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The Double-Edged Sword of Bacterial Nitric Oxide: From Fundamental Conflict to Engineered Therapeutics

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Abstract

Nitric oxide (NO) is a multifaceted signaling molecule with important roles in both prokaryotic and eukaryotic systems. In bacteria, NO is produced endogenously through several biochemical pathways, including the enzymatic activity of bacterial NO synthases (bNOS) and the respiratory denitrification enzymes known as nitrite reductases (NirK and NirS). These systems contribute to bacterial energy metabolism, stress responses, and intercellular signaling. Depending on its concentration, source, and biological context, bacterial NO may promote biofilm formation and antibiotic tolerance, whereas host-derived NO can contribute to bacterial clearance. Major bacterial genera reported to produce NO include *Bacillus subtilis*, *Deinococcus radiodurans*, *Geobacillus stearothermophilus*, *Lactobacillus fermentum*, and *Staphylococcus aureus*, as well as *Neisseria*, *Haemophilus*, *Veillonella*, *Granulicatella*, and *Prevotella* spp. Recent advances in molecular biology and synthetic biology have greatly expanded the repertoire of available approaches for engineering bacteria to produce NO at high or controllable biological levels. For example, *Escherichia coli* Nissle 1917 and *Lactobacillus* species have been engineered to express inducible NOS enzymes under tightly regulated promoters. Engineered therapeutics, including selective bNOS inhibitors, nanoparticle-delivered NO donors, and NO-antibiotic synergistic regimens, may harness this duality for therapeutic benefit. To exploit concentration-dependent effects that trigger biofilm dispersal at nanomolar levels followed by bactericidal bursts, next-generation NO-based therapies will need to achieve precise spatial and temporal control. Key areas of progress include decoding how bacteria detect NO from both host and self, engineering smart biomaterials that release NO in response to the infection microenvironment, and developing bNOS inhibitors that selectively target bacterial isoforms. In addition, modulation of NO in conjunction with other gasotransmitter systems, such as H_2S and CO, together with NO-sensing probiotic therapeutics and synthetic biology approaches, may offer promising strategies for improving NO production and therapeutic application.

Keywords: Nitric oxide, Applications, Bacteria, Engineering.

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Introduction

Nitric oxide (NO) is a gaseous signaling molecule produced in the human body through both enzymatic and non-enzymatic mechanisms. The principal enzymatic pathway for NO production involves NO synthases (NOS), a family of enzymes that catalyze the oxidation of the amino acid L-arginine to generate NO and L-citrulline. Three NOS isoforms have been identified and characterized: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (1, 2). eNOS and nNOS are constitutively expressed and activated in a

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calcium/calmodulin-dependent manner. eNOS, which is located primarily in endothelial cells lining the vasculature, generates NO to perform several essential functions, including maintaining vascular tone and fluidity through smooth muscle relaxation, thereby promoting vasodilation and regulating blood flow and pressure. nNOS is localized mainly in nerve terminals and plays an important role in neurotransmission and neuromodulatory signaling. In contrast, iNOS is expressed following immune activation, particularly in immune cells such as macrophages, and produces larger amounts of NO that contribute to cytotoxic defense against pathogens and regulation of inflammation (3).

In addition to enzymatic production, NO can also be formed non-enzymatically from dietary nitrate and nitrite ingested through food and water. This nitrate-nitrite-NO pathway is particularly important under hypoxic and/or acidic conditions, when NOS activity may be impaired. Dietary nitrates are first reduced to nitrites by beneficial bacteria in the oral cavity and are then converted to NO after systemic absorption (4, 5).

Beneficial bacteria in the mouth and gut are responsible for NO production through the nitrate reduction pathway. In the human oral cavity, particularly on the dorsal surface of the tongue, nitrate-reducing bacteria such as *Neisseria*, *Veillonella*, and *Actinomyces* species convert dietary nitrate to nitrite, which is then swallowed. Thus, the oral microbiome, together with its capacity to exploit the mild oxidative chemistry of nitrate reduction to NO, represents an important source of systemic NO homeostasis and, likely, blood pressure regulation, especially when endogenous NOS-derived NO is limited (6-8).

In microorganisms, NO is an important intermediate produced during the stepwise reduction of nitrate (NO_3^-) to dinitrogen (N_2). Enzymes involved in these redox processes include nitric oxide reductases (NOR), periplasmic nitrate reductase (Nap), respiratory nitrate reductase (Nar), and nitrite reductases (NIR), all of which support microbial respiration in low-O_2 environments (8, 9). NO-handling pathways differ across taxa, indicating substantial evolutionary divergence. For instance, denitrifying bacteria such as *Paracoccus denitrificans* and *Pseudomonas stutzeri* use NO as an electron acceptor, whereas nitrifying archaea such as *Nitrosococcus oceani* transiently produce NO during ammonia oxidation as a by-product. Some obligate anaerobes employ NO-detoxifying systems, such as flavorubredoxins and flavohemoglobins, to mitigate nitrosative stress. This use of NO as a redox intermediate, together with its potent cytotoxicity and the need for strict regulation, highlights its distinctive biological role (2, 8, 9).

Soil microorganisms, marine bacteria, methanotrophs, and gut commensals possess distinct pathways and regulatory mechanisms that reflect the ecological niches they occupy (10). For example, in marine oxygen minimum zones, denitrifying bacteria and anammox bacteria operate together metabolically to regulate nitrogen loss, thereby influencing global nitrogen budgets. In terrestrial rhizospheres, microbial consortia modulate plant-associated activities in response to plant exudates by regulating NO production, which in turn influences nutrient fluxes and plant immune responses. At the genomic level, comparative metagenomics reveals remarkable diversity in genes associated with NO production and regulation. Environmental DNA analyses show a high representation of NOR genes and flavohemoglobin genes not only in classical denitrifiers but also in unexpected taxonomic groups, including cyanobacteria and actinobacterial communities (11).

From a clinical perspective, bNOS pathways affect host physiology and disease progression. Human gut commensal bacteria can produce

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NO from dietary nitrates, thereby enhancing blood flow to the mucosa and maintaining epithelial barrier function. Conversely, pathogenic bacteria may use NO detoxification systems to evade host immune defenses, particularly because macrophages rely on reactive nitrogen species to eliminate invading organisms. A better understanding of microbial NO utilization has therefore helped direct antimicrobial research toward the identification of therapeutic targets within bacterial NO-resistance pathways (12).

Although NO is widely recognized for its importance in mammalian immunity, the discovery of bNOS has fundamentally reshaped this view. NO is not only an antimicrobial molecule produced by the host, but also a global signaling molecule exploited by many bacteria, including species that use NO for respiration, stress adaptation, and biofilm development (13). This dual nature creates an important biological paradox. On the one hand, endogenously generated NO promotes bacterial growth during oxidative stress and antibiotic exposure; on the other hand, NO also increases bacterial susceptibility to host immune attack, thereby creating an evolutionary dilemma (14, 15). In addition, pathogens are known to exploit host-derived NO as an environmental signal to activate virulence factors within macrophages. Accordingly, NO is now regarded as a communicative molecule in host-microbe interactions rather than solely as an antibacterial agent (14). At low concentrations, NO has little antibacterial activity and may even enhance virulence (Figure 1). In this article, we integrate recent advances in understanding the mechanisms underlying these opposing effects of NO on bacteria and critically examine how harnessing this duality may facilitate the development of the next generation of antimicrobial therapies. Examples of engineered therapies include: (1) nanomaterials designed to release NO in a temporally and spatially controlled manner; and (2) bNOS inhibitors designed to selectively suppress NO production in bacteria. Ultimately, these engineered strategies seek to harness, rather than oppose, bacterial NO biology in the fight against antimicrobial resistance.

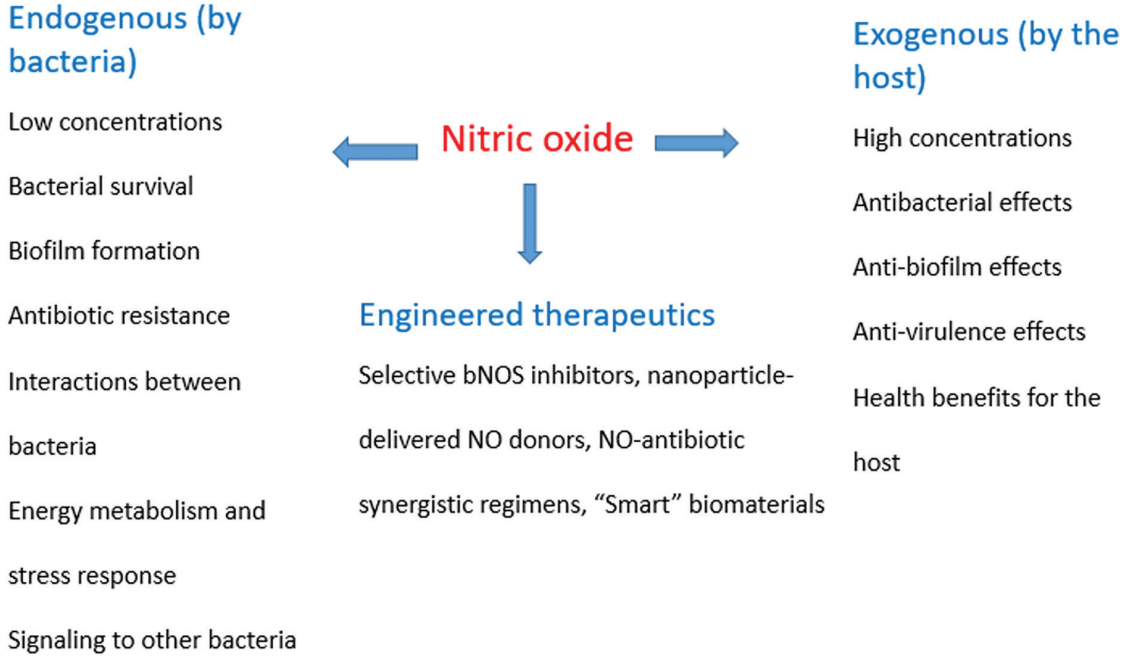

Figure 1. The effects of nitric oxide on bacteria and host populations.

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Material and Methods

The search strategy for this study was based on the keywords "nitric oxide," "bacteria," "virulence," "antibiotic resistance," and "production." The search was conducted in Google Scholar, Scopus, and PubMed. No time restrictions were applied to the search results.

Nitric oxide synthesis and health benefits

In most cases, NO is synthesized in the endothelial lining of blood vessels by eNOS. From there, NO diffuses over short distances from endothelial cells to adjacent smooth muscle cells. In smooth muscle cells, NO activates guanylyl cyclase, which in turn increases cyclic GMP concentrations (16). Upon immune activation in the context of pathogen defense, the immune system produces large amounts of NO via iNOS. NO produced by activated macrophages and other immune cells exerts antimicrobial and antiviral effects, directly targeting pathogens while also shaping the inflammatory response. In addition to its role in host defense against pathogens, NO appears to exert dual effects in inflammation by regulating cytokine production and mediating tissue repair. Moreover, NO regulates gastrointestinal motility, mucus secretion, and sphincter relaxation, thereby facilitating digestion and nutrient absorption (17). It also contributes to respiratory function by mediating bronchodilation and airflow control. At the cellular level, NO influences apoptosis, angiogenesis (neovascularization, or the formation of new blood vessels), and cell-cycle progression, all of which may be important in tissue regeneration, wound healing, and cancer biology. Notably, depending on its concentration and the surrounding microenvironment, NO may either promote or inhibit tumor development (18). From a metabolic perspective, NO interacts with mitochondrial function and affects respiration, reactive oxygen species production, and cellular energy balance. This action supports improved muscle contractility and endurance during exercise. Reduced NO levels or impaired NO bioavailability have been implicated in several disease states, including hypertension, atherosclerosis, insulin resistance, erectile dysfunction, and chronic inflammation. The widespread use of nitrates for angina and phosphodiesterase (PDE) inhibitors for erectile dysfunction underscores the pharmacological importance of NO in clinical practice (2, 19).

Bacterial production of nitric oxide

Bacterial production of NO occurs through several biochemical pathways and plays important ecological and physiological roles. Many bacteria produce NO either as an intermediate or as an end product of nitrogen metabolism. Both bNOS and respiratory denitrification pathways, represented by nitrite reductases, participate in microbial NO biosynthesis. For example, *Bacillus subtilis*, *Deinococcus radiodurans*, *Geobacillus stearothermophilus*, *L. fermentum*, and *Staphylococcus aureus* produce NO enzymatically via bNOS, which oxidizes L-arginine to citrulline and NO. This enzyme is functionally similar to mammalian NOS, although it is structurally much less complex (7, 9, 20). Furthermore, many bacteria utilize respiratory denitrification pathways in addition to bNOS. These pathways involve nitrite (NO_2^-) reductases, NirK and NirS, which reduce NO_2^- to NO as part of anaerobic respiration. Bacteria that express nitrite reductase include, but are not limited to, *Pseudomonas aeruginosa*, *P. denitrificans*, and *Phaeobacter inhibens* (11, 21, 22).

Oral and gut commensal bacteria, such as *Neisseria*, *Haemophilus*, *Veillonella*, *Granulicatella*, and *Prevotella*, convert dietary NO_3^- into NO_2^-, which can subsequently generate NO and thereby increase systemic NO bioavailability. Microbial nitrate reduction complements and augments endogenous NO production, particularly under low-O_2 conditions. The oral cavity, especially the surface of the tongue, is a critical site for this

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conversion. Although only limited studies have examined intestinal microbiota in this context, the gut microbiome may possess metabolic pathways similar to those of the oral microbiome and may contribute to digestive motility and mucosal immunity through NO signaling. NO production is also important for microbial physiology, including biofilm formation, environmental stress tolerance, and interspecies communication. Pathogenic bacteria that produce NO in the gut may influence host immune responses, which could be relevant to infection outcomes (Table 1).

NirK, NirS, and bacterial bNOS are essential enzymes in nitrogen metabolism, yet each has distinct structural and functional characteristics. NirK and NirS reduce NO_2^- to NO during denitrification, but they differ structurally and use different cofactors (8, 20). NirK is a copper-containing enzyme and belongs to the cupredoxin family. It has a homotrimeric structure, with each monomer containing a copper site coordinated by several conserved histidine residues. NirK facilitates electron transfer directly from a donor such as cytochrome c. The geometry of its active site allows efficient nitrite reduction under low-oxygen conditions, with electrons delivered to the copper centers and rapidly cycled through redox reactions during catalysis. NirS is a cytochrome cd1 nitrite reductase with a multidomain structure containing both heme c and flavin mononucleotide (FMN) cofactors. NirS reduces nitrite through a more complex electron-transfer chain, receiving electrons from donors such as quinols, passing them through the FMN domain to heme c, and ultimately reducing nitrite. Structurally, NirS is a homodimer, and each monomer contains heme and FMN domains that facilitate electron coupling and more rapid electron transfer. A further feature of NirS is its bimetallic active site, which includes both heme and a tetrahedrally coordinated center and serves as a robust electron-transfer site within the enzyme (23).

By contrast, bNOS, a homolog of mammalian NOS, catalyzes the generation of NO from L-arginine by employing tetrahydrobiopterin (BH4), flavin adenine dinucleotide (FAD), FMN, and a heme prosthetic group. Its monomeric, multidomain architecture facilitates substrate binding and electron transfer. The active site of the enzyme is analogous to that of mammalian NOS, with a heme pocket that coordinates L-arginine and O_2 and generates NO through a multistep oxidation mechanism. Unlike NirK and NirS, bNOS does not catalyze nitrite reduction; rather, it generates NO directly from

Table 1. Bacterial NO production mechanisms.

Nitric Oxide Synthetic Pathway	Primary Enzyme (s)	Bacterial Examples	Mechanism Summary	Physiological/ Environmental Role
Arginine oxidation via bNOS	Bacterial nitric oxide synthase	*B. subtilis, D. radiodurans, S. aureus*	Oxidation of L-arginine to NO and citrulline	Cellular signaling, oxidative stress resistance
Denitrification	NirK/ NirS nitrite reductases	*P. aeruginosa, Paracoccus denitrificans*	Reduction of nitrite (NO3-) to NO under anaerobic conditions	Anaerobic respiration, nitrogen cycle
Nitrate reduction (nitrate reductases Nar/Nap)	NarGHI, NapAB complexes	*Phaeobacter inhibens, Neisseriaspp.*	Reduction of nitrate (NO3-) to nitrite (NO2-), precursor to NO	Nitrate respiration, nitrate-nitrite-NO pathway
Non-respiratory nitric oxide production	bNOS	*L. fermentum, Streptomyces*	Direct enzymatic NO synthesis independent of respiration	Interaction with eukaryotic hosts, microbial communication
Anammox-related NO metabolism	Anammox enzymes	*Anammox bacteria*	Coupling ammonium oxidation to NO reduction	Global nitrogen cycle, greenhouse gas modulation

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an amino acid substrate, with electron flow through the FAD and FMN domains derived from NADPH. These structural differences underlie their functional divergence. NirK and NirS act in concert to support nitrite reduction in anaerobic environments, with NirK relying on copper chemistry and NirS using heme and c-type cytochromes, whereas bNOS mediates NO biosynthesis during oxidative stress or signaling processes through a heme-based mechanism that resembles mammalian NOS but is adapted to bacterial physiology. Overall, this comparison shows that NirK's copper-based active site enables rapid electron transfer to support denitrification, NirS's multidomain heme and FMN architecture directs controlled electron flow toward nitrite reduction, and bNOS relies on a heme-based domain (24) temsAsXml. Bacteria have been used and modified to produce NO in a variety of emerging technologies, either directly through their native enzymatic machinery or through engineered pathways designed for controlled NO production. One notable approach is the use of bNOS, an enzyme that is structurally related to mammalian NOS but simpler. For example, *B. subtilis* and *Paenibacillus nitricinens* sp. nov. naturally produce NO via bNOS. These enzymes oxidize L-arginine to generate NO and recruit reductase partners within the bacterial cell to support enzymatic activity. Researchers have also cloned bNOS genes into *E. coli* to create recombinant bacterial platforms with enhanced NO production for research and potential therapeutic applications (25).

Another technological strategy exploits bacterial denitrification pathways, which are widely distributed in species such as *P. aeruginosa* and *P. denitrificans*. These bacteria enzymatically reduce NO_3^- to NO_2^- and then to NO via NirK and NirS. Researchers have engineered bacteria with increased nitrate reductase activity to convert dietary nitrate efficiently into NO in vivo (26, 27). This microbial nitrate-nitrite-NO pathway has been harnessed in probiotic formulations and biohybrid devices to stimulate endogenous NO generation in human tissues and support therapeutic applications in tumors, cardiovascular disorders, and wound healing (28-30).

For example, CRISPR-Cas systems have enabled engineers to create bacteria that produce NO in response to specific environmental cues, thereby allowing spatiotemporal control over NO synthesis (31). These so-called smart bacteria may ultimately facilitate the delivery of nitric oxide in medicine under conditions in which a defined NO response is desirable, such as site-specific cancer therapy or the application of antimicrobial agents in which the signaling and antimicrobial effects of NO are advantageous. Related work has also examined bacteria associated with anaerobic ammonia oxidation (anammox) and the nitrogen-cycle processes of bacteria that can indirectly produce or consume NO in environmental biotechnology. For instance, investigations into how anammox bacteria produce and consume NO for environmental applications are already underway, including wastewater treatment. Engineering microbial consortia in this way may help optimize bacterial regulation of NO levels, reduce pollution, and support bioremediation (Table 2).

The challenges associated with introducing nitric oxide as an antibacterial agent into clinical studies

The introduction of NO as an antibacterial agent into clinical studies is constrained by several challenges, particularly those related to delivery and the controlled release of NO (32). Because NO is highly reactive, maintaining therapeutic concentrations at the site of infection is difficult. As a result, a range of delivery platforms, including nanoparticles, hydrogels, and surface coatings, have been developed to provide sustained and localized NO release; however, their release profiles are often inconsistent (33).

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Table 2. Bacterial species and mechanisms of NO production.

Technology Type	Bacterial Source Examples	Mechanism/Pathway	Application/Use Case
Native bNOS expression	*B. subtilis, B. anthracis*	L-arginine oxidation by bNOS	Continuous physiological NO generation, research
Recombinant bNOS systems	*E. coli* (engineered)	Cloned bNOS genes hijacking host reductases	Controlled NO production for therapeutic research
Denitrification pathway exploitation	*P. aeruginosa, Paracoccus denitrificans*	Nitrate into Nitrite into NO via NirK/NirS	Probiotic enhancement of systemic NO for cardiovascular health
Synthetic biology circuits	Various engineered strains	Environmentally triggered NO synthesis via CRISPR-Cas	Precision NO delivery for targeted therapy
Anammox and nitrogen cycle bacteria	Anammox bacteria	NO as intermediate in ammonium oxidation	Environmental biotechnology, wastewater treatment

Consequently, some of these approaches may reduce antibacterial efficacy or increase the risk of off-target cytotoxicity in surrounding tissues (34). In addition, the clinical translation of agents intended for the treatment of bacterial infections raises important safety and toxicity concerns. Although low concentrations of NO are often beneficial to the body, high doses may be cytotoxic and can induce inflammation or damage normal cells (35, 36).

Widespread acceptance of NO-based antibacterial agents (NOBAs) is also limited by economic and practical barriers. The development of NO delivery systems requires precise control over multiple parameters, including manufacturability and affordability for use in hospital or ambulatory settings (37, 38). Moreover, physicians may lack adequate familiarity with NOBAs, which may discourage their clinical use; this reluctance is likely to be greater when such therapies are not already established within routine practice. These barriers must be addressed to ensure the successful integration of NO-based applications into clinical settings as safe and effective antibacterial strategies. This will require the development of newer delivery technologies capable of releasing NO safely and effectively, as well as large-scale clinical studies to support its use as a dependable antibacterial agent.

In pathogenic bacterial species such as *P. aeruginosa* and *S. aureus*, bNO production appears to be linked to oxidative stress responses and immune evasion; however, the regulatory mechanisms involved, whether they are triggered by environmental factors such as oxygen levels, by NO itself, or by other signaling pathways, remain unclear. Considerable uncertainty also persists regarding the precise role of NO as a signaling molecule or a defensive metabolite. While some studies suggest that NO may contribute to biofilm formation and antibiotic resistance, others indicate that it primarily functions as a transient detoxification compound synthesized by NO reductases (NORs) or other reductive fermentative enzymes.

Furthermore, these reductive enzymes are subject to complex transcriptional and post-translational regulation. The regulators involved are often global, such as Nar, NsrR, and FNR-like proteins, and they respond to redox state or gasotransmitters; however, relatively little is known about their specific actions or the ways in which they integrate into broader metabolic networks. In addition, bacterial respiration adds another layer of regulation, because NO may function as a signaling molecule or, alternatively, inhibit terminal oxidases. This raises further questions about how bacteria reconcile these competing effects under fluctuating and unstable environmental

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conditions. Additional uncertainty concerns the presence, activity, and regulation of NO-scavenging and detoxification systems, such as flavohemoglobins, which appear to vary across taxa and species. This variability further complicates our understanding of how bacteria cope with NO toxicity while also benefiting from NO-mediated signaling.

Moreover, the discovery of novel non-enzymatic pathways, together with the possible production of NO through nitrite reduction under anoxic conditions, will broaden the scope of future research and may challenge the traditional enzyme-centric view of NO production. As research progresses, several fundamental questions remain to be answered: What signals and sensors regulate NO production? Which regulatory circuits determine species-specific patterns of NO regulation? And what evolutionary forces shaped these regulators? Clarifying the role of bacteria in both regulated and non-regulated NO production will continue to require interdisciplinary research spanning molecular biology, ecology, and evolutionary biology. Although substantial progress has been made, we are still only beginning to understand the full complexity of bacterial NO biology.

Innovative strategies to engineer bacterial systems for targeted nitric oxide delivery

State-of-the-art strategies for genetically engineering bacterial systems to deliver NO at specific sites of action constitute a rapidly expanding area of synthetic biology. This field is increasingly combining the precision of molecular engineering with the therapeutic versatility of NO (4). Because the biological effects of NO range from antimicrobial activity to vasodilation and immune modulation, bacteria must be engineered to release NO in a controlled manner and at the appropriate target sites, thereby maximizing therapeutic benefit while minimizing systemic toxicity, which is of paramount importance.

One of the key strategies in this area is the genetic modification of commensal or probiotic bacteria, such as *E. coli* Nissle 1917 or *Lactobacillus* species, to express iNOS enzymes under tightly regulated promoters. This inducible bacterial NOS expression system can be controlled by environmental factors, including pH, oxygen tension, quorum-sensing molecules, or disease-associated biomarkers, thereby enabling precise NO delivery to specific microenvironments, such as hypoxic tumor tissue, inflamed gut regions, or infected wounds.

Another promising direction involves synthetic gene circuits that function as logical gates, including AND, OR, and NOT gates, allowing bacteria to integrate multiple environmental signals before initiating NO production (17). Such systems can enhance specificity and safety by reducing off-target effects. In addition, advances in CRISPR-based gene editing and RNA-based regulatory systems are expected to permit more precise control over NOS gene expression and responsiveness.

Researchers are also developing bacterial chassis capable of carrying and releasing NO precursors, such as nitrate or nitrite, and coupling these systems with heterologous expression of NirK or NirS to facilitate local enzymatic conversion to NO (39). In some designs, encapsulated or biofilm-forming bacteria are being evaluated for their ability to colonize target tissues and sustain NO release within microenvironments such as solid tumors or gastrointestinal lesions. Biomaterials offer another layer of innovation; for example, bacteria may be embedded in hydrogels, microneedles, or wearable patches that serve as smart delivery platforms capable of releasing NO in response to external stimuli such as light, heat, or ultrasound.

Synthetic biology has also enabled the development of kill switches and biocontainment systems, which are essential for ensuring that engineered bacteria do not persist in, or

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spread to, non-target environments, thereby addressing important biosafety concerns. Many of these systems also incorporate biosensing functions that enable real-time monitoring of NO release and environmental conditions, such as nutrient levels and pH, through fluorescent or electrochemical signals (40, 41). Together, controlled NO synthesis, environmental sensing, and therapeutic functionality make engineered bacterial systems a powerful platform for precision medicine. These strains are currently being investigated in chronic wound care, biofilm clearance, cardiovascular disease, and cancer. With a deeper understanding of bacterial physiology and NO signaling, together with increasingly sophisticated genetic tools, the field appears poised to deliver programmable microbial therapeutics with high spatiotemporal resolution. Beyond their contribution to targeted NO therapy, these developments may also serve as a model for broader efforts to exploit engineered microbes for the in situ delivery of other gasotransmitters or small-molecule therapeutics.

Future prospects for bacterial nitric oxide production

The outlook for bacterial NO production is highly promising and multidimensional, with important implications for medicine, environmental science, and biotechnology. Our understanding of bacterial pathways for NO production, including bNOS and denitrification enzymes, has advanced substantially and now provides a foundation for the rational engineering of microbes capable of producing NO in a controlled and precise manner. This has clear implications for exploiting bacterial NO gasotransmitter functions in therapies involving vasodilation, immunomodulation, and antimicrobial activity, with potential in situ applications in cardiovascular disease, chronic wounds, infections, and cancer. In addition, recent advances in synthetic biology, particularly CRISPR-based gene editing, make it possible to modulate bacterial NO biosynthesis in response to environmental or pathological cues, thereby enhancing safety and control in therapeutic delivery.

In environmental biotechnology, microorganisms with NO metabolism can be incorporated into sustainable wastewater treatment systems to reduce greenhouse gas emissions by metabolizing harmful intermediates, such as nitrous oxide, into harmless nitrogen gas (N_2), thereby helping to mitigate climate change. Moreover, the growing body of knowledge on bacterial enzymes involved in NO metabolism across diverse microbial taxa opens the possibility of exploring ecological and biogeochemical functions that have not yet been fully realized but may prove valuable for ecosystem management.

From a commercial perspective, probiotic formulations composed of nitrate-reducing bacteria can be optimized to promote endogenous NO production and thereby support cardiovascular health and the management of metabolic disorders. Modulating the oral and gut microbiome to enhance nitrate-nitrite-NO pathways is an emerging field with potential applications in both prevention and treatment. Future research will likely focus on: (1) structural and kinetic studies of bacterial NO-synthesizing enzymes to improve biological design; (2) the development of engineered bacterial strains with greater NO output and enhanced safety; (3) the integration of NO-producing bacteria with implantable or wearable medical devices for targeted NO delivery; and (4) rigorous clinical trials to evaluate the efficacy and safety of bacteria-based NO therapies.

The future of bacterial NO research will depend on resolving the conflicting roles of NO as both a cytotoxic effector of the host immune system and an essential bacterial signaling molecule. In this context, single-cell technologies will provide new insight into the spatial and temporal

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dynamics of bacterial NO production within polymicrobial communities, including resident bacteria and opportunistic pathogens such as *Pseudomonas* and *Mycobacterium*. These methods will also help identify how opportunistic pathogens exploit NO gradients to transition between virulence and dormancy, mechanisms that are critical for persistent chronic infection. By using CRISPR-based NO biosensors, real-time mapping of bacterial NO production can be achieved during host colonization, allowing precise intervention points to be identified for the disruption of chronic infections.

From a therapeutic standpoint, engineered NO delivery platforms will continue to evolve beyond conventional donor molecules. For example, NO-targeted nanoscale carriers that release controlled bursts of NO may allow selective disruption of biofilms while minimizing collateral damage to the resident microbiota. Conversely, probiotics that sequester NO may help attenuate inflammation associated with dysbiosis, such as inflammatory bowel disease (IBD). In addition, synthetic biology approaches may be used to engineer NO-switch circuits into therapeutic bacteria so that antimicrobial agents are produced only in response to specific NO signatures generated by pathogenic bacteria, thereby reducing off-target effects. Nevertheless, interspecies interactions remain incompletely understood and require further investigation. Two major challenges also remain: (1) preventing horizontal gene transfer of antibiotic resistance genes following exposure to NO and (2) managing host-microbe NO cross-talk that may trigger immunopathology. Accordingly, by 2030, personalized modulation of NO based on patient-specific microbiome-derived NO signatures may provide a path toward precision-based anti-infective therapy and may transform ancient bacterial weapons into tunable therapeutic allies.

Conclusion

Bacterial NO represents a striking biological paradox: a molecule that functions both as a weapon in microbial competition and as a co-opted regulator of physiological processes. Certain bacterial species are capable of producing NO through specific enzymes such as NOS or nitrite reductase, thereby playing essential roles in natural biological processes and potential biomedical applications. Several bacterial genera and species produce NO enzymatically or through nitrate reduction, and bacterial NO production can also be enhanced by culture enrichment with precursors such as L-arginine or nitrite.

In this review, we have characterized the dual nature of NO, ranging from its role as a host-derived antimicrobial effector that microorganisms must detoxify through flavohemoglobins and hybrid cluster proteins to its endogenous role as a bacterial signal that can regulate biofilm dispersal, antibiotic resistance, and metabolic adaptation under stress. This fundamental duality underlies the "double-edged" nature of NO and complicates the development of therapeutic interventions because of the potential for persistent infection and damage to surrounding host tissues and normal commensal microflora. Yet, within this duality lies an important opportunity for innovation. The transition of NO research from observation to engineering has already generated a range of new applications, including NO-releasing biomaterials that disrupt resistant biofilms on medical devices, inhibitors of bacterial NO synthase that sensitize bacteria to conventional antibiotics, and synthetic circuits that use NO-responsive promoters to direct antimicrobial agents specifically to infected tissues. Ultimately, major progress will depend on a contextual understanding of NO, including its concentration, spatiotemporal dynamics, and polymicrobial interactions, all of which determine whether NO functions as a friend

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or a foe in infected hosts. Future advances in this area will be shaped by three essential developments: (1) the design of microbiome-informed NO therapeutics that preserve ecological balance during antibiotic treatment; (2) a deeper understanding of how pathogenic bacteria regulate NO synthesis; and (3) the refinement of precision platforms that translate NO biology into safe and effective interventions.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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Code of Ethics

This review study was approved by Fasa University of Medical Sciences (Code: 404171).

Authors' Contributions

Design and conceptualization: Abdolmajid Ghasemian and Mohammad Ghorbani, Supervision and final drafting: Mohammad Ghorbani and Abdolmajid Ghasemian. All authors have read and approved the final version of this manuscript.

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