REVIEW ARTICLE

MICROBIOME ENGINEERING FOR THERAPEUTIC AND ENVIRONMENTAL APPLICATIONS: A COMPREHENSIVE REVIEW

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Abstract:

Microbiome engineering is a rapidly advancing field that employs various techniques to manipulate microbial communities for therapeutic and environmental benefits. This review provides a comprehensive overview of key microbiome engineering strategies, including phage-mediated microbiome engineering, microbiome transplantation, genetic engineering, probiotic engineering, and quorum sensing modulation. These approaches are explored in the context of their applications, from treating human diseases such as gastro intestinal disorders and metabolic syndromes to addressing environmental challenges like wastewater treatment and ecosystem restoration. Additionally, this review examines the technical and ethical challenges associated with microbiome engineering, including issues of safety, efficacy, and ecological impact. By integrating these diverse techniques and applications, the review underscores the potential of microbiome engineering to drive innovation in both healthcare and environment management.

Keyword: Microbiome Engineering, Dysbiosis, Top-down approaches, Bottom-up approach, Therapeutic applications, Environmental Applications.

1. Introduction:

The microbiome, consisting of diverse microorganisms such as bacteria, fungi, viruses, and their genetic material, plays a vital role in maintaining human health and homeostasis. These microbes, although microscopic, perform essential functions, such as protecting against harmful pathogens, supporting immune system maturation, and aiding in digestion to generate energy. Moreover, the microbiome serves as a critical interface between the body and the environment, influencing how individuals respond to environmental stimuli. Some microbial species modify environmental substances in ways that increase toxicity, while others act to buffer and reduce their harmful effects. However, natural microbiomes are often subjected to dysbiosis, instability, and limitations in their functional capabilities making them less effective in certain therapeutic and environmental contexts. To address these challenges, microbiome engineering has emerged as a promising field, offering the potential to modify and optimize microbial communities for specific applications. Microbiome engineering, the deliberate manipulation of microbial communities for beneficial purposes, has emerged as a promising field with vast potential in both therapeutic and environmental applications. By harnessing the power of microbiome engineering, we can develop innovative strategies for treating diseases, enhancing agricultural productivity, and mitigating environmental pollution (Berg *et al.,* 2020).

1.1 Classification of Microbiome:

1.1.1. Human microbiome

The human body is a complex ecosystem containing a diverse community of microorganisms called the microbiome. These microorganisms, including bacteria, archaea, fungi, and viruses, live in various body sites like the skin, mouth, gut, and respiratory tract, each forming a unique community adapted to its environment. For example, the skin microbiome plays a crucial role in immune function, wound healing, and skin health; the gut microbiome breaks down complex foods, absorbing nutrients, and producing certain vitamins; the respiratory microbiome helps to filter out pathogens and maintain lung health and the urogenital microbiome helps to maintain urinary health and prevent infection (Berg *et al.,* 2020).

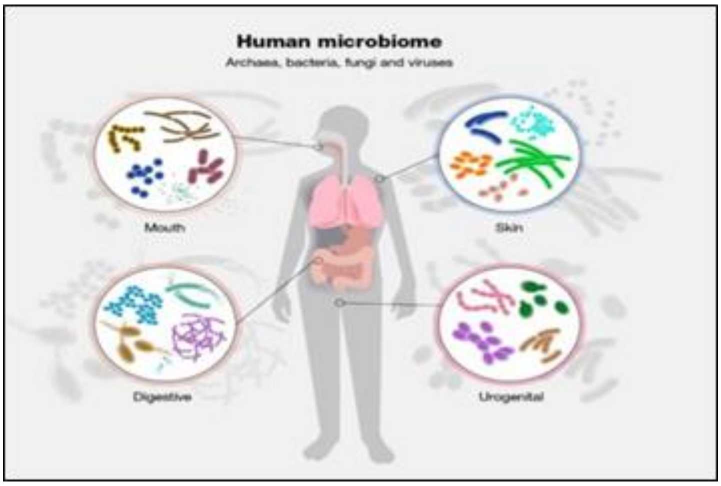

Figure 1: The Human Microbiome (https: www.genome.gov)

Key Roles of the Microbiome

i) Digestion and Nutrient Absorption: The gut microbiome is particularly important for digestion. Bacteria in the gut break down complex carbohydrates, proteins, and fats, allowing our bodies to absorb essential nutrients. They also produce vitamins, such as vitamin K and B vitamins, which are vital for our health.

ii) Immune System Development: The microbiome plays a crucial role in the development and function of our immune system. It helps to train the immune system to recognize harmful pathogens while tolerating harmless commensal bacteria. Disruptions to the microbiome have been linked to autoimmune diseases and allergies.

iii) **Protection Against Pathogens**: A healthy microbiome can help to prevent the growth of harmful pathogens. Beneficial bacteria compete with pathogens for resources and produce antimicrobial substances that can inhibit their growth.

iv) **Metabolism and Energy Regulation**: The microbiome can influence our metabolism and energy balance. Certain bacteria produce short-chain fatty acids, which have been shown to regulate appetite, insulin sensitivity, and energy expenditure (Ogunrinola *et al.,* 2020).

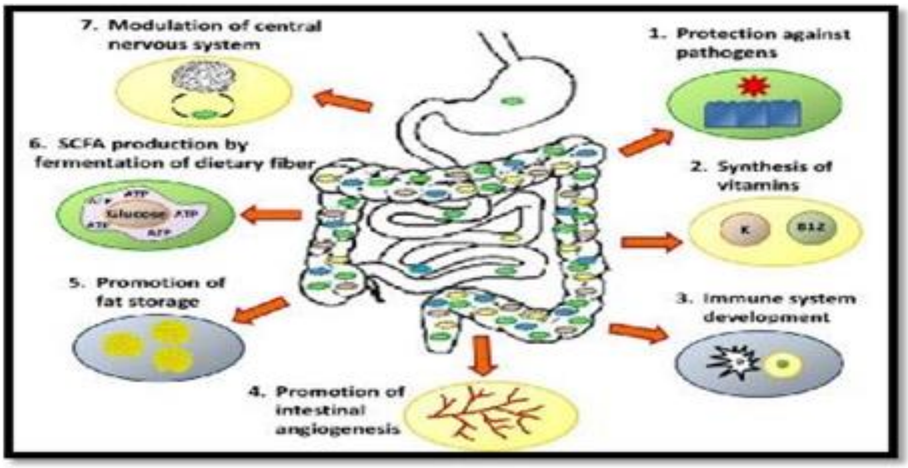

Figure 2: The human microbiome plays an important role in control of vital homeostatic mechanisms in the body. These include Enhanced metabolism, resistance to infection and inflammation, prevention against autoimmunity as well as an effect on the gut–Brain axis. SCFA, short-chain fatty acid. (Pola *et al*., 2023)

1.1.2. Plant Microbiome

The plant microbiome comprises diverse microorganisms, including bacteria, fungi, and archaea, inhabiting various plant environments. These microbes influence plant health by affecting nutrient uptake, disease resistance, and stress tolerance. These microorganismss can reside on plant surfaces, within tissues, or in the surrounding soil. The distinct communities within plant environment are:

i) Phyllosphere Microbiome

The phyllosphere includes the plant's aerial parts such as leaves, stems, and flowers. It is a nutrient-poor environment with high microbial diversity influenced by environmental factors like temperature and moisture. Key microbial residents include Proteobacteria.

ii) Rhizosphere Microbiome

The rhizosphere is the soil region around plant roots, enriched with nutrients from root exudates. The area supports a dense community of microbes that enhance plant growth, nutrient cycling, and disease resistance.

iii) Endosphere Microbiome

The endosphere refers to the internal plant tissues where microbes like endophytes reside. Thes microbes, which include both the bacteria and fungi, often form beneficial relationships with plants, aiding in nutrient acquisition and growth promotion (Khondkar *et al.,* 2020)

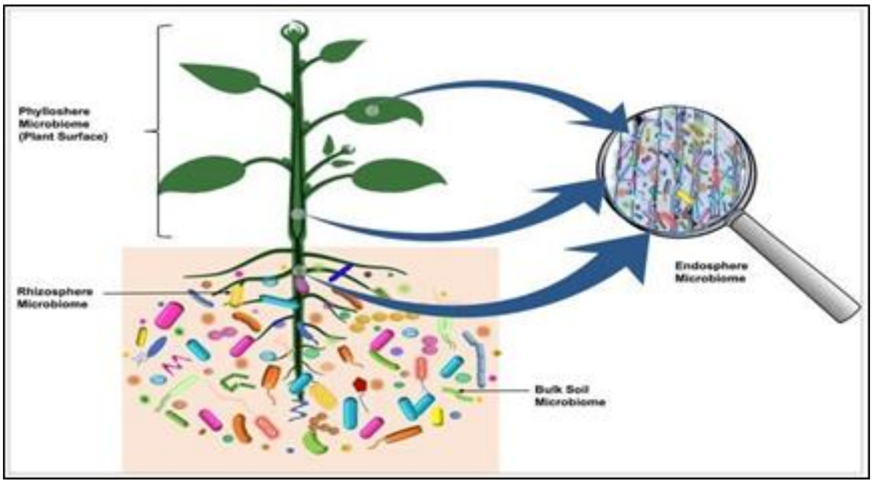

Figure 3: The Plant Microbiome. The rhizosphere, endosphere and phyllosphere constitute the major compartments of plant microbiome (Khondkar *et al.*, 2020)

1.1.3. Soil microbiome

The soil microbiome is a complex and diverse community of microorganisms living in soil, comprising bacteria, fungi, archaea, and protists. With over 50,000 species in just one gram of soil, it is the most genetically diverse community on the planet. The soil microbiome is vital for healthy soils and agricultural production. Microbes perform various functions like carbon dynamics, nutrient cycling, and soil structure maintenance, supporting ecosystem services like climate regulation and the water cycle. They act as "chemical engineers", "biological regulators", and "ecosystem engineers", but their specific contributions are not yet fully understood. Research shows that higher microbial diversity leads to greater multifunctionality, but more research is needed to understand the complex relationships between the soil microbiome and soil functions (Bhattacharjee *et al.,* 2022).

1.2. Normobiosis v/s Dysbiosis

Normobiosis refers to a state where the microbial community is in balance, promoting the overall health and stability of the host organism or environment. In humans, normobiosis is characterized by a diverse and stable gut microbiota that supports the digestion, immune function, and protection against pathogens. Similarly, in plants, normobiosis occurs when the rhizosphere harbors a balanced microbial community, enhancing nutrient uptake, growth, and resistance to diseases. In soil, normobiosis ensures soil fertility and structure, with beneficial microbes aiding in nutrient cycling, organic matter decomposition, and the suppression of harmful pathogens. A well-balanced soil microbiome contributes to the sustainability and productivity of agricultural systems.

Dysbiosis is the opposite condition, where the microbial community becomes imbalanced, often leading to negative outcomes for the host or environment. In humans, dysbiosis in the gut microbiota can contribute to various health issues, including inflammatory bowel diseases, obesity, and allergies. Dysbiosis of environmental microbiomes is related to abnormal climate change, plant diseases, harmful algal blooms, etc. This imbalance may be triggered by factors such as poor diet, antibiotics, or infections (Kamo *et al.,* 2017).

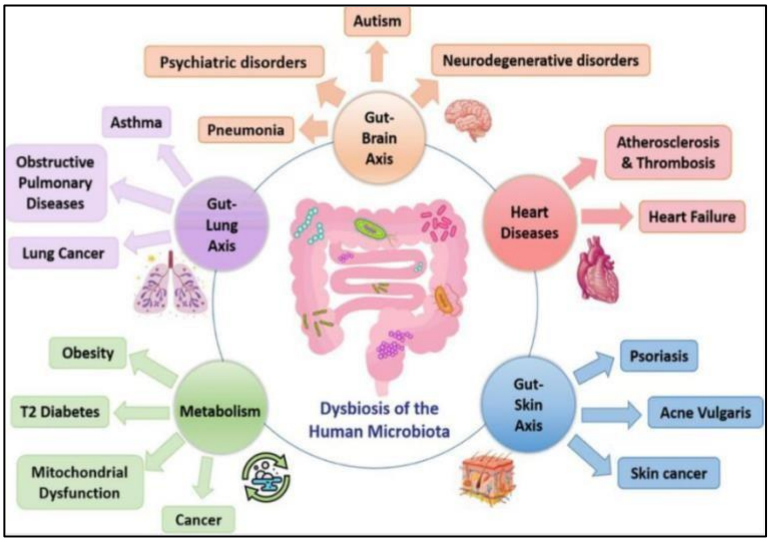

Figure 4: Diseases associated with dysbiosis in humans (Gebrayel *et al.,* 2022)

Although, dysbiosis can be treated with antibiotics to reduce harmful microbial overgrowth and restore balance to the microbiome, this approach carries the risk of promoting antibiotic resistance and complicating future treatments. Due to this, microbiome engineering offers a beneficial alternative by targeting specific microbes or modulating the microbes without the widespread effects of antibiotics, reducing the risk of antibiotic resistance.

2. Techniques for Microbiome Engineering

Microbiome engineering involves two primary strategies to manipulate microbial communities: the bottom-up and top-down approaches.

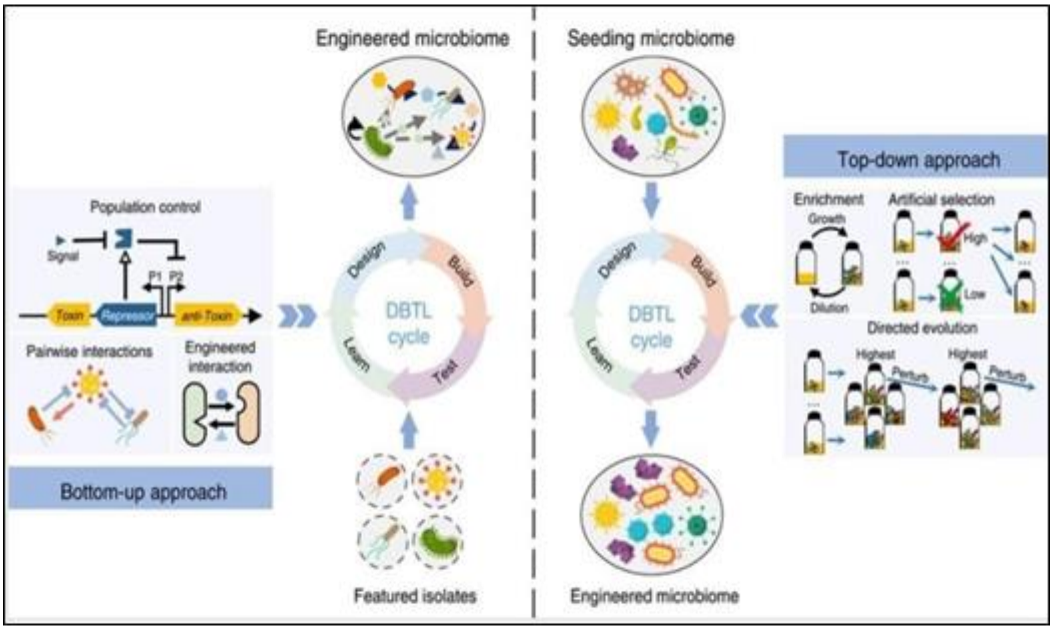

Figure 5: Bottom-up and Top-down approaches of microbiome engineering (Xiong *et al.,* 2024)

In bottom-up approach, the process begins by selecting individual microbial isolates that have been thoroughly studied for their physiological characteristics. Researchers then perform co- culture experiments to understand the interactions between these microbes, which can be altered or enhanced through genetic engineering using CRISPR-Cas systems, synthetic biology or probiotic engineering.

In contrast, the top-down approach starts with a more complex and less defined microbial community that includes uncultivated microorganisms. The goal of this approach is to guide the microbiome to self-assemble into a stable and highly functional system through methods like enrichment and direct evolution. This includes techniques like microbiome transplantation, phage therapy, etc. Both the approaches follow "design-build-test-learn" (DBLT) cycle (Xiong, *et al.,* 2024).

2.1. Bottom-up Approaches

2.1.1. Phage Mediated Microbiome Engineering:

Phage Mediated Microbiome Engineering utilizes the natural specificity of bacteriophages (viruses that infect and replicate within bacteria) to target and manipulate specific bacterial populations within a microbiome, such as the gut microbiome. This technique allows for the targeted elimination or reduction of pathogenic or otherwise undesirable strains while preserving the balance of the remaining microbial community (Tanaka *et al.,* 2024; Zelcbuch *et al.,* 2021).

Mechanism

i) Selective Targeting by Bacteriophages

Bacteriophages are host-specific and can recognize their hosts at genus, species and even at strain levels. They bind to the bacterial cell surface receptor using the distal tip of their tail. They inject their genetic material into the bacterial cell, hijack the cell's machinery to produce new phages, and eventually cause the cell to burst, releasing the new phages to target additional bacteria. This process selectively reduces specific bacterial populations without affecting other microbes in the microbiome (Tanaka *et al.,* 2024).

ii) Phage-Rebooting Technique

Phage rebooting involves genetically modifying bacteriophages to enhance their ability to combat bacterial infections, particularly antibiotic-resistant strains. This process typically takes place in a controlled laboratory setting, where phages are engineered to efficiently target specific pathogens. In cases where natural bacteriophages are unavailable or difficult to isolate, the technique can activate dormant viral DNA (prophage) within lysogenic bacteria. By triggering the prophage into the lytic cycle through molecular techniques and in-vivo DNA assembly, active virulent phages are produced. These synthetic phages can then be used to specifically reduce targeted bacterial populations within the microbiome (Zelcbuch *et al.,* 2021).

iii) Engineering of Reporter Phages

This method involves genetically modifying bacteriophages using homologous recombination or CRISPR-Cas 9 to include a reporter gene (for eg. reporter genes encoding bioluminescent proteins like NanoLuc luciferase) that produces detectable signals, such as bioluminescence or fluorescence, when the phage infects bacteria. The signal helps identify specific bacteria in the microbiome, making these engineered phages useful for diagnostic and therapeutic purposes (Zelcbuch *et al.,* 2021).

iv) Phage Cocktails

Phage cocktails are mixtures of different bacteriophages. Phage cocktails are designed to target specific bacterial strains or species. Each phage in the cocktail has a unique host range, meaning it can only infect certain types of bacteria. By combining multiple phages in a cocktail, synergistic effects can be achieved. Different phages may work together to enhance bacterial killing or prevent the development of phage-resistant bacteria. Using a mixture of phages in a cocktail increases the likelihood of effectively targeting a wider range of bacteria compared to using a single phage. Phage cocktails can adapt to changes in bacterial populations over time. If one phage in the cocktail becomes less effective due to bacterial resistance, other phages may compensate and maintain the overall efficacy of the cocktail.

Overall, the use of phage cocktails enhances the effectiveness and versatility of phage therapy by effectively targeting and controlling bacterial infections (Sanchez *et al.,* 2022).

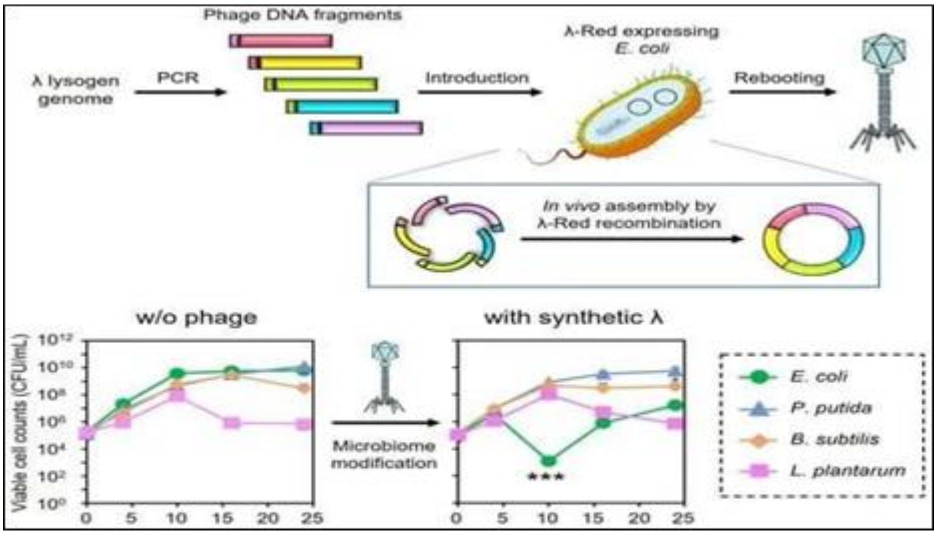

Figure 6: Mechanism of Phage Mediated Microbiome Engineering (Tanaka *et al.,* 2024)

2.1.2. Microbiome Transplantation:

Microbiome transplantation is the process of transferring beneficial microorganisms from a healthy source to a recipient to restore or modify microbial communities.This technique is increasingly recognized in both therapeutic and environmental sciences to treat diseases, improve health, and rehabilitate ecosystems by re-establishing microbial balance.

Different types of microbiome transplantation include: Fecal microbiome transplantation, soil microbiome transplantation, rhizomicrobiome transplantation, coral microbiome transplantation,etc.

i) Fecal Microbiota Transplantation (FMT)

Fecal microbiota transplantation (FMT) involves transferring fecal matter from a healthy donor into a recipient's gut to restore a balanced microbial ecosystem. This process introduces beneficial microbes that can outcompete harmful pathogens like *Clostridioides difficile,* correcting microbial imbalances (dysbiosis) (Zhang *et al.,* 2020).

The donor's stool is tested, processed, and then administered to the recipient via various methods like a nasogastric tube or colonoscopy. FMT helps reduce harmful bacteria, decrease inflammation, and promote gut health by rebalancing the microbiome and maintaining homeostasis (Lleal *et al.,* 2019; Wrzosek *et al.,* 2018).

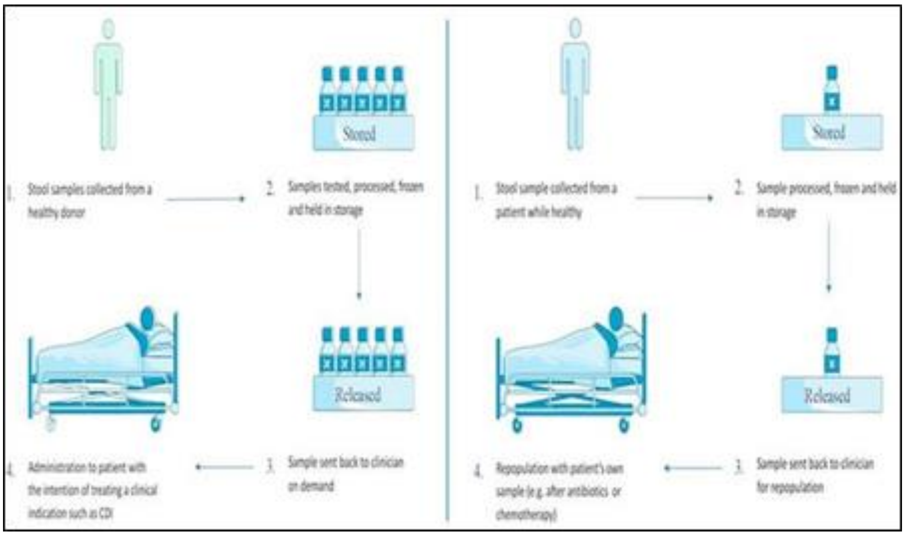

Figure 7: Schematic overview of allogenic (transfer of faecal microbiota from healthy donor to recipient's intestinal tract) and autologous (Faecal microbiota banked by a person and reinstated after medical treatment) Faecal Microbiota Transplantation (James *et al.,* 2019)

ii) Plant/ Rhizomicrobiome Transplantation (RMT)

Rhizomicrobiome transplantation involves transferring beneficial soil microorganisms from the rhizosphere (root-associated soil) of one plant to another. This method leverages the plant 'holobiont' concept, where plants and their associated microbes form an interconnected system, with microbial genomes acting as the plant's 'second genome (Bhattacharjee *et al*., 2022). These microbes play crucial roles in nutrient supply and stress protection. Transplanting a healthy, diverse rhizosphere microbiome to a plant in less diverse or sterilized soil can enhance microbial diversity, improve plant health, and increase resistance to pathogens.(Wagi *et al.,* 2023). For example, barley seedlings treated with such a microbiome showed reduced susceptibility to powdery mildew, demonstrating its potential in sustainable agriculture (Bziuk *et al.,* 2022).

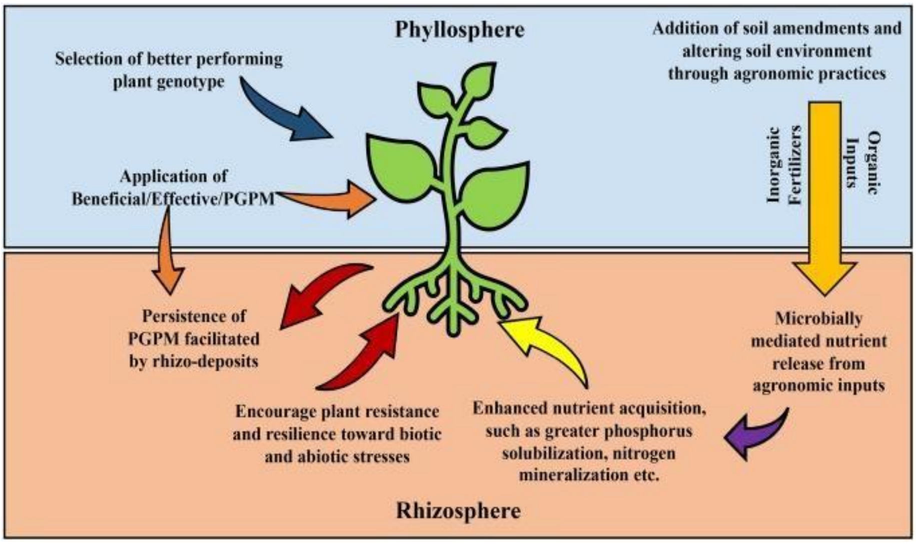

Figure 8: Rhizomicrobiome Transplantation (Kishan *et al.,* 2021)

iii) Coral Microbiome Transplantation (CMT)

Coral microbiome transplantation involves transferring beneficial microbial communities from healthy corals to stressed or diseased ones to improve their resilience. The process includes isolating bacteria, archaea, fungi, viruses, and symbiotic algae (like Symbiodiniaceae) from healthy corals. These microbes are then prepared and applied to the stressed corals either by immersion in a microbiome-rich solution or direct application to the coral's surface. Once introduced, the new microbes integrate with the coral's existing microbiome, enhancing nutrient acquisition, producing essential compounds, and providing protection against pathogens and environmental stressors (Doering *et al.,* 2021)

2.2. Top-down Approaches

2.2.1. Genetic Engineering

Genetic Engineering involves the direct manipulation of an organism's genome using biotechnological tools. This field has significantly advanced with the development of precise genetic modification techniques, which are crucial for microbiome engineering. By altering microbial genomes, researchers can create or modify microbial communities to fulfil specific functions or enhance particular traits. This approach allows for the targeted manipulation of microorganisms to improve health outcomes, optimize industrial processes, or address environmental challenges.

Mechanisms

i) Gene Editing Technologies

Gene editing technologies like CRISPR-Cas9 have revolutionized the field by providing a method for precise DNA modifications. CRISPR-Cas9 works by introducing double-stranded breaks at targeted locations within the genome. The cell then repairs these breaks, which can be harnessed to insert, delete, or modify genetic sequences. This technology has proven effective in bacterial systems for precise genome editing. Additionally, tools like TALENs (Transcription Activator-Like Effector Nucleases) and ZFNs (Zinc Finger Nucleases) also enable targeted genetic changes (Neil *et al.,* 2021).

ii) Plasmid-Based Expression Systems

Plasmids are circular DNA molecules used to introduce and express new genes in microorganisms. These plasmids often carry genes of interest along with necessary regulatory elements and selection markers. Advances in plasmid-based systems have enhanced their efficiency, allowing for high-level expression of proteins and optimization of genetic traits in microbial hosts. These systems also hold therapeutic potential, such as delivering beneficial genes to modify gut microbiota in cases of dysbiosis (Plavec *et al.,* 2021).

iii) Synthetic Biology Approaches

Synthetic biology is a revolutionary approach in microbiome engineering that involves designing and constructing new biological components or re-engineering existing ones to enhance microbiome function. This field merges biology and engineering to create microorganisms with specific, optimized functions. One key strategy is the construction of synthetic gene circuits using standardized biological parts like promoters and repressors, which are introduced into microbes to control gene expression and behavior. Another method involves engineering metabolic pathways by integrating genes for specific enzymes into the microbial genome, thereby enhancing or introducing new biochemical processes. Additionally, synthetic biology enables the creation of custom microbial communities with engineered interactions for applications in environmental remediation or therapeutics

(Contreras *et al.,* 2024).

iv) Transformation and Selection Methods

To incorporate engineered DNA into microorganisms, methods such as electroporation or heat shock shock are used. These techniques facilitate the uptake of foreign DNA by microbial cells. Following DNA introduction, selection markers, such as antibiotic resistance genes, are employed to identify and isolate successfully transformed cells. These processes are crucial for ensuring the stable expression of engineered traits in microbial populations (Neil *et al.,* 2021).

2.2.2. Prebiotic Engineering:

Prebiotic engineering is a novel approach in microbiome engineering focused on enhancing the growth and activity of beneficial gut microbes using non-digestible compounds. Unlike probiotics, which introduce live beneficial microbes, prebiotics nourish existing microbes, such as Bifidobacterium and Lactobacillus, known for their positive effects on gut health. Prebiotic compounds, like oligosaccharides and fibers, resist digestion in the upper gastrointestinal tract and reach the colon, where they are fermented by target microbes. This fermentation produces beneficial short-chain fatty acids (SCFAs) that maintain gut health by lowering the colon's pH, inhibiting harmful bacteria, and promoting beneficial microbes. Prebiotic engineering can also complement probiotic therapies, enhance the survival and effectiveness of introduced probiotics and improving gut health outcomes through a synergistic approach (Mousavinasab *et al.,* 2023).

2.2.3. Quorum Sensing

Quorum sensing (QS) is a bacterial communication system where cells produce and detect chemical signals called autoinducers. As the bacterial population grows, these signals accumulate and trigger coordinated responses when they reach a certain concentration. QS regulates various bacterial behaviors, such as biofilm formation and virulence. In microbiome engineering, QS is used to manipulate microbial communities to improve health, prevent disease, and enhance industrial processes. Mechanism.

i) Modulating Microbial Composition

One of the primary applications of QS in microbiome engineering is the ability to modulate the composition of microbial communities. By targeting specific QS pathways, researchers can selectively promote or inhibit the growth of certain bacteria within a community. This approach can be particularly useful in managing dysbiosis, where an imbalance in microbial populations leads to health issues such as inflammatory bowel disease or infections (Anton *et al.,* 2024).

ii) Controlling Biofilm Formation

Biofilms are structured communities of bacteria that adhere to surfaces and are encased in a self-produced extracellular matrix. QS is a critical regulator of biofilm formation, controlling the expression of genes involved in matrix production, adhesion, and resistance to environmental stresses. In microbiome engineering, manipulating QS can be used to either enhance or disrupt biofilm formation, depending on the desired outcome. For example, in industrial applications, promoting biofilm formation can stabilize microbial communities in bioreactors, enhancing efficiency and productivity. Conversely, in medical settings, disrupting QS-mediated biofilm formation can be used to prevent the establishment of pathogenic biofilms on medical devices or within the human body (Su *et al.,* 2023).

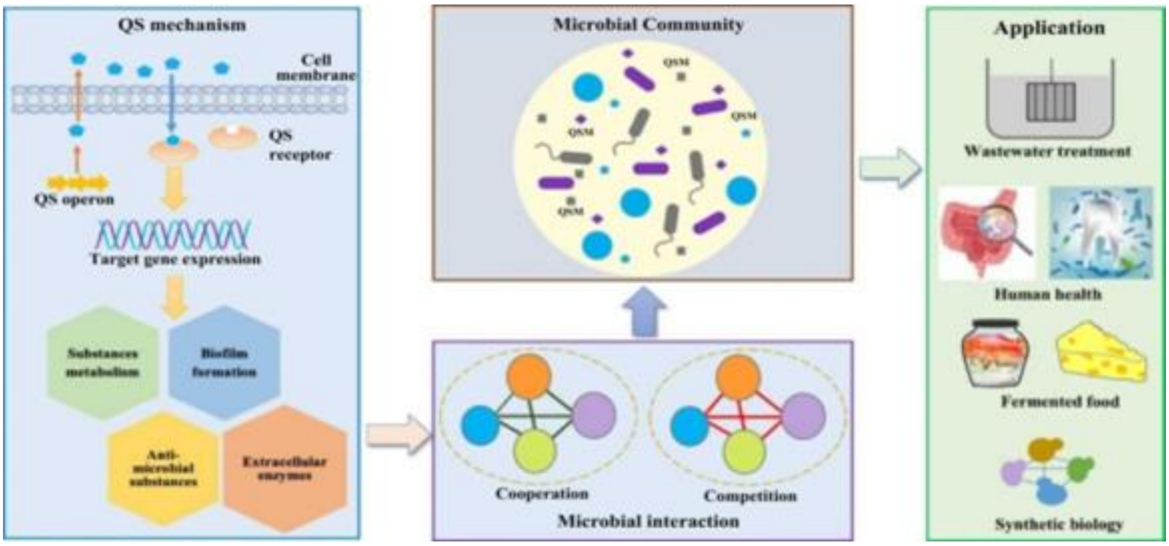

Figure 9: Quorum Sensing (X. Zeng., *et al.,* 2023)

iii) Regulating Metabolite Production

QS also plays a crucial role in regulating the production of metabolites, including antibiotics, toxins, and signaling molecules that influence host health. By engineering QS pathways, it is possible to enhance the production of beneficial metabolites while suppressing harmful ones. For instance, targeting QS pathways that regulate the production of antimicrobial peptides can boost the natural defenses of the microbiome, helping to prevent infections without the use of traditional antibiotics (Su *et al.,* 2023).

iv) Interfering with Pathogenic QS Systems

Pathogenic bacteria often use QS to coordinate the expression of virulence factors, enabling them to establish infections and evade the host immune system. In microbiome engineering, one strategy is to disrupt these QS systems, rendering the pathogens less virulent and more susceptible to treatment. This can be achieved through the use of QS inhibitors (QSI), which block the signaling pathways of pathogens, thereby reducing their ability to form biofilms, produce toxins, or resist antibiotics. QS interference thus represents a powerful tool for preventing and controlling infections, particularly in settings where antibiotic resistance is a growing concern (Anton *et al.,* 2024).

3. Therapeutic Applications of Microbiome Engineering

3.1. Gastrointestinal Disorders

Microbiome engineering offers innovative solutions for gastrointestinal disorders. Engineered *E. coli Nissle* can produce anti-inflammatory cytokines to reduce inflammation in inflammatory bowel disease (IBD), break down phenylalanine in phenylketonuria (PKU), and generate short-chain fatty acids to improve gut motility in irritable bowel syndrome (IBS). In colorectal cancer, bacteria can deliver targeted therapies like azurin to tumors, enhancing treatment effectiveness. For celiac disease, bacteria are being designed to break down gluten peptides, potentially easing the need for a gluten-free diet. Additionally, engineered bacteria can produce lactase to help digest lactose in lactose intolerance

(Isabella *et al.,* 2018).

3.2. Cancer Therapy

In cancer therapy, microbiome engineering offers innovative approaches by using engineered bacteria to deliver therapeutic agents directly to tumor sites or modulate the immune response. For example, Clostridium sporogenes has been genetically modified to release anticancer drugs specifically in the hypoxic environments typical of tumors (Allemailem *et al.,* 2021). Furthermore, Salmonella typhimurium has been engineered to produce immune-stimulating molecules like IL-2 within the tumor microenvironment, thereby enhancing the local immune response against the cancer (Mi, 2019).

3.3. Metabolic Disorders

In the management of metabolic disorders, microbiome engineering has also shown promise. For instance, in obesity, engineered bacteria can enhance the production of SCFAs, which are known to reduce fat accumulation and improve insulin sensitivity. Moreover, these bacteria can influence appetite-regulating hormones such as Ghrelin and Leptin, contributing to weight management. In diabetes, *Akkermansia muciniphila* has been engineered to improve glucose metabolism, reducing fasting blood glucose levels and improving insulin resistance (Isabella *et al.,* 2018).

3.4. Engineered Probiotics

Engineered probiotics have various therapeutic applications. They can target infections by producing antimicrobial peptides to inhibit harmful bacteria. For example, *Lactobacillus plantarum* WCSF I was developed to target *Staphylococcus aureus*, *Salmonella*, and *C. difficile*. These probiotics can also deliver therapeutic agents such as insulin (Li *et al.,* 2023). For lactose intolerance, engineered probiotics expressing lactase allow for dairy consumption without discomfort. Additionally, they can help alleviate asthma symptoms by modifying the gut microbiome and reducing inflammation (Liu *et al.,* 2021).

3.5. Mental Health

In mental health, microbiome engineering is emerging as a promising approach by modulating the gut-brain axis. Engineered probiotics, often referred to as psychobiotics, have been developed to produce neurotransmitters such as GABA, which have been shown to reduce depressive and anxiety symptoms. The gut microbiome influences sleep patterns, and disruptions in microbiota composition have been linked to sleep disorders. Engineered probiotics that modulate gut microbiota to produce sleep-promoting compounds like melatonin or improve gut barrier function are being explored as potential treatments for sleep disorders, including insomnia and circadian rhythm disruptions (Santi *et al.,* 2023).

4. Environmental Applications of Microbiome Engineering

4.1. Improving Plant Health

Microbiome engineering manipulates the microbial communities associated with plants to promote beneficial outcomes such as improved nutrient uptake, enhanced resistance to pathogens, and increased stress tolerance.

i) Biocontrol

Biocontrol involves using engineered bacteria to manage plant diseases. This includes using bacteria like *Burkholderia ambifaria* to produce antimicrobial compounds, employing RNA interference to silence pathogen genes, and engineering bacteria to produce beneficial compounds or enhance natural herbicides. Rhizosphere microbiome transplantation (RMT) was also used to study plant immune responses eggplant (*Solanum melogena*) against bacterial wilt caused by *Ralstonia solanacearum* (Jing Ke *et al.,* 2021).

ii) Biofertilization

Biofertilization centers on optimizing the availability of essential nutrients like nitrogen (N) and phosphorus (P) to plants. Engineering nitrogen-fixing bacteria, such as through the refactoring of nitrogenase gene clusters, has enabled non-leguminous crops to fix atmospheric nitrogen, reducing the reliance on chemical fertilizers. Similarly, the engineering of rhizobacteria to express diverse phytase enzymes has improved phosphate solubilization, making this nutrient more accessible to plants and potentially reducing the need for phosphate fertilizers (Sang *et al.,* 2022).

iii) Biostimulation

Biostimulation involves the enhancement of plant growth by manipulating microbial production of plant hormones. Engineered bacteria have been developed to produce auxins like indole acetic acid (IAA), which promote plant growth, as well as to degrade ethylene precursors, thereby reducing ethylene-induced growth inhibition. Furthermore, the production of other hormones such as cytokinins, abscisic acid, and gibberellins by engineered microbes can enhance plant resistance to various stresses (Jing Ke *et al.,* 2021).

iv) Enhancing Stress Tolerance

Microbiome engineering enhances plant stress tolerance by optimizing associated microbial communities. Introducing beneficial microbes or modifying existing ones can improve plant resilience to stressors like drought, salinity, and disease. For example, engineering the soil microbiome with bacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* has been shown to increase drought resistance in tomato plants by improving water retention and nutrient availability (Ali *et al.,* 2023).

4.2. Climate Mitigation

Climate change is one of the most growing challenges of our time, driven primarily by rising levels of atmospheric carbondioxide (CO_2) and other greenhouse gases. Addressing this issue requires innovative strategies for carbon sequestration and ecosystem management. One approach is the application of microbiome engineering to enhance natural processes that stabilize carbon in ecosystems. Microbes play a crucial role in carbon cycling by transforming carbon into stable forms that can be stored in soil and other ecosystems for extended periods. By strategically introducing or manipulating microbial communities within natural environments to improve their ability to sequester carbon and thus mitigate climate change (Silverstein *et al.,* 2023).

4.3. Coral Resilience

Microbiome Engineering enhances coral resilience by optimizing associated microbial communities to improve stress tolerance and health. For example, researchers have successfully used microbiome engineering to increase heat tolerance of corals by inoculating them with heat-resistant strains of symbiotic algae *Symbiodiniaceae*. This approach helps corals withstand their ability to recover from environmental stress. By improving the resilience of corals to stressors like temperature changes and diseases like black band disease, brown band disease, the white syndrome or band diseases (including stony coral tissue loss disease) and yellow band disease, microbiome engineering supports more effective coral reef restoration and conservation (Jie Li *et al.,* 2023).

4.4. Waste Biorefinery

Waste biorefinery uses microbiome engineering to convert waste into valuable products like biofuels and biochemicals through top-down and bottom-up approaches. The top-down approach utilizes natural microbial communities by manipulating environmental conditions (e.g., pH, temperature) to steer their metabolic activities toward desired outcomes. In contrast, the bottom-up approach designs synthetic consortia based on known metabolic pathways. Both approaches have been successfully used in processes such as biohydrogen production from sewage sludge and PHA production from food waste (Wu and Wang, 2024).

4.5. Wastewater Treatment

Microbiome engineering improves wastewater treatment by using natural bacterial communities to break down pollutants and contaminants more sustainably than traditional methods. It involves enhancing microbial interactions and introducing specific strains, such as *Pseudomonas putida* for degrading dyes in textile wastewater. This approach is also applied to artificial environments to manage biofouling and promote beneficial microbes for anticorrosion and self-healing (Jie Li. *et al.,* 2023).

5. Challenges

Microbiome engineering, while promising, faces several challenges that must be addressed to fully realize its potential. Key challenges include (Khan *et al.,* 2021):

i. **Complexity of Microbiome Interactions**: Microbiomes are intricate ecosystems with diverse microbial communities interacting in complex ways. Understanding these interactions and how they influence host physiology or ecosystem functions is challenging. The dynamic nature of microbiomes means that engineered modifications might have unpredictable effects, making it difficult to ensure stability and efficacy.

ii. **Variability Between Individuals**: Human microbiomes vary significantly between individuals due to factors like genetics, diet, lifestyle, and environment. This variability complicates the development of one-size-fits-all solutions and requires personalized approaches to microbiome engineering.

iii. **Ethical and Safety Concerns**: Engineering microorganisms raises ethical and safety concerns, particularly regarding the potential for unintended consequences or ecological disruptions. Ensuring that engineered microbes do not cause harm to humans, animals, or the environment is crucial.

iv. **Technical Limitations**: Current technologies for microbiome engineering, such as genetic modification and synthetic biology, are still evolving. Limitations in these technologies can hinder the precise manipulation of microbial communities and the development of effective engineered solutions.

v. **Regulatory Hurdles**: The regulatory framework for microbiome engineering is still under development. Navigating regulatory requirements for testing and approval of engineered microbes can be complex and time-consuming, potentially slowing the pace of innovation.

vi. **Understanding of Phageomes**: Although phages play a significant role in microbiome dynamics, our understanding of the phageome and its interactions with the microbiome is still limited. This gap in knowledge can impede the effective use of phages in microbiome engineering.

vii. **Long-Term Effects**: The long-term effects of introducing engineered microorganisms into microbiomes are not fully understood. Monitoring and assessing these effects over extended periods are essential to ensure that engineered solutions do not have adverse long-term consequences.

viii. **Integration with Existing Therapies**: Incorporating microbiome engineering into existing medical and agricultural practices presents challenges in terms of integration, compatibility, and overall effectiveness. Ensuring that engineered solutions complement rather than disrupt current therapies is important for successful implementation.

6. Future Prospects

i. **Precision Medicine:** Developing new methods to customize microbiome-based therapies for more effective treatment of a wider range of diseases.

ii. **Advanced Diagnostics**: Developing sophisticated tools for real-time monitoring and diagnostics of microbiome health and dynamics, leading to earlier detection of imbalances or diseases.

iii. **Integration with AI and Machine Learning**: Utilizing artificial intelligence to predict and model microbiome behaviour, interactions, and responses to various interventions, enhancing the precision and effectiveness of microbiome engineering approaches.

iv. **Microbiome-based Therapies**: Creating new forms of therapy that leverage microbiome engineering to treat a wider range of conditions, including neurological disorders, autoimmune diseases, and mental health issues.

v. **Expansion to Other Ecosystems**: Extending microbiome engineering techniques to a broader range of ecosystems beyond human health and agriculture, such as aquatic environments, deserts, and urban areas.

vi. **Enhanced Host-Microbe Interactions**: Developing methods to better understand and manipulate the interactions between hosts and their microbiomes, potentially leading to novel treatments and enhancements in host health and performance.

vii. **Long-term Impact Studies**: Conducting extensive longitudinal studies to understand the long-term effects and stability of microbiome engineering interventions on health, ecosystems, and environmental sustainability (Yadav *et al.,* 2021).

Conclusion:

Microbiome engineering represents a transformative approach to harnessing the power of microbial communities for therapeutic and environmental benefits. Understanding the diverse types of microbiomes—human, plant, and soil—provides a comprehensive perspective on how these ecosystems function and interact with their environments. The concepts of normobiosis and dysbiosis further illuminate the balance and disruption within these microbiomes, highlighting the need for precise interventions to restore and maintain microbial health.

The reviewed approaches to microbiome engineering, including phage-mediated engineering, genetic modifications, and probiotic strategies, demonstrate significant potential for addressing a range of therapeutic and environmental challenges. From enhancing human health and combating diseases to advancing ecosystem restoration and sustainability, microbiome engineering offers promising solutions.

However, the field faces several challenges, including the complexity of microbial interactions, the need for robust and scalable technologies, and ethical considerations. Future research must focus on overcoming these obstacles, improving our understanding of microbiome dynamics, and developing innovative strategies to apply microbiome engineering effectively across diverse contexts.

As we look ahead, the integration of microbiome engineering into therapeutic and environmental domains holds the potential to revolutionize our approach to health and sustainability. Continued advancements and interdisciplinary collaborations will be crucial in unlocking the full potential of microbiome engineering and addressing the pressing needs of our global society.

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