Biotechnology Advances 88 (2026) 108818

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Modulation of *Clostridioides difficile* virulence by metabolites derived from probiotic consortia and genetically edited strains

Luana Macedo Nogueira [a], Eduardo César Meurer [b], Marcos Pileggi [c,*]

[a] Department of Biotechnology, Genetics and Cell Biology, Maringá State University, Maringá 87020-900, Brazil
[b] Federal University of Paraná (UFPR), Jandaia do Sul Advanced Campus, Jandaia do Sul 86900-000, Brazil
[c] Environmental Microbiology Laboratory, Department of Evolutionary Biology, Biological and Health Sciences Sector, Ponta Grossa State University, Ponta Grossa 84030-000, Brazil

ARTICLE INFO

Keywords:
Quorum sensing
Omic approaches
Synthetic biology
Metabolic engineering
Gut microbiota

ABSTRACT

Clostridioides difficile infection (CDI) continues to pose a significant clinical and biotechnological challenge, primarily driven by antimicrobial resistance and frequent recurrence. Emerging strategies are shifting the therapeutic focus from pathobiont eradication to virulence suppression, achieved by targeting the key metabolic and regulatory networks that underpin *C. difficile* pathogenicity in the gut. This review synthesizes multi-omic data demonstrating that a synergistic approach—restoring secondary bile acid metabolism (through the bai operon), boosting short-chain fatty acid (SCFA) production, and disrupting *quorum-sensing* systems (e.g., *luxS*, agr)—can collectively suppress toxin expression, biofilm formation, and spore germination. We further examine how synthetic biology and metabolic engineering are paving the way for next-generation solutions, including engineered probiotics, designer microbial consortia, and live biotherapeutic products endowed with programmable *quorum quenching* capabilities and optimized metabolic outputs. The integration of genomics, transcriptomics, proteomics, and metabolomics, with computational modeling, now enables the predictive design and industrially scalable production of these microbiome-based interventions. Together, these advances mark a pivotal transition from empirical probiotic use to the era of precision, mechanism-driven microbiome therapeutics designed to achieve durable control of CDI recurrence.

1. Introduction

Clostridioides difficile infection (CDI) continues to present a persistent clinical and public health challenge, marked by high recurrence rates, antimicrobial resistance, and a constrained therapeutic arsenal. Conventional strategies, centered on pathobiont eradication through broad-spectrum antibiotics or non-specific probiotic supplementation, frequently cause collateral damage to the gut ecosystem. This disruption can inadvertently intensify selective pressures, thereby promoting the emergence of resistant strains. Similarly, environments contaminated by pesticides also impose intense selective pressures on microbial communities, modulating bacterial survival, metabolism, and communication networks (Silva and Pileggi, 2025). In response, a paradigm shift emerges within microbial biotechnology. The new focus moves beyond elimination toward precision virulence suppression, strategically targeting the metabolic and regulatory networks that sustain *C. difficile* in the gut environment.

Emerging multi-omic approaches are now deciphering the critical molecular interplay between *C. difficile* and commensal gut microorganisms. These studies reveal that targeted microbial interventions—specifically, the restoration of secondary bile acid metabolism (mediated by the *bai* operon), the enhancement of short-chain fatty acid (SCFA) production, and the disruption of *quorum sensing* (QS) systems like *luxS*/AI-2 and *agr*—can significantly curtail key

Abbreviations: AI-2, autoinducer-2; AMR, antimicrobial resistance; bai, bile acid–inducible operon; CDI, *Clostridioides difficile* infection; CRISPR, clustered regularly interspaced short palindromic repeats; CRISPRi, CRISPR interference; DCA, deoxycholic acid; EPS, exopolysaccharides; FMT, fecal microbiota transplantation; FXR, farnesoid X receptor; GEM, genome-scale metabolic model; GMP, Good Manufacturing Practice; LBP, live biotherapeutic product; LCA, lithocholic acid; MIC, minimum inhibitory concentration; NGP, next-generation probiotic; PaLoc, pathogenicity locus; QS, *quorum sensing*; QQ, *quorum quenching*; RBS, ribosome-binding site; SCFA, short-chain fatty acid; Spo0A, stage 0 sporulation protein A; TcdA, toxin A; TcdB, toxin B; TGR5, G protein–coupled bile acid receptor 1.

* Corresponding author.
E-mail address: mpileggi@uepg.br (M. Pileggi).

https://doi.org/10.1016/j.biotechadv.2026.108818
Received 4 December 2025; Received in revised form 12 January 2026; Accepted 30 January 2026
Available online 2 February 2026

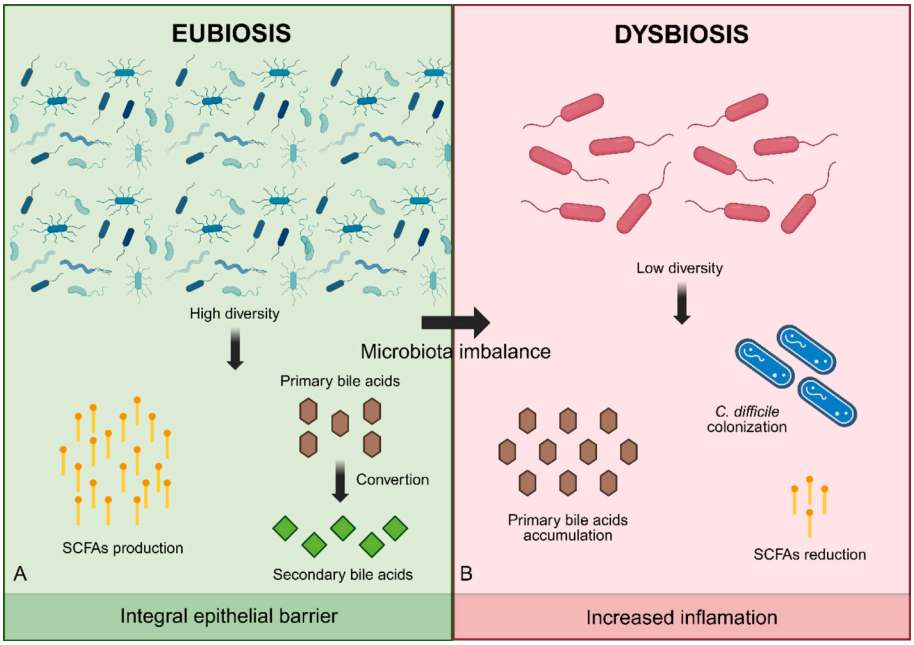

Fig. 1. Eubiosis versus dysbiosis and their metabolic consequences in the gut ecosystem.
Eubiosis (A) is characterized by high microbial diversity, adequate production of short-chain fatty acids (SCFAs), and efficient conversion of primary to secondary bile acids, thereby supporting intestinal barrier integrity. Microbiota imbalance leads to dysbiosis (B), marked by reduced diversity, accumulation of primary bile acids, decreased SCFA production, and increased susceptibility to *C. difficile* colonization. These combined alterations contribute to inflammation and loss of colonization resistance.

virulence traits, including toxin expression, sporulation, and biofilm formation. Building on these mechanistic insights, the field is advancing beyond conventional probiotics toward a new generation of designer therapeutics. This includes engineered probiotics, genetically defined consortia, and live biotherapeutic products (LBPs), all designed for targeted, mechanism-based action rather than empirical modulation of the gut ecosystem.

The integration of metabolic engineering and synthetic biology has dramatically expanded this therapeutic landscape. These disciplines now enable the design of probiotic chassis strains capable of executing targeted therapeutic functions, including the secretion of *quorum-quenching* (QQ) enzymes, the precise transformation of bile acids, and the optimized biosynthesis of SCFAs. Such engineered functionalities not only enhance the predictability and efficacy of treatments but also pave the way for robust industrial translation. Ultimately, the successful clinical deployment of these advanced biological hinges on overcoming key manufacturing challenges, particularly in scalable anaerobic fermentation, ensuring formulation stability, and establishing clear regulatory pathways.

This review synthesizes recent advances (2020–2025) in strategies to modulate *C. difficile* virulence, with a specific emphasis on mechanistic breakthroughs, their translational and industrial potential, and critical future research directions. By charting the integration of multi-omic discovery with established engineering principles, we underscore the emergence of precision microbiome therapeutics as a transformative and promising paradigm for the durable management of CDI.

1.1. Main text

1.1.1. Gut microbiota, dysbiosis, and the gateway for Clostridioides difficile

The intestinal microbiota operates as a dynamic biochemical network, essential for maintaining host metabolism, immune tolerance, and colonization resistance against pathogens. Crucially, its functional output—extending beyond mere taxonomic diversity—is defined by key metabolites like short-chain fatty acids (SCFAs) and bile acid derivatives, which are fundamental to intestinal homeostasis (Vital et al., 2014; Ruff et al., 2020; De Vos et al., 2022). These molecules act as pivotal regulatory signals that shape microbial community interactions and directly influence virulent pathways of opportunistic residents (Fig. 1). As multi-omic studies now elucidate, it is precisely through these metabolic signals that the microbiota tunes critical *C. difficile* pathogenicity modules, including toxin production, biofilm formation, and sporulation (Buddle and Fagan, 2023).

When this homeostatic balance is disrupted, a state of dysbiosis emerges, characterized by the loss of key commensals (e.g., *Faecalibacterium prausnitzii*), diminished SCFA production, and a shift in bile acid metabolism (Alagiakrishnan et al., 2024). This dysregulation is frequently triggered by broad-spectrum antibiotic exposure, dietary Westernization, chronic inflammatory states, or recurrent hospitalization, all of which selectively deplete obligate anaerobes while favoring aerotolerant opportunists. The resulting collapse of butyrate-producing Firmicutes and bile-acid–transforming Clostridia disrupts colonization resistance at both metabolic and immunological levels, creating permissive conditions for *C. difficile* spore germination and niche dominance (Claesson et al., 2011; Alagiakrishnan et al., 2024). This altered metabolic environment collectively creates a niche that is highly permissive for *C. difficile* spore germination and subsequent expansion (Claesson et al., 2011; Schirmer et al., 2019). Critically, recent research has established a more precise link: the specific depletion of Firmicutes bacteria harboring the *bai* operon, which is responsible for the 7-α-dehydroxylation of primary bile acids, correlates strongly with depleted secondary bile acid pools and a consequently higher risk of CDI recurrence (Ridlon et al., 2022; Blair, 2024).

The restoration of secondary bile acid metabolism mediated by the bai operon in commensal bacteria represents a critical mechanism for suppressing *C. difficile* spore germination and vegetative outgrowth. However, accumulating evidence suggests that the therapeutic application of this approach requires careful consideration of host epithelial safety (Ridlon et al., 2022; Blair, 2024). Secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA), although effective inhibitors of *C. difficile* germination, have been demonstrated to exert cytotoxic effects on intestinal epithelial cells at supraphysiological

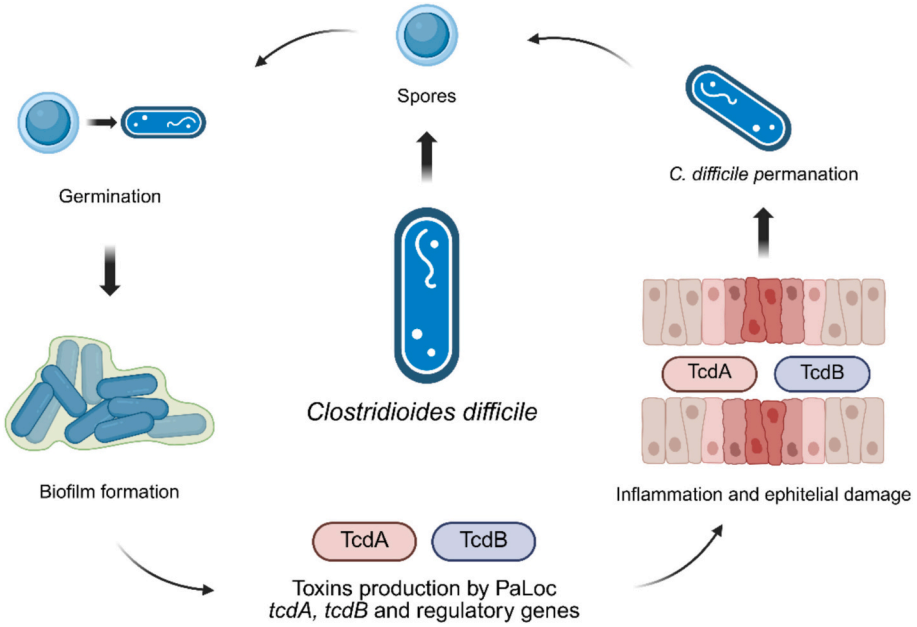

Fig. 2. Key steps in the *Clostridioides difficile* life cycle and virulent pathway.
Sporulation enables environmental persistence, and germination restores vegetative growth under permissive gut conditions. Vegetative cells form biofilms and activate the PaLoc locus, producing the major toxins TcdA and TcdB, which induce epithelial damage and inflammation. These processes together enable colonization, persistence, and virulence within the host intestinal environment.

concentrations. These effects include membrane destabilization, oxidative stress, and the induction of pro-inflammatory signaling pathways (Ridlon et al., 2022; McMillan et al., 2024). Notably, these cytotoxic effects are strongly dose-dependent and are influenced by host-related factors, including the integrity of the mucus layer, epithelial cell turnover, and bile acid receptor signaling via the FXR and TGR5 pathways (McMillan et al., 2024; Blair, 2024).

Under physiological conditions, commensal-mediated bile acid transformation typically produces spatially confined and tightly regulated pools of secondary bile acids that maintain epithelial homeostasis while providing effective colonization resistance against *C. difficile* (Ridlon et al., 2022). Multi-omic analyses of LBPs and defined microbial consortia further indicate that clinical efficacy is linked not to the maximal accumulation of secondary bile acids, but to the restoration of a balanced bile acid composition capable of achieving inhibitory concentrations against *C. difficile* without causing epithelial damage (McMillan, 2024a; Pettit et al., 2024). Collectively, these findings underscore that precision metabolic modulation—rather than indiscriminate enrichment of bile acids—should inform the design and translational application of microbial therapeutics based on the bai operon therapeutics.

Computational modeling integrated with metabolomic profiling provides a crucial insight: the restoration of specific metabolic fluxes is a more reliable predictor of colonization resistance recovery than a mere increase in microbial alpha-diversity (McMillan, 2024). This principle is supported by multi-omic co-culture studies demonstrating that commensals, such as *Bifidobacterium longum*, exert reproducible metabolic stress on *C. difficile*. These interactions trigger critical shifts in the pathogen's energy and nutrient pools—such as ATP/nucleotide balance and amino acid/proline pathways—that are directly concordant with reduced toxin synthesis (Jo et al., 2023). Collectively, this evidence underscores the necessity of a metabolite-centric design framework. It informs both the engineering of therapeutics and the development of industrial process conditions—including fermentation and formulation—that are specifically optimized to preserve the production of these protective metabolites under both manufacturing and physiological challenges (Jo et al., 2023; McMillan, 2024).

In this refined framework, dysbiosis is understood not merely as a loss of taxonomic diversity, but as a fundamental breakdown in the gut's metabolic signaling networks. This pivotal concept provides the foundational rationale for modern strategies aimed at suppressing *C. difficile* virulence. It directly underpins the shift toward interventions that are informed by multi-omic insights and enabled by biotechnological engineering (Ridlon et al., 2022; Buddle and Fagan, 2023; Blair, 2024; Pettit et al., 2024).

1.1.2. Clostridioides difficile: morphology, virulence, and resistance

The pathogenicity of *C. difficile* is initiated by a sophisticated regulatory network that integrates spore germination, QS, and toxin expression within a susceptible gut environment. While the vegetative form can persist asymptomatically, dysbiosis induced by broad-spectrum antibiotics creates a permissive niche for spore germination and subsequent colonization of the intestinal epithelium (Guh et al., 2020; Mada and Alam, 2024; Ronish et al., 2024; Meza-Torres et al., 2025). Host-derived and microbiota-modified signals mediate the decision to germinate in response to chemical stimuli. Primary bile acids, such as taurocholate, function as potent germinants by binding to the pseudoprotease receptor CspC. This binding initiates a proteolytic cascade involving CspB and SleC, culminating in cortex hydrolysis and spore outgrowth (Fig. 2) (Kevorkian et al., 2017). In direct opposition, secondary bile acids—notably deoxycholic and lithocholic acids, which are produced by commensals harboring the *bai* operon—act as potent inhibitors of this same germination process, thereby maintaining a critical barrier to colonization (Sorg and Sonenshein, 2008; Ridlon et al., 2022).

The inhibitory role of secondary bile acids and SCFAs in *C. difficile* pathogenesis is well established; however, their biological effects are highly dependent on concentration. Experimental studies have demonstrated that secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA), inhibit *C. difficile* spore germination and vegetative growth at micromolar to low millimolar concentrations. Reported minimum inhibitory or suppressive thresholds generally range between 0.05 and 0.5 mM, varying according to strain background and experimental conditions (Sorg and Sonenshein, 2008; Ridlon et al., 2022).

Table 1
Representative inhibitory threshold of bile acids and SCFAs against *Clostridioides difficile*.

Metabolite	Reported inhibitory range	Primary effect	Reference
Deoxycholic acid (DCA)	~ 0.05–0.5 mM	Inhibition of spore germination	Sorg and Sonenshein, 2008
Lithocholic acid (LCA)	~ 0.1–0.5 mM	Suppression of vegetative growth	Ridlon et al., 2022
Butyrate	~ 10–30 mM	Reduced toxin expression and sporulation	Vital et al., 2014; Jo et al., 2023
Propionate	~ 10–25 mM	Impaired growth and toxin regulation	Jo et al., 2023
Acetate	> 20 mM (context-dependent)	Metabolic stress, indirect inhibition	Vital et al., 2014

Notably, at concentrations exceeding these physiological ranges, bile acids may exert deleterious effects on both bacterial cells and host epithelial cells, highlighting the importance of defining functional rather than maximal inhibitory doses.

SCFAs, including acetate, propionate, and butyrate, exert dose-dependent effects on the physiology of *C. difficile*. Both in vitro and in vivo studies demonstrate that butyrate and propionate, at concentrations comparable to those observed in a healthy colon (typically 10–30 mM), suppress toxin gene expression, reduce sporulation efficiency, and modify cellular energy metabolism. In contrast, concentrations below this threshold may yield limited or inconsistent effects (Vital et al., 2014; Jo et al., 2023). These quantitative relationships underscore the importance of restoring physiological metabolite concentrations, rather than exceeding them, to achieve consistent suppression of virulence.

Collectively, the existing data underscore the necessity of incorporating quantitative metabolite thresholds into the development and assessment of microbiome-based interventions. Defining target concentration ranges for secondary bile acids and SCFAs establishes a mechanistic link between multi-omic findings and translational outcomes, thereby guiding therapeutic dosing strategies and potency assays for LBPs (Ridlon et al., 2022; McMillan et al., 2024). Representative inhibitory concentration ranges and their primary biological effects on *C. difficile* are summarized in Table 1.

Virulence is governed, at the transcriptional level, by the pathogenic locus (PaLoc), a genomic island that encodes the major toxins TcdA and TcdB, alongside their regulatory partners TcdR (an alternative sigma factor), TcdC (a putative anti-sigma factor), and TcdE (a holin-like protein) (Buddle and Fagan, 2023). Upon secretion, these toxins act synergistically to glucosylate Rho GTPases, dismantle the host acting cytoskeleton, and trigger potent inflammatory responses (Chen et al., 2015; Alam and Madan, 2024). Beyond this direct cytotoxicity, *C. difficile* employs QS systems—specifically LuxS/AI-2 and Agr—to population-density-dependently coordinate collective behaviors, including toxin synthesis, biofilm formation, and sporulation (Carter et al., 2005; Rutherford and Bassler, 2012). Critically, recent mutational and transcriptomic analyses (2023–2025) have demonstrated that the downregulation of key QS and regulatory components (e.g., *luxS*, *agrA*, or *tcdR*) directly attenuates virulence. These findings firmly establish these nodes as prime, druggable targets for innovative QQ therapeutic strategies (Guh et al., 2020; Gunaratnam et al., 2021).

The pronounced resistance to antibiotic therapy and the high frequency of CDI recurrence are primarily attributable to the bacterium's capacity to form resilient spores and biofilms. These structures provide a physical sanctuary for vegetative cells, shielding them from both immune-mediated clearance and antimicrobial agents (DiCandia et al., 2024; Meza-Torres et al., 2025). Furthermore, biofilms are not merely inert barriers; they function as metabolically active niches where QS signaling and stress-response pathways are upregulated, thereby reinforcing bacterial persistence even in the face of environmental challenges. This understanding has guided therapeutic efforts to inhibit these critical regulatory nodes. Consequently, strategies utilizing engineered probiotic enzymes (e.g., lactonases, acylases) or specific metabolic inhibitors that disrupt essential processes, such as ATP and nucleotide biosynthesis, have emerged as an auspicious approach to cripple *C. difficile* communication and undermine its fitness in situ (Grandclement et al., 2016; Hwang et al., 2017; Gunaratnam et al., 2021; Jo et al., 2023).

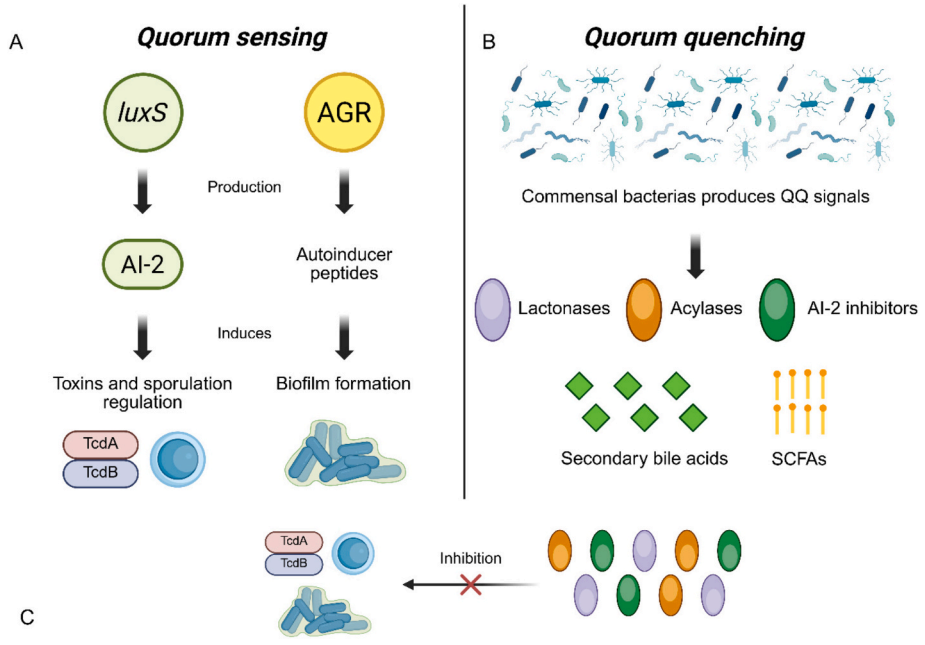

Fig. 3. *Quorum sensing* and *quorum quenching* pathways involved in *Clostridioides difficile* virulence.
The LuxS/AI-2 and Agr quorum-sensing systems regulate toxin expression, sporulation, and biofilm formation. (B) Commensal bacteria produce QQ activities, including lactonases, acylases, AI-2 inhibitors, secondary bile acids, and SCFAs, which disrupt QS signaling. (C) These QQ mechanisms collectively inhibit toxin production and biofilm development, reducing *C. difficile* virulence.

From a biotechnological perspective, these collective insights have fundamentally reframed our understanding of *C. difficile* pathogenesis. It is no longer viewed primarily as an antibiotic target but as a network of interconnected metabolic and regulatory vulnerabilities. The integration of multi-omic data has been instrumental in clarifying the causal links between bile acid imbalance, quorum-sensing circuit activity, and toxin gene transcription. This causal understanding, in turn, directly enables the rational design of precision probiotics and defined microbial consortia. These next-generation microbial therapeutics are engineered to act through targeted metabolic interference and regulatory silencing—strategies that circumvent the evolutionary pressures of direct bacterial killing (Blair, 2024; Pettit et al., 2024; Raeisi et al., 2025). Ultimately, these approaches represent a pivotal shift in therapeutic philosophy, aligning with the global imperative to move beyond conventional antibiotics. They herald a new era of mechanism-driven, evolvability-aware microbial therapeutics, designed to achieve a durable reduction in recurrence while preserving the stability of the gut ecosystem (Spigaglia, 2024). These regulatory and structural traits make *C. difficile* uniquely susceptible to targeted disruption through QS interference, which is explored in the next section.

1.1.3. Quorum sensing, quorum quenching, and potential targets

Among the complex mechanisms governing *C. difficile* virulence, cell-to-cell communication via QS stands out as a decisive factor for coordinating collective behaviors such as toxin synthesis, sporulation, and biofilm formation (Fig. 3). The pathogen primarily employs two major QS systems—LuxS/AI-2 and Agr—which modulate distinct transcriptional programs in a population density–dependent manner (Carter et al., 2005; Buddle and Fagan, 2023). The LuxS system, which produces the universal signaling molecule autoinducer-2 (AI-2), plays a key role in regulating extracellular matrix production and enhancing bacterial persistence in the challenging gut environment (Rutherford and Bassler, 2012). In a complementary role, the Agr system utilizes autoinducing peptides to create a positive feedback loop that amplifies toxin gene expression. The critical nature of this system is underscored by evidence showing that *agrA* mutations consistently lead to attenuated pathogenicity and significantly reduced production of the major toxins TcdA and TcdB (Rutherford and Bassler, 2012; Gunaratnam et al., 2021).

In direct contrast, *Quorum Quenching* (QQ) mechanisms represent a promising therapeutic and industrial strategy by interfering with or degrading these very signaling molecules. QQ operates through multiple modalities: it can be enzymatic, employing lactonases and acylases that cleave AI-2 or peptide autoinducers, or it can function via competitive receptor inhibition and the production of antagonistic metabolites (Grandclement et al., 2016). By disrupting this bacterial crosstalk, these processes effectively suppress toxin expression, destabilize biofilms, and limit sporulation, thereby collectively reducing *C. difficile* virulence and the likelihood of recurrence (Hwang et al., 2017; Gunaratnam et al., 2021). The translational potential of this approach is strongly supported by recent in vitro and animal studies (2021–2024), which demonstrate that native probiotics like *Lactobacillus acidophilus* and *Bifidobacterium longum* can naturally downregulate key virulence genes (*luxS, tcdA, tcdB*) and reduce AI-2 activity. These findings provide compelling evidence that targeted microbial QQ activity offers a viable and sustainable substitute for direct antibiotic pressure (Guh et al., 2020; Gunaratnam et al., 2021).

The comprehensive omic characterization of these QS and QQ pathways has been instrumental in pinpointing precise molecular targets for controlling *C. difficile*. Genomic and metabolomic datasets consistently identify the LuxS/AI-2 and Agr systems as critical regulatory hubs that directly link the pathogen's metabolic state to its virulence output (Jo et al., 2023; McMillan, 2024). Furthermore, proteomic analyses provide a mechanistic understanding, demonstrating that QQ-active commensal strains can suppress the key sporulation initiation factor Spo0A and its downstream σ-factor cascade, thereby directly reducing sporulation efficiency (DiCandia et al., 2022; DiCandia et al., 2024). This integrative, multi-omic understanding forms a solid foundation for the rational design of next-generation therapeutics. It enables the creation of engineered probiotics and synthetic microbial consortia that are programmed to deploy QQ enzymes or secrete inhibitory peptides directly within the gut environment, offering a targeted and sustainable intervention strategy (Raeisi et al., 2025).

Although QQ represents a promising strategy to mitigate *C. difficile* virulence, accumulating evidence suggests that interference with QS signals may extend beyond the targeted pathobiont, affecting broader microbial communication networks within the gut microbiota. Notably, the LuxS/AI-2 system serves as a conserved interspecies signaling mechanism among diverse gut commensals, contributing to metabolic coordination, biofilm formation, and community stability (Rutherford and Bassler, 2012; Grandclement et al., 2016). Therefore, indiscriminate degradation or inhibition of AI-2 signals by QQ enzymes could inadvertently disrupt beneficial microbial interactions, potentially altering cross-feeding relationships and compromising ecological resilience within the gut microbiome (Zhu et al., 2024).

Experimental and ecological investigations indicate that the effects of QQ activity are highly context-dependent, influenced by factors such as enzyme specificity, local concentration, spatial confinement, and the metabolic state of the surrounding microbial community (Grandclement et al., 2016; Markowska et al., 2024). Although targeted QQ can inhibit toxin expression, biofilm formation, and sporulation in *C. difficile*, excessive or non-selective signal interference may disrupt mutualistic signaling among commensal microorganisms, potentially compromising microbiota homeostasis and long-term stability (Ziegert et al., 2024).

These considerations highlight the critical importance of precision-guided QQ strategies in the design of therapeutic interventions. Emerging methodologies seek to spatially or temporally restrict QQ activity by employing inducible promoters, metabolite-responsive biosensors, or chassis-specific deployment. Such approaches aim to minimize off-target ecological impacts while maintaining anti-virulence efficacy (Bober et al., 2018; Hao et al., 2023). Collectively, these findings emphasize that QQ-based interventions should be optimized not merely for maximal signal disruption but for the selective attenuation of *C. difficile* communication within a complex and interconnected microbial ecosystem.

These characterized regulatory systems offer precise molecular levers for the development of next-generation microbiome therapeutics, from a biotechnological and translational perspective. Leveraging advances in synthetic biology and metabolic engineering, researchers can now insert QQ genes (e.g., *aiiA*, *pvdQ*) or deploy CRISPRi modules into probiotic chassis organisms. This enables the targeted silencing of *luxS* or *agr* circuits, resulting in predictable, self-regulating therapeutic strains with defined anti-virulence functions (Hwang et al., 2017; Raeisi et al., 2025). From an industrial manufacturing standpoint, these bioengineered probiotics are compatible with scalable anaerobic fermentation processes. This paves the way for their incorporation into diverse product formats, such as functional foods, oral biotherapeutics, or antimicrobial coatings. This pipeline offers environmentally safe, mechanism-based alternatives posed to supplant traditional antibiotic interventions (Grandclement et al., 2016; Blair, 2024; Pettit et al., 2024).

Despite the conceptual appeal and demonstrated experimental efficacy of QQ strategies, accumulating evidence suggests that disruption of QS pathways does not consistently result in robust anti-virulence effects across diverse biological contexts. Multiple in vitro and in vivo studies have reported partial, strain-dependent, or transient reductions in toxin production, biofilm formation, or sporulation, highlighting that QS interference alone may be insufficient to effectively suppress *C. difficile* pathogenicity within complex gut ecosystems (Grandclement et al., 2016; Markowska et al., 2024). Variability in QS circuit architecture, redundancy among regulatory pathways, and compensatory metabolic responses can mitigate or circumvent QQ-mediated inhibition, thereby producing negligible or modest phenotypic effects.

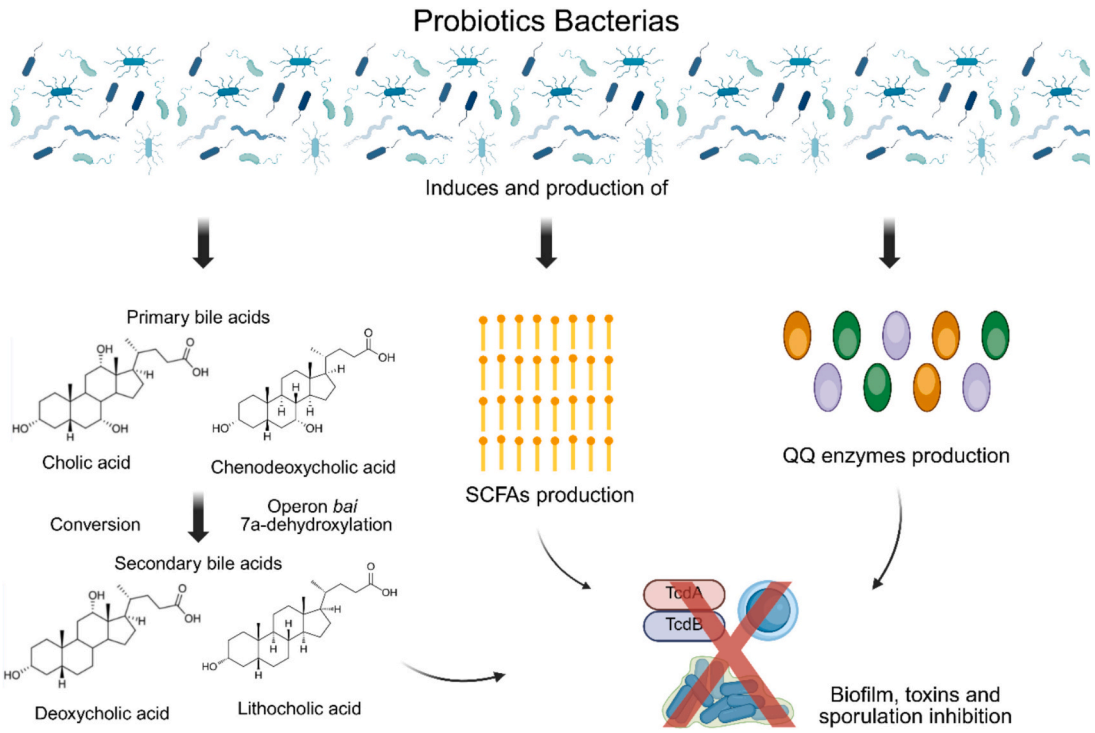

Fig. 4. Probiotic-driven modulation of bile acid metabolism, SCFA production, and *quorum quenching* activities in the gut.
Probiotic bacteria stimulate critical protective pathways, including conversion of primary bile acids into inhibitory secondary bile acids via *bai*-operon 7α-dehydroxylation, enhanced production of short-chain fatty acids (SCFAs), and secretion of QQ enzymes. Together, these mechanisms suppress *C. difficile* virulence by inhibiting toxin expression, biofilm development, and sporulation.

Furthermore, the effectiveness of QQ enzymes and engineered probiotics is highly dependent on the ecological context, including factors such as microbial community composition, substrate availability, and host-derived selective pressures. In polymicrobial environments, the suppression of QS in *C. difficile* may be offset by alternative signaling pathways or by fitness advantages unrelated to quorum regulation, thereby limiting the long-term efficacy of QQ-based interventions (Rutherford and Bassler, 2012; Markowska et al., 2024). These findings underscore that QQ should not be considered a universal or standalone approach; rather, it should be integrated into multifaceted intervention strategies that encompass metabolic modulation, bile acid restoration, and ecological stabilization to ensure consistent clinical outcomes benefit.

In summary, QS and QQ systems represent a critical convergence between molecular microbiology and microbial biotechnology. Environmental studies further demonstrate that herbicide exposure can modulate QS signaling profiles, reshaping antioxidant responses and biofilm development in Gram-negative bacteria (Freitas et al., 2021). They embody a fundamental paradigm shift in which targeted interference with bacterial communication supersedes broad-spectrum pathogen eradication as the central therapeutic strategy. This foundational transition directly fuels the development of omics-guided, enzyme-producing probiotics and defined microbial consortia. These advanced biologics are meticulously designed to achieve a dual objective: the precise suppression of *C. difficile* virulence and the active preservation of the gut microbiota's ecological integrity (Gunaratnam et al., 2021; Blair, 2024; Pettit et al., 2024; Raeisi et al., 2025).

1.1.4. Probiotics, next-generation probiotics, gene editing, bacterial consortia, and live biotherapeutic products

The strategy of modulating *C. difficile* virulence through probiotics and microbial consortia has undergone a significant evolution, transitioning from empirical supplementation to a mechanism-driven biotechnology. Conventional probiotics, including strains like *Lactobacillus acidophilus*, *L. fermentum*, *Bifidobacterium breve*, *B. bifidum*, and L. *paracasei*, have demonstrated value through multifaceted actions such as competition for adhesion sites, secretion of antimicrobial compounds (e.g., organic acids and bacteriocins), and modulation of host mucosal immune responses (Plaza-Diaz et al., 2019; Yong et al., 2019; Hamo et al., 2025). However, moving beyond these general mechanisms of colonization resistance, modern multi-omic investigations reveal a more targeted capability: specific probiotic species can directly interfere with the core virulence networks of *C. difficile*, leading to a measurable reduction in toxin expression, sporulation, and biofilm formation (Guh et al., 2020; Gunaratnam et al., 2021). Comparable patterns of QS-mediated adaptation have also been observed in environmental *Enterobacter* strains exposed to herbicide stress, reinforcing the role of QS molecules in coordinating metabolic resilience and stress responses (Martins et al., 2025).

Translationally, the mapping of this dysbiotic metabolic architecture has enabled a new generation of defined bacterial consortia and engineered probiotics. These therapeutics are designed to perform specific, restorative functions—reconstituting bile-acid conversion, enhancing SCFA synthesis, and rebalancing interspecies signaling (Fig. 4). This represents a fundamental shift in CDI management: from empirical recolonization to mechanism-guided reprogramming of the gut ecosystem (Blair, 2024; Pettit et al., 2024; Menon et al., 2025). Ultimately, these strategies reframe the gut from a static microbial community into a dynamic, programmable metabolic circuit, opening the door to precision interventions guided by predictive design.

Designer microbial consortia present a promising approach to mitigating *C. difficile* virulence through coordinated metabolic activities; however, their therapeutic efficacy within the gut environment is fundamentally influenced by ecological and metabolic constraints. Beyond synergistic interactions, the effective design of such consortia must consider metabolic division of labor, nutrient competition, and the maintenance of long-term community stability amid fluctuating intestinal conditions (Bober et al., 2018; Raeisi et al., 2025). In this regard,

functional specialization—where distinct strains perform complementary roles such as secondary bile acid conversion, SCFA production, and quorum quenching—can improve system robustness while reducing direct competition for shared substrates (Pettit et al., 2024; Menon et al., 2025).

Metabolic overlaps among consortium members may induce competitive pressures for limited resources, such as simple carbohydrates, amino acids, and electron acceptors, which can potentially result in shifts in dominance or functional collapse over time (McMillan et al., 2024). Multi-omic and modeling investigations suggest that stable consortia generally depend on structured cross-feeding networks, wherein metabolic by-products produced by one species function as essential substrates for another, thereby enhancing interdependence and ecological persistence (Gasparrini et al., 2020; Menon et al., 2025). This metabolic interdependence has been demonstrated to be a more reliable predictor of in vivo stability than taxonomic diversity alone.

The long-term functionality of engineered or defined microbial consortia is contingent upon their capacity to endure host-mediated selective pressures, such as immune surveillance, bile acid gradients, and dietary variability (McMillan, 2024; Pettit et al., 2024). Neglecting these factors may lead to strain loss, altered metabolic profiles, or diminished therapeutic reliability. Therefore, rational design of microbial consortia increasingly incorporates genome-scale metabolic modeling, flux balance analysis, and longitudinal omics monitoring to predict competitive constraints and optimize functional specialization prior to clinical application (Bober et al., 2018; Raeisi et al., 2025). Collectively, these considerations underscore that the evaluation of designer consortia must extend beyond short-term anti-virulence efficacy to include ecological resilience and sustained functional performance within the complex gut ecosystem.

The research by Gunaratnam et al. (2021) confirmed that specific *Lactobacillus* and *Bifidobacterium* strains can repress key virulence genes—including *luxS*, *tcdA*, *tcdB*, and *txeR*—thereby attenuating *C. difficile* pathogenicity without resorting to direct pathogen elimination (Gunaratnam et al., 2021). In a complementary study, proteomic and metabolomic profiling of *B. longum* co-cultured with *C. difficile* uncovered a multifaceted metabolic assault. This interaction was characterized by increased lactate dehydrogenase (LDH) activity, altered proline metabolism, and reduced nucleotide pools, collectively resulting in an energy-limited state that reduced toxin synthesis (Guh et al., 2020). Together, these findings demonstrate that probiotic–pathobiont interactions frequently operate through sophisticated metabolic interference rather than simple direct antagonism. This crucial insight establishes a rational foundation for the precision metabolic engineering of next-generation therapeutic strains.

Despite these promising advances, the clinical translation of conventional probiotics for CDI remains constrained by significant hurdles, including variable efficacy, a lack of standardized formulations, and a scarcity of longitudinal safety and efficacy data (Goldenberg et al., 2017; Carr et al., 2024; Kristina and Putri, 2025). To directly overcome these limitations, the field is increasingly turning into advanced biotechnological strategies. By integrating synthetic biology and precision gene editing, researchers can now design optimized probiotic chassis engineered to express defined therapeutic assets—such as QQ enzymes, bile-acid–modifying operons (e.g., *bai*, *bsh*), or antitoxin peptides (Bober et al., 2018; Raeisi et al., 2025). This shift to engineered biologics enables targeted, predictable modulation of the gut metabolic landscape, offering a viable path to reduce recurrence rates while minimizing collateral damage from broad-spectrum antibiotic pressure.

Parallel development efforts are advancing next-generation probiotics (NGPs) and LBPs, including species such as *Akkermansia muciniphila*, *Bacteroides fragilis*, and *Faecalibacterium prausnitzii*. These strains are selected for their specialized immunomodulatory and metabolic functions that are naturally deficient in the dysbiotic gut (O'Toole et al., 2017; Singh and Natraj, 2021). This shift toward defined microbial therapeutics is exemplified by consortia such as VE303 and SER-109. Clinical trials demonstrate that these formulations work through a concerted mechanism: they restore secondary bile acid metabolism, elevate SCFA levels, and, consequently, suppress *C. difficile* spore germination (Blair, 2024; Pettit et al., 2024; Menon et al., 2025). Multi-omic profiling of patients receiving these treatments provides mechanistic validation, confirming the reactivation of *bai*-operon expression, the recovery of genes responsible for 7-α-dehydroxylation, and the direct transcriptional repression of the major toxin genes *tcdA* and *tcdB* (Ridlon et al., 2022; Jo et al., 2023).

Reductions in toxin expression, sporulation, or biofilm formation observed in in vitro systems and animal models offer valuable mechanistic insights, these surrogate endpoints do not necessarily correspond directly to clinically meaningful outcomes in humans. Experimental studies often report percentage reductions in tcdA/tcdB expression or toxin activity in murine models, typically on the order of ~30%, following probiotic or microbiota-based interventions; however, the relationship between these molecular measures and definitive clinical endpoints—such as recurrence rate, length of hospital stay, or CDI-associated mortality—remains complex and non-linear (Guh et al., 2020; Feuerstadt et al., 2023).

Clinical evidence suggests that achieving durable reductions in recurrence necessitates not only the attenuation of toxin production but also the sustained restoration of colonization resistance and microbiome functionality over time. Phase III trials of defined LBPs indicate that clinical benefit is most accurately measured by recurrence-free survival rather than by isolated molecular markers. This finding underscores the limitations of extrapolating quantitative toxin suppression observed in animal models to patient-level outcomes (Blair, 2024; Pettit et al., 2024).

Accordingly, surrogate experimental endpoints ought to be regarded as indicators of mechanistic plausibility rather than as direct predictors of therapeutic efficacy. The integration of these molecular readouts with longitudinal clinical data and validated outcome measures is crucial for the accurate assessment of translational efficacy and for preventing the overinterpretation of preclinical findings within the context of recurrent CDI (Guh et al., 2020; Feuerstadt et al., 2023).

Defined microbial consortia, such as SER-109 and VE303, have demonstrated substantial efficacy in reducing recurrent *C. difficile* infection (CDI). However, their clinical performance should be evaluated in comparison to conventional fecal microbiota transplantation (FMT), which remains the standard intervention for refractory CDI. Meta-analyses and controlled clinical trials report that FMT achieves recurrence-free rates ranging from approximately 70% to 90%, contingent upon factors such as donor selection, delivery method, and patient comorbidities (Goldenberg et al., 2017; Feuerstadt et al., 2023). Nonetheless, FMT is inherently heterogeneous, as it depends on donor-derived microbial communities characterized by variable composition, undefined functional outputs, and carries non-negligible risks of pathobiont transmission as well as regulatory challenges (Schmidt et al., 2018; Pettit et al., 2024).

In contrast, SER-109 and VE303 are standardized, compositionally defined LBPs developed to restore critical metabolic functions essential for colonization resistance, specifically secondary bile acid conversion and short-chain fatty acid production. Phase III clinical trials have demonstrated that SER-109 significantly reduces the recurrence of CDI compared to placebo, with recurrence rates of approximately 12–15% and a favorable safety profile, thereby supporting its regulatory approval and clinical implementation (Blair, 2024; Pettit et al., 2024). Similarly, VE303 has exhibited robust efficacy in late-stage clinical studies, achieving sustained suppression of *C. difficile* colonization through targeted metabolic restoration and microbiome reprogramming (Menon et al., 2025).

Although direct head-to-head clinical trials comparing defined LBPs and FMT are currently limited, emerging evidence indicates that standardized microbial consortia may provide comparable efficacy while enhancing safety, reproducibility, and regulatory oversight. Notably, in

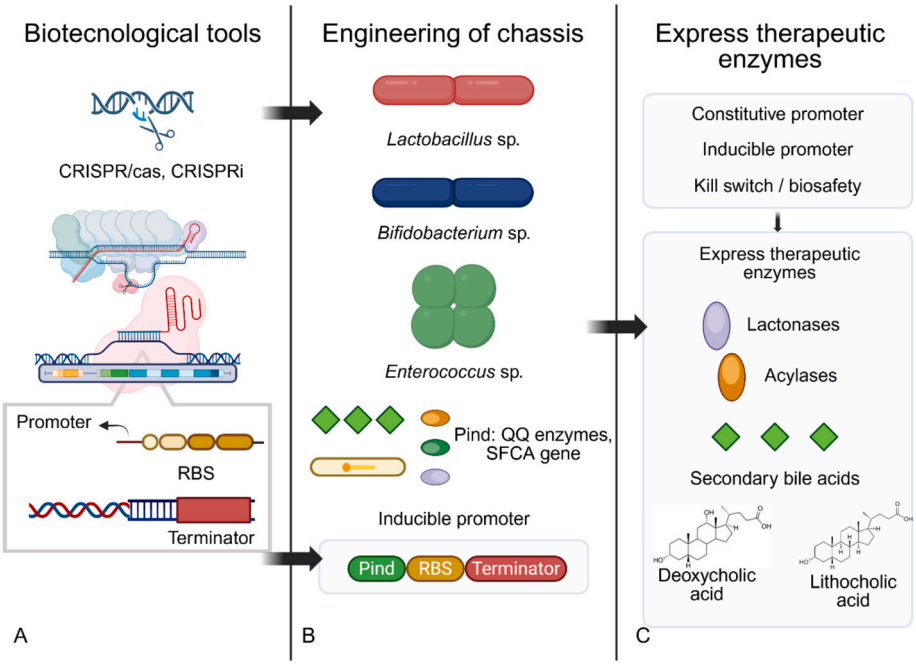

Fig. 5. Synthetic biology tools and engineered probiotics chassis for targeted therapeutic functions.
(A) CRISPR/Cas and CRISPR interference (CRISPRi) systems enable precise genetic modification of probiotic genomes, allowing gene insertion, deletion, or transcriptional repression. (B) Engineered microbial chassis—including *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* species—are designed to harbor therapeutic genetic modules organized as defined expression cassettes composed of promoters, ribosome-binding sites (RBS), therapeutic genes, and transcriptional terminators. Gene expression can be regulated by constitutive or inducible promoters, and biosafety elements such as kill switches may be incorporated to enhance biocontainment. (C) These engineered strains can express functional enzymes, such as lactonases and acylases, or promote secondary bile acid synthesis, thereby attenuating *C. difficile* virulence.

contrast to FMT, defined microbial therapeutics allow for precise mechanistic elucidation, consistent batch-to-batch production, and scalable manufacturing processes. These attributes position them as a promising translational advancement in microbiota-based interventions for recurrent CDI (Schmidt et al., 2018; Pettit et al., 2024).

The successful industrial translation of these advanced microbiome therapeutics hinges on overcoming key manufacturing and regulatory challenges, particularly in scalable anaerobic fermentation, ensuring formulation stability, and achieving regulatory harmonization (Blair, 2024). Critical process optimization—encompassing advanced encapsulation technologies, maintaining viability under oxygen exposure, and developing potency assays linked to functional metabolic endpoints—will ultimately determine the commercial feasibility of LBPs as standardized, reliable drugs (Blair, 2024). To this end, the field is increasingly adopting multi-omic profiling as a rigorous quality-control metric (McMillan, 2024). This approach directly links specific genetic and metabolite markers (e.g., expression of *bai*, *but*, and *buk* genes; SCFA flux rates) to product consistency and potency, providing a scientific foundation for batch-to-batch standardization and regulatory approval (McMillan, 2024).

Ultimately, the field is converging on a new paradigm of rationally designed microbial consortia. These therapeutics are selected or engineered based on integrative genomic, proteomic, and metabolomic criteria (Raeisi et al., 2025), moving beyond taxonomy to a functional definition of a healthy gut ecosystem (Pettit et al., 2024). Their overarching aim is to restore eubiosis by executing a coordinated set of tasks: re-establishing a protective bile-acid profile, enhancing SCFA synthesis, and silencing pathobiont quorum-sensing systems (Blair, 2024). By uniting multi-omic discovery with the precision tools of metabolic engineering and robust bioprocess innovation, next-generation probiotics and LBPs represent a pivotal advance (Raeisi et al., 2025). They mark the transition from broad-spectrum interventions to a future of personalized, mechanism-based, and industrially scalable microbiome therapeutics for the durable management of CDI (Pettit et al., 2024; Menon et al., 2025).

1.1.5. Synthetic biology and precision engineering in microbiome therapeutics

The convergence of synthetic biology, metabolic engineering, and systems microbiology is fundamentally redefining the landscape of microbiome-based therapeutics. By integrating genetic programmability with multi-omic insights, researchers are now constructing precision-engineered microbial chassis capable of executing targeted metabolic and regulatory functions within the complex gut environment (Bober et al., 2018; Raeisi et al., 2025). These foundational innovations are enabling a pivotal transition: from the empirical use of probiotic formulations to the deployment of rationally engineered, smart biotherapeutics that can dynamically and predictably respond to host and environmental cues.

Recent advances in CRISPR/Cas and CRISPRi technologies have enabled site-specific modification of probiotic genomes, enabling targeted enhancement of traits such as environmental stress tolerance, metabolite synthesis, and QQ activity (Fig. 5) (Bober et al., 2018; Raeisi et al., 2025). This precision editing is exemplified by engineering chassis strains such as *Bacillus subtilis*, *Lactobacillus plantarum*, and *Bifidobacterium longum* to express therapeutic enzymes—including lactonases, acylases, and bile salt hydrolases. These modifications directly disrupt *C. difficile* quorum-sensing circuits and restore a protective bile-acid profile (Grandclement et al., 2016; Hwang et al., 2017). Moving beyond single-strain engineering, the field is now leveraging computational metabolic modeling to design sophisticated synthetic consortia. These multi-species communities are rationally balanced to optimize cross-feeding interactions, maximize SCFA production, and enhance toxin inhibition, thereby ensuring both robust ecological stability and superior therapeutic efficacy (Bober et al., 2018; Raeisi et al., 2025).

Despite the therapeutic potential of CRISPR-based and genetically

engineered probiotics, ensuring the long-term stability of introduced functions remains a significant challenge for achieving in vivo efficacy. Plasmid-based expression systems, although experimentally convenient, are especially vulnerable to segregationally loss, structural rearrangements, and selective silencing when exposed to gut environmental pressures such as nutrient limitation, immune-mediated stress, and fluctuating bile acid concentrations (Bober et al., 2018; Raeisi et al., 2025). These factors can accelerate the loss of engineered traits, resulting in inconsistent therapeutic outcomes and diminished clinical reliability.

To mitigate these risks, contemporary strategies increasingly prioritize the chromosomal integration of therapeutic gene modules, which enhances genetic stability across multiple bacterial generations and reduces the probability of plasmid loss (Liu et al., 2023; Martínez-Porchas et al., 2025). Concurrently, the implementation of genetic stabilization systems—such as toxin–antitoxin modules, essential gene coupling, or metabolic selection circuits—has been demonstrated to impose a fitness cost on reversion events, thereby preserving engineered functionality under selective pressure (Srivastava et al., 2024; Martínez-Porchas et al., 2025).

Additional approaches encompass the application of CRISPR interference (CRISPRi) to reversibly suppress virulence or regulatory genes without inducing permanent genomic alterations, thereby mitigating the evolutionary pressure for escape mutations (Liu et al., 2023). Collectively, these strategies emphasize that robust genetic design, rather than the editing tool per se, constitutes the principal factor determining the functional durability of engineered probiotics within the complex and competitive gut ecosystem.

The clinical application of genetically engineered probiotics requires the implementation of robust biocontainment strategies to ensure environmental safety, prevent uncontrolled dissemination, and comply with regulatory standards. One of the most established approaches involves auxotrophic designs, wherein engineered strains are made dependent on specific nutrients or metabolites that are exclusively available within the host intestinal environment, thereby restricting their survival outside the target niche (Bober et al., 2018; Martínez-Porchas et al., 2025). These strategies effectively mitigate ecological risks while maintaining therapeutic efficacy in vivo.

Complementary biocontainment systems encompass genetically encoded kill switches that induce cell death upon detection of specific environmental stimuli, such as oxygen exposure, temperature fluctuations, or the absence of host-derived signals (Srivastava et al., 2024; Frutos-Grilo et al., 2024). Recent developments have further integrated receptor-mediated and metabolite-responsive circuits, facilitating the conditional activation or repression of essential genes in response to gut-specific biochemical markers, including bile acids, short-chain fatty acids (SCFAs), and quorum sensing (QS) molecules (Hao et al., 2023; Raeisi et al., 2025).

Contemporary biocontainment strategies increasingly integrate multiple safeguard mechanisms—such as auxotrophy, inducible lethality, and context-dependent gene regulation—to reduce the likelihood of evolutionary escape while preserving therapeutic effectiveness (Bober et al., 2018; Srivastava et al., 2024). This multilayered approach not only improves biosafety but also aligns engineered LBPs with evolving regulatory standards for genetically modified microbiome interventions.

The increasing availability of high-resolution multi-omic datasets has driven a transition from empirical probiotic engineering to predictive, model-guided design of microbial chassis. Integrative analyses that combine genomics, transcriptomics, proteomics, and metabolomics enable the reconstruction of genome-scale metabolic models (GEMs), which capture strain-specific metabolic capabilities and regulatory constraints within the gut environment (Bober et al., 2018; Díaz-Ruiz et al., 2024). These models support flux balance analysis and constraint-based simulations to predict the responses of engineered strains and consortia to host-derived selective pressures, nutrient availability, and interspecies interactions.

In the context of *C. difficile* control, computationally guided design has demonstrated significant value in optimizing critical therapeutic functions, such as secondary bile acid conversion, short-chain fatty acid (SCFA) biosynthesis, and QQ efficiency (Gasparrini et al., 2020; McMillan et al., 2024). Additionally, multi-omic profiling facilitates the identification of metabolic bottlenecks, cross-feeding dependencies, and unintended trade-offs that, if unaddressed, may undermine in vivo stability or therapeutic consistency (Menon et al., 2025; Raeisi et al., 2025).

Recent advancements have increasingly integrated machine learning methodologies with multi-omic and metabolic modeling frameworks to improve strain selection, predict functional resilience, and guide iterative chassis optimization prior to clinical application (Díaz-Ruiz et al., 2024; Srivastava et al., 2024). By correlating genotypes, metabolic fluxes, and phenotypic outcomes, these predictive pipelines enhance both the instructional and translational potential of microbiome engineering, thereby facilitating the rational development of LBPs with predefined and reproducible therapeutic performance.

The implementation of programmable genetic circuits, at the molecular level, is enabling unprecedented dynamic control over probiotic behavior. Strains can now be engineered with sophisticated biosensors that detect specific intestinal cues—such as AI-2 concentration, pH shifts, or bile acid levels—and subsequently activate or repress therapeutic gene expression in response (Bober et al., 2018; Raeisi et al., 2025). This closed-loop, sense-and-response capability allows for context-dependent actions, such as localized QQ or on-demand metabolite release. This targeted approach significantly reduces the risk of off-target effects and enhances overall biosafety. Ultimately, this sophisticated level of environmental awareness and autonomous control marks the arrival of a new generation of microbiome therapeutics: self-regulating probiotics capable of fine-tuned, real-time virulence modulation within the complex gut ecosystem.

From an industrial perspective, these advanced engineered systems are increasingly compatible with the rigorous demands of Good Manufacturing Practice (GMP) and large-scale production. The establishment of standardized genetic parts, modular vector systems, and optimized anaerobic fermentation protocols now enables the reproducible and compliant manufacturing of engineered probiotics under regulatory scrutiny (Blair, 2024; McMillan, 2024). At the metabolic and molecular levels, the adoption of standardized biological parts—including well-characterized promoters, ribosome-binding sites, and regulatory cassettes—is enabling increasingly sophisticated control. These components are engineered to respond to key gut metabolites such as lactate, acetate, butyrate, and bile acid derivatives (Hao et al., 2023), allowing engineered strains to dynamically modulate therapeutic gene expression in response to local biochemical fluctuations. Furthermore, modular vector systems facilitate the stable integration or plasmid-based expression of functional genes responsible for bile acid transformation (e.g., *baiCD/H*) (Wang et al., 2020), SCFA biosynthesis (e.g., *buk/but* for butyrate; *pdu/fab* for propionate) (Singh and Natraj, 2021), and QQ (e.g., lactonases and acylases) (Zhu et al., 2024). Collectively, these engineered capabilities empower probiotics to directly target critical *C. difficile* virulence mechanisms, including the repression of *tcdR* activation, disruption of Spo0A-driven sporulation cascades, and attenuation of LuxS/AI-2–mediated quorum signaling (Liu et al., 2023). Critically, integrating these robust bioengineering tools with multi-omic analytics and computational modeling (Díaz-Ruiz et al., 2024) paves the way for a fully predictive design framework. This powerful synergy is accelerating the development of microbial therapeutics with predefined and consistent metabolic outputs, marking a definitive advance toward a new era of precision, scalable, and sustainable microbiome-based interventions for *C. difficile* and related infectious diseases (Bober et al., 2018; Blair, 2024; Pettit et al., 2024; Raeisi et al., 2025).

1.1.6. Social impact and future perspectives

The rising incidence and recurrence of *C. difficile* infection (CDI) underscore an urgent, unmet need for sustainable, microbiota-centered therapeutic strategies that move beyond reliance on conventional antibiotics (Valdes et al., 2018; Liu et al., 2020; Di Tommaso et al., 2021). CDI represents not only a challenging clinical condition but also a mounting public health and economic crisis. High relapse rates and associated extended hospitalizations generate substantial and growing costs for healthcare systems globally (Schmidt et al., 2018; Ruff et al., 2020; Feuerstadt et al., 2023). Furthermore, the societal impact of CDI extends deeper: the antibiotic-driven dysbiosis that predisposes individuals to CDI concurrently fuels the broader dissemination of antimicrobial resistance (AMR), creates long-term ecological imbalance in the gut, and diminishes the efficacy of future standard therapeutic regimens, creating a vicious cycle of treatment failure (Claesson et al., 2011; Schirmer et al., 2019).

Emerging multi-omic and systems biology approaches are fundamentally redefining CDI management by catalyzing a paradigm shift: from a narrow focus on pathogen elimination to a holistic strategy of metabolic and regulatory virulence modulation (Buddle and Fagan, 2023; Pettit et al., 2024). These robust integrative analyses are uncovering specific microbial and metabolic biomarkers with the potential to predict disease severity and stratify recurrence risk, paving the way for more personalized patient management (Ridlon et al., 2022; McMillan, 2024). As these mechanistic insights mature, they provide a rigorous scientific foundation for the rational development of a new class of precision microbiome therapeutics. This includes engineered probiotics, defined bacterial consortia, and LBPs that are explicitly optimized for the critical trifecta of clinical stability, therapeutic efficacy, and industrial scalability (Blair, 2024; Pettit et al., 2024; Menon et al., 2025).

The successful translation of omic discoveries into manufacturable therapeutics demands robust bioprocess engineering and global regulatory harmonization, from an industrial perspective. Key advances in anaerobic fermentation, microencapsulation, and the stabilization of oxygen-sensitive species now enable the large-scale production of LBPs with guaranteed viability and consistent potency (Blair, 2024; McMillan, 2024). Furthermore, integrating machine learning and mechanistic metabolic modeling into formulation pipelines is revolutionizing the development process. These computational tools enable the prediction of critical parameters, such as strain behavior, metabolic fluxes, and community resilience, under both industrial manufacturing and in vivo gastrointestinal conditions (Raeisi et al., 2025). The establishment of such predictive frameworks is becoming indispensable for ensuring the reproducibility, standardization, and ultimately, the successful clinical translatability of next-generation microbiome-based therapeutics.

Beyond clinical efficacy, the widespread implementation of microbiota-based therapies for recurrent *C. difficile* infection is significantly influenced by pharmacoeconomic and logistical considerations. Although conventional FMT is effective, it entails considerable hidden costs associated with donor screening, pathogen testing, individualized preparation, and complex regulatory requirements. These factors collectively constrain scalability and hinder the standardization of reimbursement processes (Schmidt et al., 2018; Feuerstadt et al., 2023). Furthermore, variability in donor material contributes to uncertainty in therapeutic outcomes, thereby complicating health-economic evaluations and acceptance by payers (Pettit et al., 2024).

In contrast, standardized LBPs such as SER-109, present more transparent pharmacoeconomic profiles due to their batch-controlled manufacturing processes, defined dosing regimens, and predictable safety monitoring. Although LBPs entail costs related to anaerobic fermentation, cold-chain storage, and distribution, these expenditures may be offset by reductions in recurrence rates, shorter hospital stays, and diminished requirements for repeated antibiotic treatments or rehospitalization (Feuerstadt et al., 2023; Blair, 2024). Preliminary real-world evidence and modeling studies indicate that preventing recurrent CDI through sustained microbiome restoration could be cost-effective compared to repeated antibiotic-based therapies, particularly among high-risk patient populations.

Cold-chain requirements continue to pose significant logistical challenges for LBPs, as preserving the viability of obligate anaerobes necessitates stringent control of temperature and oxygen exposure during storage and transportation (Blair, 2024; McMillan, 2024). Nevertheless, recent advancements in encapsulation technologies, lyophilization methods, and formulation engineering are progressively mitigating these challenges by enhancing shelf life and facilitating incorporation into existing pharmaceutical supply chains (Frutos-Grilo et al., 2024). Taken together, these factors highlight that economic viability, reimbursement strategies, and logistical feasibility are critical determinants of successful clinical translation, thereby emphasizing the importance of incorporating pharmacoeconomic modeling early in the development process of microbiome-based therapeutics.

Future research must prioritize bridging the critical gap between omic data interpretation and robust functional validation, with a dedicated focus on long-term biosafety, genetic stability, and the colonization dynamics of therapeutic strains. Synthetic biology provides a robust toolkit to address these challenges, enabling the design of sophisticated probiotic chassis capable of auto-regulated QQ, intelligent metabolic feedback control, and context-dependent therapeutic synthesis (Bober et al., 2018; Raeisi et al., 2025). These advanced, self-regulating strategies pave the way for scalable and highly customizable solutions. While their immediate promise lies in transforming CDI management, their underlying principles are poised to expand the reach of microbiome engineering to a broad spectrum of microbiota-related diseases. This progression marks a pivotal shift toward a new paradigm of environmentally conscious, evolvability-aware biotechnological interventions for global health (Ridlon et al., 2022; Blair, 2024; Pettit et al., 2024).

Ultimately, the deep integration of multi-omic discovery with the principles of industrial biotechnology is poised to fundamentally transform CDI therapy. This evolution will shift the paradigm from empirical modulation to a new era of mechanistic, data-driven, and sustainable microbiome engineering. This powerful convergence of microbiology, synthetic biology, and systems engineering defines a new frontier in infection control—one uniquely capable of safeguarding human health and promoting ecological balance simultaneously (Ridlon et al., 2022; Blair, 2024; Pettit et al., 2024; Raeisi et al., 2025).

1.2. Perspectives

The effective control of *C. difficile* virulence necessitates innovative strategies that transcend traditional antibiotic dependency and directly address the profound ecological and molecular complexity of the gut ecosystem (Ridlon et al., 2022; Buddle and Fagan, 2023; Spigaglia, 2024). Multi-omic analyses have been pivotal in illuminating the critical interdependence between bile acid metabolism, QS/QQ regulation, and the restoration of protective metabolic fluxes (Bosnjak et al., 2023; DiCandia et al., 2024; Markowska et al., 2024; McMillan, 2024; McMillan et al., 2024). These insights provide a clear set of mechanistic targets for the rational design of next-generation microbial therapeutics (Pettit et al., 2024; Raeisi et al., 2025). By systematically integrating genomic, transcriptomic, proteomic, and metabolomic data, we can now engineer interventions that precisely modulate pathobiont behavior rather than attempting to eradicate it (Bosnjak et al., 2023; Jo et al., 2023). This sophisticated approach minimizes selective pressure for resistance and promotes the restoration of resilient, host-protective eubiosis (McMillan et al., 2024; Spigaglia, 2024).

The emergence of genetically engineered probiotics defined microbial consortia, and versatile synthetic biology platforms mark a transformative leap toward the precision control of microbial virulence (Srivastava et al., 2024; Raeisi et al., 2025). These advanced systems are designed to execute a dual therapeutic strategy: they restore host-protective metabolic pathways—such as those for secondary bile acids

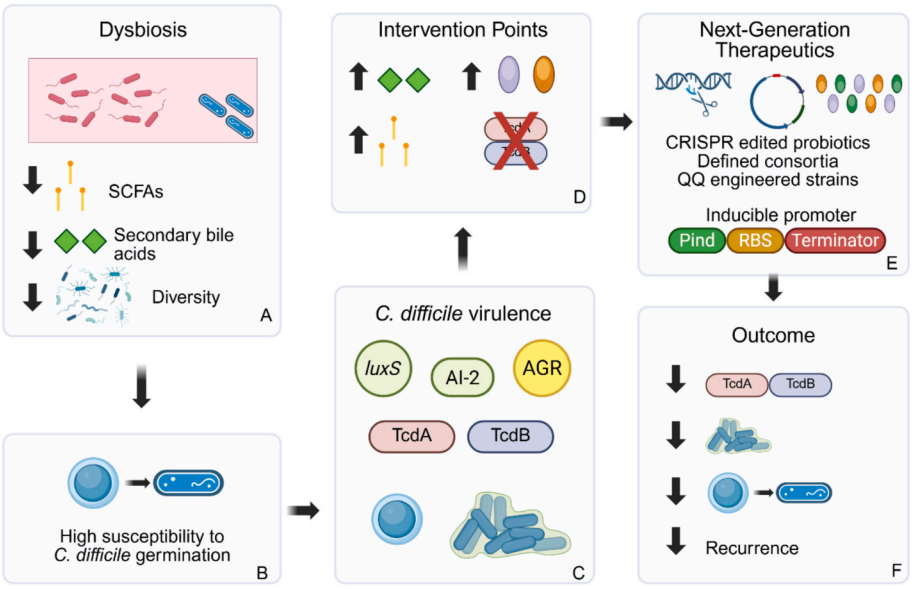

Fig. 6. Integrated overview of dysbiosis-driven *Clostridioides difficile* virulence and therapeutic intervention points.
(A) Dysbiosis is characterized by reduced SCFAs, diminished secondary bile acids, and loss of microbial diversity. (B) These alterations increase susceptibility to *C. difficile* spore germination and vegetative growth. (C) Virulence is driven by QS pathways (LuxS/AI-2 and Agr) and toxin production (TcdA/TcdB). (D) Key intervention points include enhancing secondary bile acids, restoring SCFAs availability, and deploying QQ enzymes to disrupt pathobiont communication networks. (E) Next-generation therapeutics—such as CRISPR-engineered probiotics, defined microbial consortia, and QQ-optimized strains— implement these strategies through engineered genetic circuits under controlled expression, including inducible regulatory elements. (F) The combined effect reduces toxin levels, biofilm formation, sporulation, and ultimately recurrence of infection.

and SCFAs—while simultaneously and dynamically repressing key quorum-sensing circuits (e.g., *luxS*, *agr*) and virulence genes (*tcdA/B*) (Gunaratnam et al., 2021; Markowska et al., 2024; Ziegert et al., 2024). This allows for context-specific modulation of pathogenicity that is not achievable with conventional antibiotics. Looking forward, the powerful convergence of metabolic engineering, robust bioprocess design, and predictive computational modeling is poised to accelerate the translation of these multi-omic discoveries into a new generation of scalable, GMP-compliant LBPs (Bober et al., 2018; Blair, 2024; Frutos-Grilo et al., 2024; Srivastava et al., 2024), ultimately reshaping our approach to infectious disease.

Ultimately, the advancement of next-generation CDI therapy hinges on the seamless harmonization of deep mechanistic insight, cutting-edge biotechnological innovation, and scalable industrial implementation (Pettit et al., 2024; Spigaglia, 2024). By strategically bridging multi-omic discovery with the precision tools of synthetic and systems biology, microbiome engineering solidifies its role as a sustainable, evolvable, and precision-driven paradigm to suppress *C. difficile* virulence (Bober et al., 2018; Srivastava et al., 2024; Raeisi et al., 2025). This integrated framework does not merely offer new treatments; it sets a transformative new stage for a future generation of intelligent, microbiome-based interventions in infectious disease management (Fig. 6) (Frutos-Grilo et al., 2024; Martínez-Porchas et al., 2025; Tian et al., 2025).

2. Conclusions

This review demonstrates that suppressing *C. difficile* virulence—rather than pursuing pathobiont eradication—represents a more sustainable and mechanistically grounded strategy for preventing recurrence. By integrating multi-omic evidence, we show that restoring key metabolic pathways—particularly secondary bile acid conversion and SCFA synthesis—coupled with targeted disruption of QS circuits, can reliably attenuate toxin production, biofilm formation, and sporulation. Advances in probiotic engineering, defined microbial consortia, and LBPs further validate that these protective functions can be rationally programmed into microbial chassis with increasing precision. Importantly, by acting on virulence and metabolic regulation rather than broad microbial killing, these strategies also impose markedly lower selective pressure for antibiotic resistance, addressing a primary global health concern linked to recurrent antimicrobial exposure. Collectively, these findings confirm that a mechanism-driven, metabolite- and signaling-centric framework provides a promising foundation for next-generation CDI therapeutics designed for durable clinical efficacy.

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.

Acknowledgments

This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES – Financial Code 001), granted to Luana Macedo Nogueira. The author Marcos Pileggi expresses gratitude to the National Council for Scientific and Technological Development (CNPq) for providing financial support (Process No. 307602/2025-7) through a Research Productivity Grant.

Data availability

All data discussed in this paper are available in the published literature cited herein.

References

Alagiakrishnan, K., Morgadinho, J., Halverson, T., 2024. Approach to the diagnosis and management of dysbiosis. Front. Nutr. 11, 1330903. https://doi.org/10.3389/fnut.2024.1330903.

Alam, M.Z., Madan, R., 2024. *Clostridioides difficile* toxins: host cell interactions and their role in disease pathogenesis. Toxins (Basel). 16 (6), 241. https://doi.org/10.3390/toxins16060241. PMID: 38922136; PMCID: PMC11209539.

Blair, H.A., 2024. SER-109 (Vowst™): A Review in the Prevention of Recurrent *Clostridioides Difficile* Infection. Drugs.

Bober, J.R., Beisel, C.L., Nair, N.U., 2018. Synthetic biology approaches to engineer probiotics and members of the human microbiota for biomedical applications. Annu. Rev. Biomed. Eng. 20, 277–300. https://doi.org/10.1146/annurev-bioeng-062117-121019 (Epub 2018 Mar 12. PMID: 29528686; PMCID: PMC6100750).

Bosnjak, M., Branković, M., et al., 2023. Multi-omics analysis of hospital-acquired diarrhoeal disease reveals interactions between host, microbiome and pathogens, including *Clostridioides difficile*. Nat. Commun. 14, 7867. https://doi.org/10.1038/s41467-023-43671-8.

Buddle, J.E., Fagan, R.P., 2023. Pathogenicity and virulence of *Clostridioides difficile*. Virulence 1, 2150452. https://doi.org/10.1080/21505594.2022.2150452. PMID: 36419222; PMCID: PMC9815241.

Carr, A., Baliga, N.S., Diener, C., Gibbons, S.M., 2024. Personalized *Clostridioides difficile* engraftment risk prediction and probiotic therapy assessment in the human gut. bioRxiv. https://doi.org/10.1101/2023.04.28.538771, 2023.04.28.538771.

Carter, G.P., Purdy, D., Williams, P., Minton, N.P., 2005. Quorum sensing in *Clostridium difficile*: analysis of a luxS-type signaling system. J. Med. Microbiol. 119-27. https://doi.org/10.1099/jmm.0.45817-0.

Chen, S., Sun, C., Wang, H., Wang, J., 2015. The role of rho GTPases in toxicity of *Clostridium difficile* toxins. Toxins 7, 5254–5267.

Claesson, M.J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H., Flannery, E., Marchesi, J.R., Falush, D., Dinan, T., Fitzgerald, G., Stanton, C., van Sinderen, D., O'Connor, M., Harnedy, N., O'Connor, K., Henry, C., O'Mahony, D., Fitzgerald, A.P., Shanahan, F., Twomey, C., Hill, C., Ross, R.P., O'Toole, P.W., 2011. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc. Natl. Acad. Sci. USA 108, 4586–4591. https://doi.org/10.1073/pnas.1000097107.

De Vos, W.M., Tilg, H., Van Hul, M., Cani, P.D., 2022. Gut microbiome and health: mechanistic insights. Gut 71, 1020–1032. https://doi.org/10.1136/gutjnl-2021-326789.

Di Tommaso, N., Gasbarrini, A., Ponziani, F.R., 2021. Intestinal barrier in human health and disease. Int. J. Environ. Res. Public Health 18, 12836. https://doi.org/10.3390/ijerph182312836. PMID: 34886561; PMCID: PMC8657205.

Díaz-Ruiz, G., Martinez, A., Torres, J.P., 2024. Multi-omic engineering strategies for next-generation live biotherapeutics. Microb. Biotechnol. 17 (5), e70057. https://doi.org/10.1111/1751-7915.170057.

DiCandia, M.A., Wetzel, D., Shen, A., 2022. Identification of functional Spo0A residues critical for sporulation and toxin regulation in *Clostridioides difficile*. mBio 13. https://doi.org/10.1128/mbio.03266-22, 03266–22.

DiCandia, M.A., Wetzel, D., Shen, A., 2024. A conserved switch controls virulence, sporulation, and transmission in *Clostridioides difficile*. Nat. Microbiol. 9, 1358–1371.

Feuerstadt, P., Theriault, N., Tillotson, G., 2023. The burden of CDI in the United States: a multifactorial challenge. BMC Infect. Dis. 23, 132. https://doi.org/10.1186/s12879-023-08096-0.

Freitas, P.N.N., Rovida, A.F.S., Silva, C.R., Pileggi, S.A.V., Olchanheski, L.R., Pileggi, M., 2021. Specific quorum sensing molecules are possibly associated with responses to herbicide toxicity in a *Pseudomonas* strain. Environ. Pollut. 289, 117896. https://doi.org/10.1016/j.envpol.2021.117896.

Frutos-Grilo, E., et al., 2024. Bacterial live therapeutics for human diseases: recent advances and future directions. EMBO Mol. Med. 16 (9), e00067. https://doi.org/10.1038/s44320-024-00067-0.

Gasparrini, A.J., Wang, B., Sun, X., Kennedy, E.A., Hernandez-Leyva, A., Ndao, I.M., et al., 2020. Metabolic network modulation and SCFA biosynthesis in engineered gut microbial communities. Cell Host Microbe 28 (6), 103–119. https://doi.org/10.1016/j.chom.2020.10.001.

Goldenberg, J.Z., Yap, C., Lytvyn, L., Lo, C.K., Beardsley, J., Mertz, D., Johnston, B.C., 2017. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. Cochrane Database Syst. Rev. 12. https://doi.org/10.1002/14651858.CD006095.pub4. CD006095. (PMID: 29257353; PMCID: PMC6486212).

Grandclement, C., Tannieres, M., Morera, S., Dessaux, Y., Faure, D., 2016. Quorum quenching: role in nature and applied developments. FEMS Microbiol. Rev. 40, 86–116. https://doi.org/10.1093/femsre/fuv038.

Guh, A.Y., Mu, Y., Winston, L.G., Johnston, H., Olson, D., Farley, M.M., 2020. Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. N. Engl. J. Med. 382, 1320–1330. https://doi.org/10.1056/NEJMoa1910215.

Gunaratnam, S., Millette, M., McFarland, L.V., DuPont, H.L., Lacroix, M., 2021. Potential role of probiotics in reducing *Clostridioides difficile* virulence: interference with quorum sensing systems. Microb. Pathog. 153, 104798. https://doi.org/10.1016/j.micpath.2021.104798. Epub 2021 Feb 18. PMID: 33609647.

Hamo, Z., Ninio, H., Azrad, M., 2025. Probiotics Ameliorate Histological Alterations and Prevent Increases in Cytokine and Toxin Levels in Mice Infected with Various *Clostridioides Difficile* Strains. Probiotics & Antimicro. Prot. https://doi.org/10.1007/s12602-025-10586-3. Epub ahead of print. PMID: 40531412.

Hao, Z., Zhang, L., Chen, G., 2023. Metabolite-responsive biosensors for microbial engineering and gut metabolic monitoring. Curr. Opin. Biotechnol. 85, 103020. https://doi.org/10.1016/j.copbio.2023.103020.

Hwang, I.Y., Koh, E., Wong, A., March, J.C., Bentley, W.E., Lee, Y.S., Chang, M.W., 2017. Engineered probiotic *Escherichia coli* can eliminate and prevent *Pseudomonas aeruginosa* gut infection in animal models. Nat. Commun. 11, 815028. https://doi.org/10.1038/ncomms15028. PMID: 28398304; PMCID: PMC5394271.

Jo, S.H., Jeon, H.J., Song, W.S., Lee, J.S., Kwon, J.E., Park, J.H., Kim, Y.R., Kim, M.G., Baek, J.H., Kwon, S.Y., Kim, J.S., Yang, Y.H., Kim, Y.G., 2023. Unveiling the inhibition mechanism of *Clostridioides difficile* by *Bifidobacterium longum via* multiomics approach. Front. Microbiol. 8 (14), 1293149. https://doi.org/10.3389/fmicb.2023.1293149. PMID: 38029200; PMCID: PMC10663266.

Kevorkian, Y., Shen, A., Schneewind, O., 2017. Revisiting the role of Csp family proteins in regulating *Clostridium difficile* spore germination. J. Bacteriol. 199 (22). https://doi.org/10.1128/JB.00266-17.

Kristina, Y., Putri, I., 2025. A comprehensive review of probiotic mechanisms and their implementation in gastrointestinal health management. Science Get Journal. 39–48.

Liu, S., Gao, J., Zhu, M., Liu, K., Zhang, H.L., 2020. Gut Microbiota and Dysbiosis in Alzheimer's Disease: Implications for Pathogenesis and Treatment. Mol. Neurobiol. 57, 5026–5043. https://doi.org/10.1007/s12035-020-02073-3. Epub 2020 Aug 23. PMID: 32829453; PMCID: PMC7541367.

Liu, L., Singh, R., Lee, S.Y., 2023. CRISPRi-enabled programmable repression of virulence regulators in anaerobic gut bacteria. BioDesign Research. 2023, 0017. https://doi.org/10.34133/bdr.0017.

Mada, P.K., Alam, M.U., 2024. *Clostridioides difficile* infection. StatPearls.

Markowska, K., Nowak, A., Krawczyk, A., et al., 2024. Understanding quorum-sensing and biofilm forming in anaerobic bacteria: molecular mechanisms and therapeutic perspectives. Int. J. Mol. Sci. 25 (23), 12808. https://doi.org/10.3390/ijms252312808.

Martínez-Porchas, M., et al., 2025. Genetic improvement of a synthetic microbiota: CRISPR-based strategies for safety and functional stability. Front. Microbiol. 16, 1619874. https://doi.org/10.3389/fmicb.2025.1619874.

Martins, J.G., Iurk, V.B., De Oliveira, E.P., De Oliveira, E.L., Silva, C.R., Tonin, A.P.P., Pileggi, S.A.V., Meurer, E.C., Olchanheski, L.R., Pileggi, M., 2025. Adaptation of *Enterobacter* sp. to herbicides is correlated with distinct patterns of quorum sensing molecules. J. Hazard. Mater. 496, 139324. https://doi.org/10.1016/j.jhazmat.2025.139324.

McMillan, A.S., 2024. Metagenomic, metabolomic, and lipidomic shifts associated with fecal microbiota transplantation for recurrent *C. Difficile*. mSphere 9 (10), e0070624. https://doi.org/10.1128/msphere.00706-24. Epub 2024 Oct 8. PMID: 39377587; PMCID: PMC11520286.

McMillan, A.S., Branković, M., Bosnjak, M., et al., 2024. Bile acids impact the microbiota, host, and *Clostridioides difficile* infection. Gut Microbes 16 (1), 2393766. https://doi.org/10.1080/19490976.2024.2393766.

Menon, R., Bhattarai, S.K., Crossette, E., 2025. Multi-omic profiling a defined bacterial consortium for treatment of recurrent *Clostridioides difficile* infection. Nat. Med. 31, 223–234. https://doi.org/10.1038/s41591-024-03337-4.

Meza-Torres, J., Tinevez, J.Y., Crouzols, A., Mary, H., Kim, M., Hunault, L., 2025. *Clostridioides difficile* binary toxin CDT induces biofilm-like persisting microcolonies. Gut Microbes 17, 2444411. https://doi.org/10.1080/19490976.2024.2444411 (PMID: 39719371).

O'Toole, P.W., Marchesi, J.R., Hill, C., 2017. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. Nat. Microbiol. 2, 17057. https://doi.org/10.1038/nmicrobiol.2017.57.

Pettit, N.N., Shaeer, K.M., Chahine, E.B., 2024. Live biotherapeutic products for the prevention of recurrent *Clostridioides difficile* infection. Ann. Pharmacother. 58, 1204–1217. https://doi.org/10.1177/10600280241310646 (PMID: 38546138).

Plaza-Diaz, J., Ruiz-Ojeda, F.J., Gil-Campos, M., Gil, A., 2019. Mechanisms of action of probiotics. Adv. Nutr. 1, 10. https://doi.org/10.1093/advances/nmy063.

Raeisi, H., Leeflang, J., Hasan, S., Woods, S.L., 2025. Bioengineered probiotics for *Clostridioides difficile* infection: an overview of the challenges and potential for this new treatment approach. Probiot. Antimicrob. Proteins. 17, 763–780. https://doi.org/10.1007/s12602-024-10398-x. Epub 2024 Nov 12. PMID: 39531149.

Ridlon, J.M., Kang, D.J., Hylemon, P.B., 2022. Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. 63, 100–116. https://doi.org/10.1194/jlr.TR120001003.

Ronish, L.A., Biswas, B., Bauer, R.M., Jacob, M.E., Piepenbrink, K.H., 2024. The role of extracellular structures in *Clostridioides difficile* biofilm formation. Anaerobe 88, 102873. https://doi.org/10.1016/j.anaerobe.2024.102873.

Ruff, W.E., Greiling, T.M., Kriegel, M.A., 2020. Host–microbiota interactions in immune-mediated diseases. Nat. Rev. Microbiol. 18, 521–538. https://doi.org/10.1038/s41579-020-0367-2.

Rutherford, S.T., Bassler, B.L., 2012. Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb. Perspect. Med. 2, 012427. https://doi.org/10.1101/cshperspect. a012427.

Schirmer, M., Garner, A., Vlamakis, H., Xavier, R.J., 2019. Microbial genes and pathways in inflammatory bowel disease. Nat. Med. 25, 601–612. https://doi.org/10.1038/s41591-019-0402-7.

Schmidt, T.S.B., Raes, J., Bork, P., 2018. The human gut microbiome: from association to modulation. Cell 172, 1198–1215. https://doi.org/10.1016/j.cell.2018.02.044.

Silva, C.R., Pileggi, M., 2025. Environments contaminated by pesticides are hotspots for the selection of bacterial chassis for biotechnological applications. Biotechnol. Adv. 86, 108723. https://doi.org/10.1016/j.biotechadv.2025.108723.

Singh, T.P., Natraj, B.H., 2021. Next-generation probiotics: A promising approach towards designing personalized medicine. Crit. Rev. Microbiol. 47, 479–498. https://doi.org/10.1080/1040841X.2021.1902940.

Sorg, J.A., Sonenshein, A.L., 2008. Bile salts and glycine as cogerminants for *Clostridium*. J. Bacteriol. 190, 2505–2512.

Spigaglia, P., 2024. *Clostridioides difficile* and gut microbiota: from colonization to infection and treatment. Pathogens 13, 646. https://doi.org/10.3390/pathogens13080646. PMID: 39204246; PMCID: PMC11357127.

Srivastava, R., Frutos-Grilo, E., et al., 2024. Living engineered bacterial therapeutics: emerging technologies and clinical translation. Microb. Biotechnol. 17 (5), e70057. https://doi.org/10.1111/1751-7915.70057.

Tian, S., et al., 2025. A designed synthetic microbiota provides insight into host–microbe interactions and suppresses *Clostridioides difficile* virulence. Cell Host Microbe. https://doi.org/10.1016/j.chom.2025.01.005 (in press).

Valdes, A., Walter, J., Segal, E., Spector, T.D., 2018. Role of the gut microbiota in nutrition and health. BMJ 361, 2179. https://doi.org/10.1136/bmj. k2179.

Vital, M., Howe, A.C., Tiedje, J.M., 2014. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. mBio 5. https://doi.org/10.1128/mBio.00889-14, 00889–14.

Wang, W., Chen, L., Zhou, R., Xiong, M., Yang, H., 2020. Regulation of bile acid biotransformation by gut microbial bai operon genes. Nat. Microbiol. 5, 465–478. https://doi.org/10.1038/s41564-020-0678-0.

Yong, C.C., Lim, J., Kim, B.K., Park, D.J., Oh, S., 2019. Suppressive effect of *Lactobacillus fermentum* Lim2 on *Clostridioides difficile* 027 toxin production. Lett. Appl. Microbiol. 68, 386–393. https://doi.org/10.1111/lam.13124.

Zhu, J., Wang, S., Zhang, Q., 2024. Quorum-quenching mechanisms in the intestinal microbiome and implications for pathogen control. Front. Microbiol. 15, 1411320. https://doi.org/10.3389/fmicb.2024.1411320.

Ziegert, Z., et al., 2024. Targeting quorum sensing for manipulation of commensal microbiota and control of bacterial virulence. Front. Microbiol. 15, 1411320. https://doi.org/10.3389/fmicb.2024.1411320.