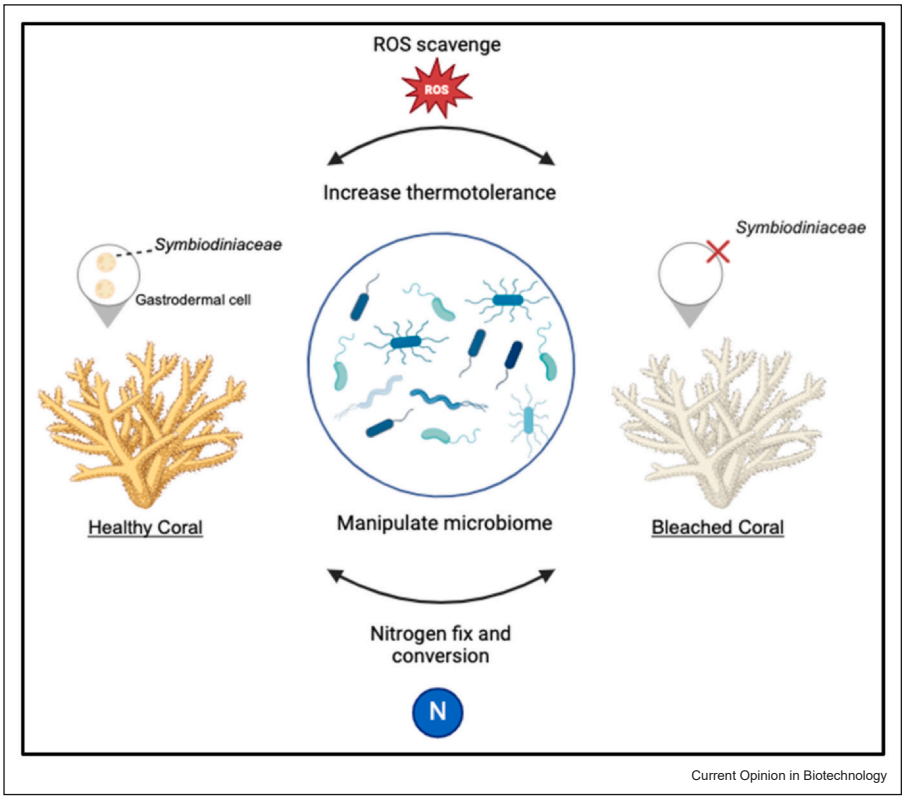
Coral reefs are vital for coastal protection, biodiversity, and socio-economic activities. Corals form a symbiotic relationship with Symbiodiniaceae algae, providing crucial nutrients through photosynthesis. Environmental stressors such as rising sea temperatures lead to coral bleaching, compromising their primary energy source [18]. Given the essential role of coral reefs, coral restoration and preservation has garnered substantial attention [19]. Other than Symbiodiniaceae, coral-associated bacteria play a multifaceted role in host organic and inorganic nutrient acquisition and protection from pathogens [20]. Microbiome engineering and microbial community transplantation have emerged as strategies to mitigate environmental stressors against corals [21–23].

Beneficial microorganisms enhance coral fitness through their symbiotic relationships [24,25]. Doering et al. conducted coral microbiome transfer (CMT) for coral preservation. They identified donor corals in reefs with high environmental variability. Fresh homogenates from coral donor tissues were used to inoculate heat-susceptible recipients. The bleaching response and microbiomes of recipients were documented using 16S rRNA gene metabarcoding, revealing the successful transmission of 128 donor-specific bacterial species [26]. During heat bleaching assays, the presence of beneficial microorganisms for corals reduced "post-heat stress disorder," leading to higher survival rates and stable photosynthesis from their endosymbiotic algae [24,26].

When environmental changes lead to increased levels of oxidative stress, reactive oxygen species (ROS) from Symbiodiniaceae are hypothesized to enter host cells, triggering coral bleaching [19]. To test the impact of an engineered microbiome scavenging ROS on coral heat tolerance, researchers inoculated the coral model *Exaiptasia diaphana* with ROS-scavenging bacteria [19,20]. They found a high relative abundance of free radical bacteria after inoculation, demonstrating successful incorporation of some species of bacteria into the coral holobiont. Further research on the prolonged integration of engineered bacterial consortia into the coral holobiont is necessary before deploying this strategy.

Excess nitrogen, often from agricultural runoff, is another stressor between corals and their endosymbiotic algae. A current hypothesis suggests that an increase of nitrogen is detrimental to coral reproduction and growth,

Figure 3



Engineered microbes have the capacity to assist corals in alleviating environmental stresses.

but if maintained at stable levels, it may be beneficial and necessary for specific coral species [27]. To assess nutrient exchange between bacteria and corals, coral larvae were incubated with ^{15}N-labeled coral-associated bacteria [21]. An increase in detected nitrogen isotopes within corals suggests coral larvae acquire nutrients from bacteria, potentially improving their survival in early-life stages [21]. Engineered bacteria or bacterial communities could be developed to assist coral larvae in critical stages such as settlement and polyp growth.

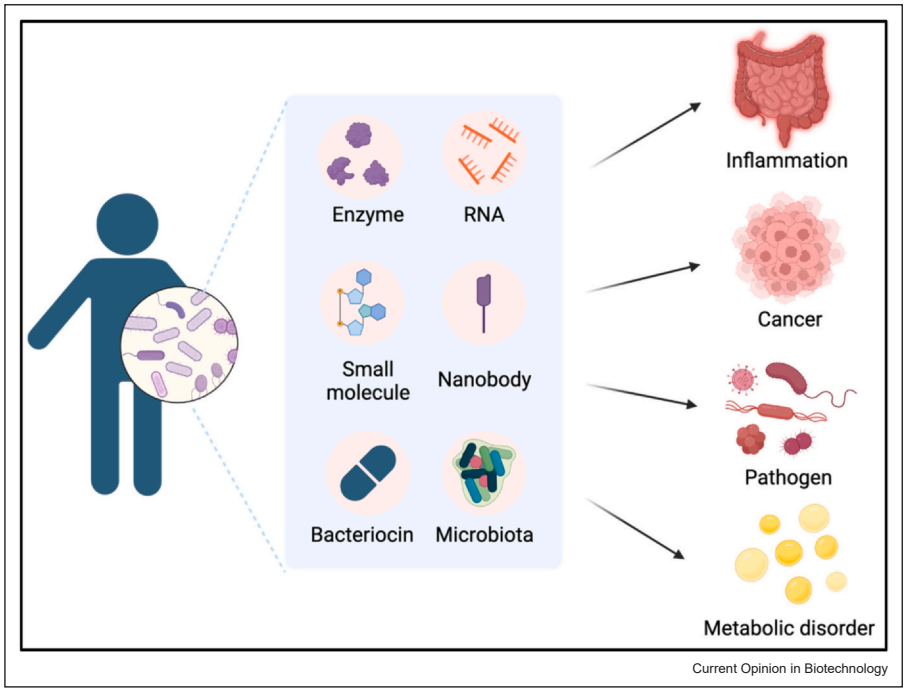
Fragoso et al. incubated fragments of *Mussismilia harttii* with a bacterial consortium capable of degrading water-soluble oil fractions [28]. The inoculated coral exhibited a reduction in the adverse effects of a simulated oil spill. This demonstrates that utilizing bacterial communities from the environment enables acquisition of enhanced survivability for host corals.

Microbial supplementation has the potential to address multiple stressors simultaneously and positively influencing the entire reef ecosystem [29], but deploying microbes at scale has inherent challenges that must be overcome. The intricate interactions between introduced and native microorganisms in coral reef ecosystems are difficult to predict, and interventions must be carefully designed to avoid harm to these sensitive organisms. Coral reefs can occupy large areas of the ocean floor, thus, strategies to physically deploy microbes must be developed. Currents and tides could be used strategically to help distribute microbes across the reef. Ultimately, it may be infeasible to deploy interventions across a whole reef, but a strategy of targeted deployment could be used to save distributed coral colonies that can then serve as founders to reseed the reef after a bleaching event. Despite the challenges, exploring innovative approaches is crucial for developing sustainable methods to improve coral reef health. Synthetic biology plays a significant role in leveraging microbial communities to amplify positive interactions and mitigate the impact of harmful elements, including pathogenic microorganisms among coral reefs.

Enhancing human health outcomes through disease diagnosis and therapeutics delivery

Beyond programming the model animal *C. elegans* and improving coral health, bacterial product modalities such as RNAs and proteins, as well as microbiota transplantation have enabled engineered bacteria to offer therapeutic benefits to a range of human health conditions. There are

Figure 4



Engineered microbes can provide therapeutic benefits through multiple bacterial product modalities.

numerous comprehensive reviews in this area [30–33], and here we highlight some of the representative studies and recent advancements using different modalities for various conditions, including inflammatory diseases, cancer, infections, and metabolic disorders [Figure 4].

In the context of inflammatory bowel disease (IBD), bacteria have been engineered to detect inflammation signals, including tetrathionate and thiosulfate [34–37]. Engineering lactic acid bacteria (LAB) and nonpathogenic *E. coli* to produce cytokines such as IL-10 and IL-35 has shown promise in modulating inflammation as indicated by up to 50% reduction in disease activity index (DAI) [38–40]. Additionally, LAB and engineered *E. coli*-producing nanobodies and short-hairpin RNA have been effective in reducing inflammation markers such as TNF-α and COX-2 in murine models and reducing DAI by up to sixfold [11,41–43]. An alternative method is to engineer bacteria to produce antioxidant enzymes, providing a long-lasting therapeutic impact [44,45].

In cancer therapy, engineered bacteria have been used to deliver cytokines and nanobodies targeting immune checkpoints leading to tumor regression [46–49]. Bacteria have also been engineered to act as vaccines, producing tumor-associated antigens to stimulate adaptive immune responses. For example, engineered *Lactobacillus casei* producing modified full-length HPV16 E7 protein reduced cervical intraepithelial neoplasia grade 3 (CIN3) to CIN2 in 70% of patients. Similarly, engineered *E. coli*-producing antigen-bearing outer-membrane vesicles (OMVs) decreased lung metastases from over 150 to less than 50 in animals during tumor rechallenge [46,50]. Additional approaches involve engineering bacteria to produce prodrug enzymes. For instance, *Salmonella typhimurium* and *Bifidobacterium longum* can convert nontoxic prodrugs such as 5-fluorocytosine into the active antitumor agent 5-fluorouracil, inhibiting tumor growth effectively [51,52].

A modified lactic acid-free *Streptococcus mutans* strain prevented dental caries by replacing traditional acid-producing mutants crucial to the pathogenic process [53]. Engineered strains of *L. lactis* and *E. coli* with bacteriocin secretion inhibited the growth of pathogens such as *Enterococcus faecalis* and *Pseudomonas aeruginosa* [54,55]. Additionally, biofilms composed of inactivated *Lactobacillus casei* showed significant antibacterial effectiveness against methicillin-resistant *Staphylococcus aureus* [56]. Besides engineering specific bacterial strains, RBX2660 (Rebyota) and SER-109 (Vowst) — both fecal microbiota-based live biotherapeutic products — have demonstrated efficacy in preventing the recurrence of *Clostridioides difficile* infection, and received approval from the Food and Drug Administration (FDA) in 2022 and 2023, respectively [57,58].

In treating metabolic disorders, engineered bacteria equipped with optimized enzymes can help digest specific metabolic by-products that are toxic when accumulated at high levels. One of the most exciting advancements for engineered bacteria is the development of SYNB1934, an engineered *E. coli* Nissle 1917 producing phenylalanine ammonia lyase with enhanced activity [59]. This engineered strain breaks down phenylalanine, a compound that patients with phenylketonuria (PKU) cannot metabolize naturally [59]. In a Phase-2 clinical trial, PKU patients receiving SYNB1934 treatment exhibited a substantial 43% reduction in phenylalanine levels from baseline, paving the way for SYNB1934 to advance into a Phase-3 clinical trial in 2023 [59].

In summary, these engineered bacteria not only offer novel treatments for chronic conditions such as IBD and cancer but also provide a potent weapon against drug-resistant pathogens and many other diseases. By integrating synthetic biology tools with natural bacterial metabolic pathways, researchers can target specific disease markers.

Discussion

Engineered microbes exhibit versatile capabilities, impacting host organisms in diverse ways. In model organisms such as *C. elegans*, engineered bacteria can sense and respond to signals, delivering biomolecules for reporting or manipulation. Beyond model organisms, engineered microbiomes have demonstrated significant promise in environmental applications. For example, they help corals adapt to changing environments and neutralize harmful stressors. Finally, bacteria have been engineered for therapeutic applications, detecting disease markers, and delivering targeted therapies to enhance efficacy without undesired side effects.

While engineered bacteria have demonstrated diverse functions across a range of applications, their effectiveness varies significantly outside the controlled conditions of a laboratory, influenced by the unique characteristics of individual hosts and fluctuating environments. Dynamic control mechanisms that enable engineered bacteria to consistently localize and function within various hosts are worth exploring [60]. Such advancements would ensure more uniform and predictable outcomes across different individual hosts. The use of animal models such as *C. elegans*, offers a rapid and high-throughput method for testing these dynamic controls, serving as a crucial step before transitioning to broader environmental and health-related applications.

Additionally, safety concerns also require careful consideration, including the biocontainment of engineered bacteria, the potential for unforeseen repercussions on host ecosystems, and the emergence of resistant strains. To address these concerns, new artificial systems to contain synthetically engineered microbes, such as a kill switch developed in *E. coli* Nissle 1917 [61], are currently being explored to aid in the safety concerns proposed by FDA.

Synthetic biology's influence spans a wide range of disciplines, underscored by its essential role in various applications of biotechnology. By fostering creativity and integrating interdisciplinary insights, synthetic biology stands to drive significant advancements in our understanding and manipulation of biological systems.

CRediT authorship contribution statement

Q.S. and R.J. did the conceptualization. B.G. and D.R. drafted the review paper. Q.S. and R.J. supervised the project and revised the review paper. H.C. helped with reference collection. Q.S. and R.J. did the funding acquisition.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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