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ASSESSING THE VARIOUS TECHNIQUES OF MICRO BIOME ENGINEERING IN TREATING GASTROINTESTINAL DISEASES: A REVIEW

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Abstract: Microbiomes : microbial communities exist consistently in or on living hosts, such as humans, animals, plants, in soil, water and air. The microbiome includes micoorganisms, like bacteria, fungi, viruses, and their genes. Microorganisms in microbiomes interact dynamically with their hosts and among themselves to form synergistic relationships. These interactions determine the composition of the microbiome and influence not only the features of the ecosystems where they reside, but can also have effects affecting the physiology of the hosts. The disease development is often correlated with microbial dysbiosis, or a shift in the microbiota. Gut dysbiosis may lead to Neurological, Gastrointestinal diseases, Celiac disease, Liver diseases, Allergies etc. Other reasons for dysbiosis and gut disorders are medications, unhealthy lifestyles, and genetic conditions. Current review focuses on studying effectiveness of Microbiome techniques in treating gut disorders as Microbiome engineering gaining more attention from few decades. Microbiome engineering probably applied most extensively on the human microbiome due to the potential of manipulating microbiota in humans for treatment of diseases. Allopathy often focuses on symptom suppression and acute intervention, while microbiome therapy aims to restore ecosystem (human) balance to address root causes. Microbiome techniques like Probiotoics, FMT, Phage therapy, omics studies are proving their effectiveness in treating the GI conditions. Engineering methods like FMT, Genetically engineered bacteria, prebiotic, probiotics are gaining success. Products like **Rebyota,** *SER-109 / Vowst* are successful for treatment of *C. difficile* infection. Although certain limitations of microbiome engineering are like poor Engraftment, Colonization Challenges & risks of altering the human microbiome detailed studies and research in combination with smart tools will lead to new trend in disease treatment. Microbiome techniques are thus effective, safe & have clinical applications in treating GI disorders.

Key words: Microbiome, Microbiome techniques/ engineering, Microbes, Gut disorders.

Introduction

Microbiome: The microbiome is collection of all microbes, such as bacteria, fungi, viruses, and their genes, that naturally reside on human bodies and inside body. Microbiome (in Greek micro means small' and bios means life) i.e. the community of microorganisms that can usually be found living together in any given habitat. It was defined more precisely in 1988 by Whipps *et al.* as "a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatre of "activity". The microbiota consists of all living micro organisms forming the microbiome. Most microbiome researchers agree bacteria, archaea, fungi, algae, and small protists should be considered as members of the microbiome. Although microbes are so small that they require a microscope to see them, they contribute in big ways to human health and wellness. The microbiome can affect metabolism, development, immunity, and other aspects of human health. Imbalances in the microbiome may alter an individual's health status. The 'hygiene hypothesis' suggests, that loss of microbial diversity in the human microbiome may underlie the increase in the incidence of asthma. Bacteria exert their effects not only locally where they reside, but also at other sites of the human body; they release molecules that travel to other tissues, such as the liver or brain. It is estimated that gut microbial metabolites represent 10% of the metabolites found in mammalian blood and their effects on hosts are only starting to be identified. As a result of these novel insights, animals are no longer considered autonomous units but, rather, 'holobionts', the ensemble of the host and its microorganisms.[1]

The unique characteristic of each ecosystem is a result of the complex network of interactions within the microbial communities, between the microorganisms and the hosts, as well as responses to the metabolites that are produced by the microbial communities. Thus, perturbation to microbiomes can cause extensive changes to the hosts and environment, such as reduced host fitness and soil fertility. By engineering microbiomes, microbial compositions can be altered to improve host phenotypes, illness and benefit ecosystems.[2] Employing these engineering methods for treating diseases can prove much effective as they aim to modulate the composition and functions of the gut microbiome to develop targeted therapies as compared to medicines. Microbiome engineering is an emerging field; to develop targeted biotherapies for ailments. Novel advancements in this field for certain ailments can emerge or be competitive to pharmaceutical products.

Microbiome Engineering: Microbiome is multifarious and dynamic ecological element in which different species such as bacteria, fungi, archea, protozoa & viruses are in continual flux. Microbiome engineering is an artificial experimental method for analyzing and developing existing microbiomes/ microbial compositions to improve host phenotype and benefit ecosystem. Microbiome engineering encompasses a range of approaches, such as faecal microbiota transplantation, synthetic biology techniques, and the use of engineered live biotherapeutic products. These strategies leverage genetic engineering tools to precisely manipulate the gut microbiome, introducing beneficial microbial strains or altering the functions of existing pathogenic microbes. [3]

Different Techniques of Microbiome Engineering [4]

S.N.	Techniques/ approaches of Microbiome Engineering		
	Additive **Aim**- To supplement existing gut micro biota with beneficial natural or engineered microorganisms	**Substractive** **Aim**- To eliminate deleterious members of the micro biome	**Modulatory** **Aim**-Altering the composition or activity of endogenous micro biota
1.	Probiotics	Antibiotics	Feed enzyme
2.	Synthetic biology	Chemicals	Physical / material-based delivery systems**.**
3.	Microbial consortia	Peptides	Acceptance & maintenance of foreign DNA,
4.	Prebiotics	Bacteriophages	Genome editing,cellular/genetic engineering**,** horizontal gene transfer
5.	Targeted delivery system	Fecal micro biota transplantation,	Signalling Molecules, phage-mediated techniques**,**

Table 1: Different Techniques of Microbiome Engineering

Human microbiota

Microbiome research is advancing fastly in biotechnological, agricultural field and also in pharmaceutical sector over the past few decades and has now become a topic of great scientific and health interest. The field of microbiome research emerged from environmental microbiome research and later evolved into viewing eukaryotes as inseparable from the microbial community with which they share space. Microbiome is related to ecosystem which mainly involves soil and the human body. Human body is system where trillions of micro organisms coexist with the host. The scientific term "microbiome" therefore refers to the set of genes of all microorganisms that inhabit almost all human body parts. The microbiome is thus considered as a second genome that has a symbiotic relationship with the host. This relationship may be positive or beneficial, negative or pathogenic, or neutral; hence, microbiome interactions play a key role in human health. There is a symbiotic relationship between human cells and the microbial community that dwells in the human body.[5] In humans, there are approximately 39 trillion microbial cells, encoding nearly 20 million microbial genes. In contrast, human bodies with approximately 30 trillion human cells possess a little more than 20,000 human genes. This vast difference of a factor of 102 to 103 in microbial gene number has an impact on immunity, behavior and health in humans as shown below.[6]

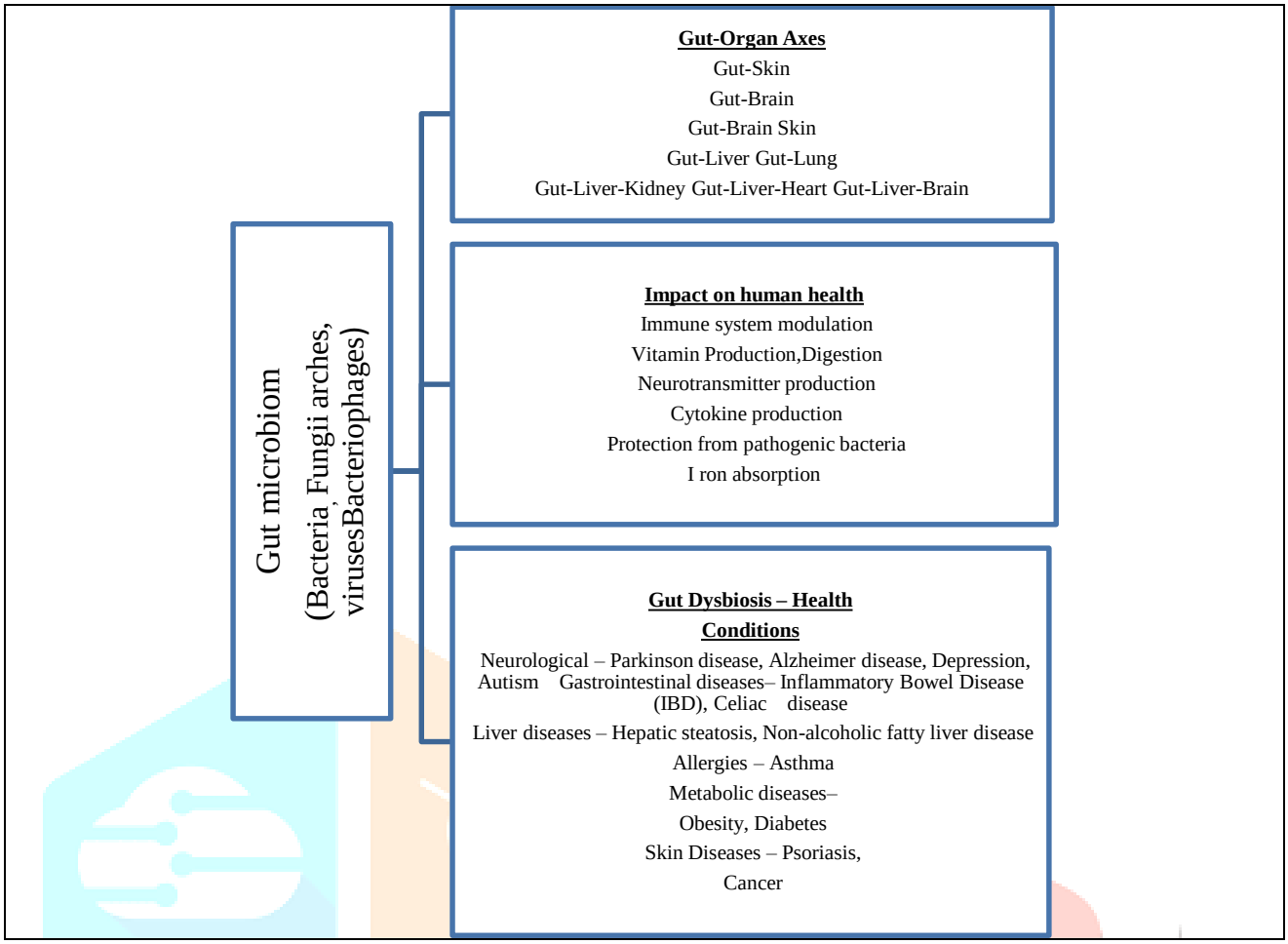

Figure 1: Impact of Microbiota on Human health

The complex and diversified microbiome operates as a functional expansion of host genomes with an estimate of 50- to 100-fold more genes. These extra genes contribute to the regulation of host physiology by possessing various types of enzymatic proteins, influencing the produced metabolites and thus affecting host metabolism.The beneficial interplay of the host and its microbiome is responsible for maintaining the host's health, whereas disease development is often correlated with microbial dysbiosis, or a shift in the microbiota. As such, pathogens therefore represent only a tiny fraction of microorganisms, whereby the altered composition of the microbiome promotes the emergence and outbreak of pathogens. The vast majority of microbes are crucial for ecosystem functioning as well as beneficial interactions with other microbes, contributing to population dynamics and functional activities. Thus, opportunistic pathogens show that host–microbe interactions depend not only on the host but also on the entire microbiome.

The microbiota comprises all living members that form the microbiome, which encompasses bacteria, archaea, fungi, algae, and small protists. The members of microbiome also extend to viruses, phages, and mobile genetic elements─one of the most controversial inclusions in the definition of a microbiome. The microbiome has been defined to pertain to not only the community of microorganisms but also the whole spectrum of molecules produced by microorganisms, including their structural elements, metabolites, and molecules produced by the coexisting host. Microbial composition varies individually and amongst different anatomical parts; it is highly personalized. The exact definition of a healthy microbiota has yet to be defined, but studies have shown that the use of probiotics, prebiotics, and synbiotics are beneficial by maintaining healthy body flora or by altering the microbiome toward a healthy microbial ecosystem.

Beneficial bacteria, primarily from the *Firmicutes* and *Bacteroidetes* phyla and key beneficial bacteria like *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium* promote a strong gut barrier, efficient digestion, and immune regulation are considered to be an ideal gut microbiome. The core microbiota is the microbial community that is constantly associated with a given host genotype or a specific environment, whereas transient microbiota changes over time. By identifying these differences, an appropriate experimental, methodological,

and statistical design can be applied to refine the approach taken in microbiome studies for therapeutic applications.

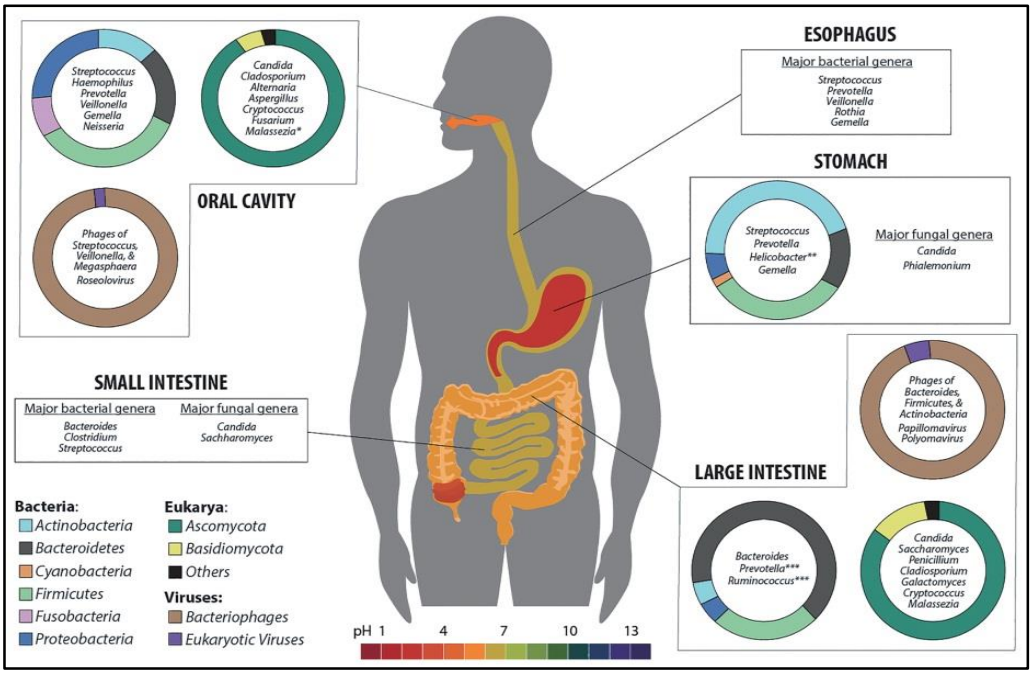

Figure 2: Different Microbial flora present in humans [7]

S. n.	Body site	Predominant microbes
1.	Mouth	Bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, and Spirochaetes
		Fungal genera: *Candida*, *Cladosporium*, *Saccharomycetales*, *Fusarium*, *Aspergillus*, and *Cryptococcus*
2.	Stomach	Bacterial phyla: Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria
3.	Intestines	Bacterial phyla: Firmicutes and Bacteroides
		Archaeal species: *Methanosphaera stadtmanae* and *Methanobrevibacter smithii*
4.	Nose	Bacterial phyla: Actinobacteria, Firmicutes, and Proteobacteria
5.	Airway and lungs	Bacterial phyla: Firmicutes, Proteobacteria, and Bacteroidetes
		Fungal species: *Candida albicans*, *Ceriporia lacerata*, *Saccharomyces cerevisiae*, and *Penicillium brevicompactum*
		Viruses: *Herpesviridae*
6.	Skin	Bacterial phylum: Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria
7.	Bladder	Bacterial phylum: Firmicutes
8.	Vagina	Bacterial phylum: Firmicutes (*Lactobacillus*)

Table 2: List of different Microbial phylas in various organ system[7]

1.1. Gut Microbiome.[7,8]

The gut microbiome is composed of bacteria, viruses, archaea, protists, and fungi, with a total cell number even higher (by around 12%) than the host itself. These microorganisms typically inhabit the small intestine and colon in the lower GI tract, where they perform a dynamic and diverse array of metabolic activities. Across the mucosa, biomolecules are under constant flux between the host and the microorganism, which eventually connects the gut microbiota to the whole body.

Such bidirectional communication and host-microbiome interactions are initiated immediately after birth with microbial consortia delivered from the mother. Gut microbiota continues to diversify, followed by a second stage that is relatively resilient to environmental factors (e.g., antibiotics and age.) The composition of the gut microbiota changes at three stages in life: from birth to weaning; from weaning to obtaining a normal diet; and finally, during old age. After birth there are rapid changes in gut microbiota i.e. the stage of initiation of formation of microbiome. At birth Facultative anaerobes are the first to colonize the gut followed by obligate anaerobes (like *Bifidobacterium* and *Bacteroides* spp). More abundance and variety of *Bifidobacterium* spp

Are found in 3days. When child is subjected to solid food gut microbiome is diversified with the abundance of Firmicutes. From the age of three children microbiome develops fully like an adult. With the increase in age there is decline in microbial diversity and at old age reduction in number of microbiome. Due to these developmental differences, the gut microbiota exhibit high variability among different individuals and also at different locations in the GI tract. Greater similarities exist among family members, possibly originating from shared environmental factors and/or genetic relatedness.

- **Oral Cavity -** The oral cavity is the principal entry point to the inner human microbiome, and thus, microbes residing in this area can potentially spread to different body sites and cause disease. The composition of the oral microbiome therefore plays a vital role in providing immunity for human health. The human oral cavity has versatile microbiomes, including bacteria, fungi, viruses, and protozoa, among others. There are two regions in the oral cavity colonized by microorganisms─dentures, or the hard surfaces of the teeth, and the soft tissue of the oral mucosa.
- **Dental caries-** The bacteria involved are *Streptococcus mutans, Streptococcus sobrinus*, and Lactobacillus acidophilus, Bifidobacterium, Actinoimyces, Atopobium etc. When acid-producing bacteria residing in the oral cavity interact with the fermentable carbohydrate found in food it starts dental decay. Tooth decay / cavities are also caused by demineralization of the tooth as a result of low pH /acidic environment.
- **Gingivities-** In Prolonged Gingivities the subgingival plaque accumulation rearranges the microflora causing damage to the supporting connective tissue and the bone that fixes the teeth to the jaws.
- **Defence -** Nitrate metabolism by the microbiome reduces nitrate to nitrite. Nitrite is then converted to nitric oxide, which has an antimicrobial effect and is crucial for vascular health. Some oral microorganisms such as *Streptococcus salivarius* strain K12 produces a bacteriocin that restrains the growth of Gram-negative species associated with periodontitis disease contribute to host defense.

As oral cavity is entry gate for microorganisms it contributes to several chronic diseases such as endocarditis, osteoporosis, and rheumatoid arthritis and also in the development and progression of noncommunicable diseases such as obesity, diabetes, cancers and neuropsychiatric disorders. Thus, it has been proposed that the oral microbiome could potentially be used to assess the risk for certain diseases.

- **Gastric/stomach-** The stomach prominently has acidic environment, highly presented phyla in the gastric mucosa under normal conditions are Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria. The gastric juice has a diverse microbial community that differs from the gastric mucosa. The phyla in gastric juice are Firmicutes, Actinobacteria, and Bacteroidetes, whereas Proteobacteria and Firmicutes are dominant in the gastric mucosa. Bacteria found in the oral cavity and duodenum such as *Veillonella*, *Lactobacillus*, and *Clostridium* can transiently colonize the stomach.
- **Gastric infection-** *H. pylori* is the dominant bacterium in the stomach of *H. pylori*-infected patients, and most *H. pylori* strains can modulate the gastric environment, thus altering the habitat of resident microorganisms. Furthermore, alterations in the gastric microbiome community can increase the risk for developing gastric cancer.
- **Intestines-** The gut is the most densely and diversely colonized organ a vast majority of commensal bacteria reside in the colon, whereas a lower bacterial population is found in the stomach and small intestine. The main bacterial phyla present in the gut are Firmicutes and Bacteroides, which make up 90% of the gut

microbiota. Others are Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Meanwhile, the predominant genera in Bacteroidota are *Bacteroides* and *Prevotella*. The less abundant Actinobacteria phylum is largely represented by *Bifidobacterium*. Under the Proteobacteria phylum, some well known pathogens include *Enterobacteria, Helicobacteria, Shigella, Salmonella & E. Coli*

- **Microbiome for digestion-** Gut bacteria regulate digestion by processing nutrients and metabolites such as short-chain fatty acids (SCFAs), bile acids, amino acids, etc. and leads to facilitate host energy harvesting and metabolic efficiency. Metabolic output depends on microbes as well as extrinsic factors like diet, medications, lifestyle etc.
- **Immunity-** Some of these members act against pathogenic bacteria and prevent bacterial invasion by maintaining intestinal epithelium integrity. Microbe-synthesized metabolites potentially mediate crosstalk between the metabolic, immune, and neuroendocrine systems, thus governing host wellness. Imbalance in gut microbiome changes the permeability allowing opportunistic pathogens to invade and colonize empty places, changing the gut environment. This may lead to the production of dys regulated metabolites that are potentially harmful to the host, causing a range of diseases. Increased gut permeability also permits the entrance of microbe-derived products such as metabolites, virulence factors, and other luminal components, disrupting the gut microbiome's normal function and contributing to aberrant immune-inflammatory responses such as inflammation, allergy, and autoimmune disorders mediated by molecular mimicry and a dys regulated T cell response.

Other microbiome community leaving in gut are viruses and bacteriophages that make up the vast majority of gut microbiota's viral components. Dominant archaeal species such as *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* are also found in the gut microbiome. Studies of the gut have shown that specific species of an individual's microbiota are very stable and persist for a year or more.

Factors Influencing the Human Microbiome[9]

Presence of Microorganisms in different parts of body can be contributed to their favourable growth conditions. They are present on the human body's external and internal parts as well as entrance sites. The external sites like skin, eyes, and exposed area under the nails houses microorganisms. The microorganisms can enter body via are the respiratory tract (mouth and nose), gastrointestinal tract (oral cavity), urogenital tract, and broken surfaces on the skin. While internal parts of the body include the lungs, gut, bladder, kidneys, and vagina occupied by microbes. The specific communities of human gut microbiomes are influenced by interindividual and intraindividual variation throughout the life cycle. Some of the factors that affect variations in the microbiome include the intestine's anatomical regions, mode of delivery, method of milk feeding, weaning period, age, diet, and antibiotic treatments etc. other than this factors that can contribute to alter the microbiome are as follows:

- **Physiologoical factors** - Residence of these microorganisms to the specific body site is because of their adaptation to the favorable conditions in the human microbiome that resemble their preferred natural environment. Environmental factors such as temperature, pH, oxygen concentration, pressure, osmolarity, and nutrient source contribute to the diversity and abundance of microorganisms at different sites of the body. For instance, our body temperature is optimal for housing many different types of microbes. Also the gut environment varies between different anatomical regions in terms of physiology, digest flow rates, substrate availability, host secretions, pH, and oxygen tension. Factors like the presence of nutrient sources like sebum change the skin's pH and also act as a carbon source, facilitating the growth of certain groups of microbes. Prominent location rich in microbial flora in humans is small intestine. Intestine is lines with dense layer of mucus that covers the intestinal epithelium which not only serves as a carbon source for microbes but also provides attachment sites for bacterial adhesion.

Physiology- The abundance and diversity of the human microbiota is dependent on intrinsic and extrinsic factors. Intrinsic factors include the nature of body environments, as previously described, as the physiology of habitat sites facilitates the growth of some microbes. Moreover, the mode of delivery during birth has been shown to influence the microbiome. For example, newborns delivered via the vaginal versus Caesarean delivery possess different groups of dominating gut microbiome. However, at the age of 3, the gut microbiome changes

to resemble that of the adult's gut microbiome. As people reach beyond the age of 70, the ability to digest food and absorb nutrients in the gut changes, affecting the composition of the gut microbiome. With decreasing immune activity in older adults, this also contributes to changes in the overall microbiome as they are more susceptible to pathogens─thereby influencing the core microbiome. As *Bifidobacterium* spp. stimulates the immune system and metabolic processes, a decrease in Bifidobacteria may result in malnutrition and low systemic inflammatory status in older adults. Altogether, the human microbiome thrives in optimal growth conditions, depending on the natural environment of the body. When the natural environment of the body is altered, this results in microbial composition and diversity shifting to adapt to the changing environment, potentially resulting in disease.

- **Genetics -** Intrinsic factors that contribute to the microbiome's composition include genetics, ethnicity, gender, and age. The human microbiome is generally stable and resistant once the microorganism has adapted to the environment but may cause a shift in the microbiome over time.

- **Environmental exposure –** Geographic location, pollution, climate, and exposure to pets or bacteria in the environment shape the skin and gut microbiome.

- **Disease and medications –** Medicines like NSAIDs (Aspirin, Ibuprofen), Anticholinergics, antipsychotics, Antibiotics (Penicillin, Clindamycin), Proton Pump Inhibitors (PPIs) upon frequent administration can cause dysbiosis as these medications may cause conditions like Constipation (Anticholinergics, Antipsychotics, Calcium channel blockers, and Iron), Diarrhea (Metformin, Fibrates, ACE inhibitors, and PPIs), Colitis/Inflammation (Antibiotics, NSAIDs, and some immunosuppressants), Gastroesophageal Reflux Disease (Drugs weaken the lower esophageal sphincter).

Engineering of human microbiome

The most diverse and largest population of microbiota resides in gastrointestinal tract. Human microbiome consist mainly of Bacteriodes (45%), Fermicutes (40%) majorly whereas Proteobacteria and Actinobacteria along with other phyla are present in less proportion. These phyla play critical roles in digestion, immune modulation, and maintaining gut homeostasis. An imbalance in their ratio is often associated with diseases such as obesity and inflammatory bowel disease. Microbiome involved in Immune modulation, metabolic regulation, Gut-brain communication, Vitamin synthesis, Pathogen resistance etc. These physiological roles when disrupted can lead to autoimmune diseases, allergies, Obesity, Type 2 diabetes Depression, Parkinson disease, Vitamin deficiency (e.g., B12, K). It highlights the microbiome's essential contribution to health and its potential as a target for therapeutic interventions.[10]

Extensive experimentation can be done on the human microbiome due to the potential of manipulating microbiota in humans for treatment of diseases, given that the human microbiome has been found to influence the physiology of the host. Dysbiosis to the gut microbiome causes gastrointestinal diseases, such as ulcerative colitis, *Clostridium difficile* infection (CDI) and inflammatory bowel disease (IBD), Crohns disease, ulcerative colitis, and irritable bowel syndrome as well as non-gastrointestinal conditions, such as diabetes, autism and metabolic syndrome. Dysbiosis can trigger systemic inflammation and metabolic endotoxemia. Certain gut microbes produce trimethylamine-Noxide (TMAO), which has been linked to atherosclerosis and cardiovascular events. The involvement of gut microbiota in neurodevelopmental and neurodegenerative diseases is gaining evidence, with alterations noted in Alzheimer's, multiple sclerosis, and schizophrenia. The administration of beneficial microbial strains (probiotics) or dietary substrates that favor their growth (prebiotics) shows promise in restoring microbial balance and mitigating disease symptoms.[10]

Microbiome Engineering techniques like Prebiotics, probiotics, synthetic biology, genome editing, FMT, targeted delivery systems, designer microbiome etc. are getting successful. Fecal microbiota transplantation (FMT) has gained much attention for microbiome engineering to re-establish healthy microbial composition in patients with gastrointestinal diseases. This method involves acquiring fecal material from healthy donors and administering microbiota-laden suspension obtained from the fecal material to the gastrointestinal tract of the

patients. Most notably, FMT has shown great potential for treating recurrent CDI. Traditionally, CDI has been treated by using broad-spectrum antibiotics such as metronidazole and vancomycin to eliminate *C. difficile*.

FMT has demonstrated efficacy in treating recurrent *Clostridioides difficile* infections, with ongoing research in metabolic and neurological disorders. Postbiotics (microbial metabolites) and synbiotics (combination of probiotics and prebiotics) represent next generation interventions with potential for safer, more targeted therapies. Advancements in metagenomic and metabolomic profiling have enabled the identification of microbial biomarkers for early disease detection and personalized therapeutic strategies. While the microbiome presents exciting opportunities for preventive and therapeutic healthcare, several challenges remain.

Gut Disorders/ diseases & Microbiome Techniques used therein:[11-22]

Gut disorders like CDI, IBD, UC, CD, Pouchitis, Refractory Immunotherapy-Associated Colitis, *Helicobacter pylori* Infection, Irritable Bowel Syndrome (IBS), Small Intestine Bacterial Overgrowth (SIBO), Hepatic Encephalopathy, and Chronic Constipation are now seen in many people around. Allopathic treatment often provides highly effective, evidence-based (lab investigations) treatments that can induce long-term remission, managing symptoms, improve quality of life. Also prevent complications using pharmaceuticals, biologics, and, when necessary, surgery and in some specific cases "cure" the acute infection or condition, many of these are chronic, relapsing diseases that are managed rather than permanently cured. Allopathy often focus on symptom suppression and acute intervention, while microbiome therapy aims to restore ecosystem (human) balance to address root causes. Allopathic treatments, such as antibiotics and non-antibiotic drugs (e.g., Rifaximin, Metronidazole, PPIs, metformin), can disrupt the gut microbiome, whereas microbiome treatments (probiotics, prebiotics, fecal transplantation) aim to restore it. Microbiome techniques aims to increase beneficial bacteria (e.g., *Bifidobacterium*) and modulate the immune system thus potentially reduces dependency on drugs, offers personalized solutions based on individual bacterial composition, and aims at long-term resolution of chronic issues.

Gut microbiota in the GI tract is an important component of the host's health because it controls the cells of the local and distant organs including the brain. The gut-brain axis (GBA) means bidirectional communication between the gastrointestinal tract and the neurological system. This is possible through brain signals, hormones, the immune system, and the gastrointestinal microbiota. Bidirectional transmission in the GBA plays a major role in modulating brain dysfunction, maintaining symbiosis with the host and modulating innate as well as adaptive immune responses. The normal gut microbiota synthesizes microbial metabolites and neurotransmitters that interact with host cells including intestinal epithelial cells and immune cells. Diet-induced alterations in gut microbial composition and the products generated by the microbiota affect immune-mediated neurological conditions including developmental disorder, neurodegenerative disorder, and emotional dysregulation.

The human microbiome shows early signs of being able to reprogram malnutrition, increase nutrient absorption, and utilize energy from a range of food stuff. Apart from that, microbes play a role in the metabolism of xenobiotics. Various human gut bacteria alter the chemical forms of drugs, toxins, and many insecticides during xenobiotic metabolism. The gut microbiota and host immunity are interconnected, complex, and variable. The gut microbiome has been linked to many intestinal and extra-intestinal diseases. Most extensive research into the relationship between gut microbiota and its role has been done in primary GI disorders including IBDs, irritable bowel syndrome (IBS), colorectal cancer (CRC), chronic liver diseases or pancreatic disorders.

Microbiome-based therapies are used for gut disorders because an imbalanced gut, or dysbiosis, is a primary driver of illnesses like IBD, IBS, and infections such as *C. difficile*. Targeting the microbiome restores healthy bacteria, strengthens the gut lining, modulates the immune system, and repairs metabolic function, rather than just treating symptoms.

The most successful and established application is for recurrent *Clostridioides difficile infection (CDI),* with therapies like Vowst and Rebyota approved to prevent relapse.

Other GI diseases and conditions being treated or studied using microbiome technologies include:

Microbiome technology—encompassing fecal microbiota transplantation (FMT), specialized probiotics (live biotherapeutic products), prebiotics, and synthetic biology (engineered bacteria)—is increasingly used to treat gastrointestinal (GI) diseases by correcting dysbiosis and restoring a healthy gut environment.

Infectious & Inflammatory Conditions

I. Recurrent *Clostridioides difficile* Infection (CDI)

The opportunistic pathogens comes from the resident site of the microbiome, when the healthy nondisease state of the gut microbiome is disturbed, causing the failure of colonization resistance against the pathogenic member. An example is *Clostridium difficile*, which exists in the normal gut microbiota but becomes pathogenic when the healthy nondisease microbiome state is disrupted.

Symptoms- Include diarrhea, pseudo membranous colitis, sepsis, and death. *C. difficile* may damage the cytoskeleton and colonic epithelial barrier integrity, inducing aberrant inflammatory response and cell death.

Microbiome Engineering Curative Techniques for CDI

1. Fecal Microbiota Transplant (FMT) / Fecal Microbiota Products (FMPs):

Mechanism: Involves transferring screened donor feces into a patient's colon via colonoscopy, enema, or capsule to increase microbial diversity, promote nutrient competition, and restore metabolic functions, such as producing secondary bile acids.

Recent advances include the development of FDA-approved products like **Rebyota (RBX2660)**, a standardized, lab-manufactured, filtered, and frozen suspension of fecal microbiota.

2. Live Biotherapeutic Products (LBPs) & Defined Consortia:

Mechanism: These are "designer" microbiome therapies consisting of specific, cultured bacterial strains (often Firmicutes spores) rather than a whole-stool mixture.

e.g. (SER-109 / Vowst): FDA-approved, it uses purified Firmicutes spores to compete with *C. difficile* for nutrients and restore the bile acid metabolism needed to inhibit spore germination.

e.g. (VE303)*:* A defined consortium of eight *Clostridia* strains designed to combat *C. difficile*.

3. Genetically Engineered Probiotics:

Mechanism: Uses synthetic biology to equip bacteria with circuits that detect the inflamed gut environment and act on it.

Researchers have engineered *Escherichia coli* Nissle 1917 to detect sialic acid (a marker of dysbiosis) and produce the enzyme bile salt hydrolase **(Cbh)**. This enzyme converts taurocholate (a germinant) into cholate (an inhibitor), directly reducing *C. difficile* growth.

4. Targeted Strain Approach (Non-Toxigenic *C. difficile*):

Mechanism: Administering non-toxigenic *C. difficile* (NTCD) strains to colonize the gut and compete for the same niche, preventing the toxigenic strains from establishing, which has shown promise in trials (e.g., NTCD-M3).

Mechanisms by which *C. difficile* infection can be controlled

a. **Metabolic Restoration:** Restoring bacteria that convert primary bile acids into secondary bile acids, which inhibit *C. difficile* germination.

b. **Nutrient Competition:** Introducing bacteria that consume essential amino acids (e.g. proline) required for *C. difficile* growth.

c. **Antimicrobial Production:** Using bacteria that produce bacteriocins (e.g., thuricin CD) that target *C. difficile*.

d. **Biofilm Disruption:** Engineered probiotics can reduce the formation of biofilms, which protect *C. difficile* and lead to recurrence.

These techniques are particularly focused on treating recurrent CDI **(rCDI)**, where antibiotic treatments have failed and the normal protective microbiome has been destroyed.

II. Inflammatory bowel disease (IBD):

Inflammatory bowel disease (IBD) is a group idiopathic, chronic, and relapsing gastrointestinal inflammation with two common forms: ulcerative colitis (UC) and Crohn's disease (CD).

CD- Inflammation occurs at any location along the entire GI tract.

UC- Inflammation is restricted to the large intestine.

Symptoms- Recurring fever, diarrhea, and abdominal pain results in gut dysbiosis.

Microbiome Engineering Curative Techniques for IBD:

a. **Ulcerative Colitis (UC):** FMT and engineered probiotics are used to treat intestinal health but FMT regimens have shown ability to induce remission.

b. **Crohn's Disease (CD):** Probiotics are used to manage symptoms, while FMT is used in specialized cases.

c. **Pouchitis:** Probiotic combinations (specifically, 8-strain formulations) are used to prevent relapse after ileal pouch-anal anastomosis.

d. **Refractory Immunotherapy-Associated Colitis:** FMT has successfully treated colitis caused by cancer immunotherapy.

e. **Helicobacter pylori Infection:** Probiotics (e.g., *Lactobacillus* and *Saccharomyces boulardii*) are used as adjuncts to standard triple therapy to improve eradication rates and reduce antibiotic-induced diarrhea.

f. **Synthetic biology:** Genetically modified bacteria (e.g., *Lactococcus lactis*) are designed to colonize the gut and produce anti-inflammatory factors like cytokines (IL-10, IL-35) or trefoil factors to repair the mucosal barrier.

g. **Genome edition for engineered bacteria:** Bacteria are engineered with "sense-and-respond" circuits that detect inflammation markers, such as specific metabolites or excessive Reactive Oxygen Species (ROS), and only release drugs precisely when and where needed.

h. Other techniques involve Targeting therapeutic delivery, Microbiome modulation by bacteriophages, Engineered yeast, Engineered bacteria out-membrane nanovesicles.

III. Irritable Bowel Syndrome (IBS)

Functional & Metabolic Disorders

Irritable bowel syndrome (IBS) - A common, chronic functional disorder of the gut-brain interaction.

Symptoms- Recurring abdominal pain, cramping, bloating, and altered bowel habits (diarrhea, constipation, or both)

Microbiome Engineering Curative Techniques for IBS:

Microbiota-Based Therapies:

a. **Probiotics-** Probiotics are living microorganisms which commonly comprises gut-friendly bacteria and sometimes also yeast, and are ingested in the form of foodstuffs and supplements. With the known pathophysiology of IBS, consistent use of probiotics has been demonstrated in previous studies to improve symptoms associated with IBS particularly with the Biofidobacterium and Lactobacillus strains[19] . Other bacteria which are helpful as probiotics are combinations of strains Bifidobacterium, Lactobacillus, and Streptococcus genera, the yeast Saccharomyces boulardii[20] , bacteria B. infantis[22] and Lactobacillus

rhamnosus GG [21] albeit with differing results. S. boulardii, L. rhamnosus.

B. infantis in combination probiotics was able to reduce IBS symptoms (bloating and abdominal pain), but not B. infantis alone; probiotics seem to have beneficial effects on improving IBS, how they function is still relatively unknown.

A disadvantageous feature of probiotics is that most have a short lifespan and thus repeated doses are necessary.

b. Prebiotics- Are compounds in food that promote growth or activity of beneficial microorganism such as bacteria and fungi. The most common environment concerning their effects on human health is the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome.

Example is lactulose, which is shown to increase gut bacteria, enhance water retention in stools, and is thus associated with laxative effects. Other prebiotics include fructo-oligosaccharides (FOS), soybean oligosaccharides, galacto-oligosaccharides (GOS), isomalto-oligosaccharides, xylo-oligosaccharides, and transgalacto-oligosaccharides (TGOS). The fructan inulin, cellulose, hemicellulose, reflux starch, and pectin are polysaccharide prebiotics.

Many sources of prebiotics exist in nature including cereals, fruits and vegetables. Lactulose, lactosaccharose, FOS, GOS, and cyclodextrins are artificially synthesized prebiotics that can be commonly found as food additives or components in food production. The benefits of prebiotics to gut health are multi-pronged. Commensal bacteria in the colon can ferment prebiotics to produce short chain fatty acids (SCFAs) such as acetate, butyrate and propionate. For instance, most strains of Bifidobacterium and Lactobacillus can utilize FOS.

c. Synbiotics- Synbiotics refer to the combination of probiotics and prebiotics in food ingredients or supplements in a form of synergism. In theory, synbiotics should be more potent or efficacious than their probiotics or prebiotics components used in singularity.

IV. Small Intestine Bacterial Overgrowth (SIBO) - Occurs when there is an excessive bacteria grow in the small intestine.

Symptoms- Chronic bloating, abdominal pain, diarrhea, and nutrient mal absorption.

Microbiome Engineering Curative Techniques for SIBO: Microbiome modulation (diet + antibiotics) is used to control bacterial overgrowth and associated diarrhea.

a. **Targeted Non-absorbable Antibiotics:** The primary approach is the use of non-absorbable, narrow-spectrum antibiotics such as **rifaximin**, which eradicates small bowel bacteria while largely preserving the colonic flora. For methane-dominant SIBO (IMO), a combination of rifaximin and neomycin is used.

b. **Strain-Specific Probiotic Therapy:** Contrary to the idea that adding bacteria to an overgrowth is harmful, specific probiotics are used to out compete pathogens.

e.g Saccharomyces boulardii: A yeast probiotic frequently used because it is unaffected by antibiotics, allowing it to be administered concurrently to mitigate symptoms.

***e*.g. Lactobacillus and Bifidobacterium Strains**: Specific strains such as *Lactobacillus casei* and *Lactobacillus acidophilus* have demonstrated effectiveness in reducing H2 levels and alleviating symptoms.

c. Synbiotic Therapy: A combination of probiotic bacteria and prebiotics is used to support the growth of beneficial bacteria, for instance, a combination of *Bacillus coagulans* and FOS.

d. **Prokinetics and Motility Support:** Since low motility is a root cause of SIBO, prokinetic agents (5-HT4 receptor agonists) are used to enhance the migrating motor complex (MMC), acting as a physical, rather than chemical, engineering of the microbial habitat.

e. **Nutritional/Dietary Modification:** The low-FODMAP (Fermentable Oligosaccharides, Disaccharides,

Monosaccharides, and Polyols) diet is used to reduce the fuel source for bacteria in the small intestine, thus reducing fermentation and symptoms.

f. **Botanical (Herbal) Antimicrobials:** Studies have shown that herbal preparations, such as berberine or oregano, can be as effective as rifaximin in resolving SIBO, particularly for those who fail standard antibiotic therapy.

These techniques are increasingly used in combination, with studies showing that probiotics can increase the success rate of antibiotic therapy from 41% to 62%.

V. Non-Alcoholic Fatty Liver Disease (NAFLD) & NASH:

NAFLD -Excessive fat buildup in the liver unrelated to alcohol,

NASH- NASH is the form of NAFLD in which there is inflammation of the liver and liver damage, in addition to fat in liver i.e. from simple steatosis to Non-alcoholic Steatohepatitis.

Symptoms- Generally silent, but can cause fatigue or right upper quadrant discomfort.

Microbiome Engineering Curative Techniques for NAFLD & NASH:

Probiotics and FMT are used to reduce liver enzymes, hepatic steatosis, and inflammation.

a. Fecal Microbiota Transplantation (FMT): Replaces a dysbiotic (imbalanced) microbiome with one from a healthy donor to restore gut microbial diversity, which can improve intestinal barrier permeability and reduce intrahepatic lipid accumulation.
b. Probiotics & Next-Generation Probiotics: Administration of live beneficial bacteria, such as *Lactobacillus*, *Bifidobacterium*, and *Akkermansia muciniphila*, to reduce hepatic fat, decrease lipopolysaccharide (LPS) levels, and improve liver enzyme levels (ALT/AST).
c. Prebiotics: Indigestible carbohydrates (e.g., inulin, fructo oligosaccharides) that selectively promote the growth of beneficial, short-chain fatty acid (SCFA)-producing bacteria.
d. Synbiotics: Combinations of probiotics and prebiotics designed to synergistically restore gut balance, reducing liver fat and fibrosis.
e. Bacteriophage Therapy: Highly specific viruses used to target and eliminate pathogenic bacteria, such as cytolytic *Enterococcus faecalis* or high-alcohol-producing *Klebsiella pneumoniae*, to prevent liver injury.
f. Engineered Probiotics: Genetically modified bacteria, such as *Lactobacillus reuteri* engineered to secrete IL-22, designed to enhance intestinal barrier integrity and reduce liver inflammation and steatosis.
g. Postbiotics: Bioactive metabolites produced by bacteria (e.g., butyrate, indole) or heat-killed bacteria, which provide the benefits of probiotics without the risk of colonization, helping to maintain intestinal homeostasis.
These methods aim to address the "gut-liver axis" dysfunction by reducing endotoxemia, lowering endogenous ethanol production, and increasing the levels of beneficial short-chain fatty acids like butyrate.

VI. Hepatic Encephalopathy- Is a temporary, reversible decline in brain function caused by severe liver disease, where the liver fails to filter toxins—primarily ammonia—from the blood.

Symptoms- range from mild confusion (covert) to coma (overt), including behavior changes, slurred speech, and motor issues. It is generally treated with medication Lactulose, Rifaximin and prebiotics, probiotics and FMT are used to reduce toxins.

Microbiome Engineering Curative Techniques for Hepatic Encephalopathy:

Probiotics are used to reduce ammonia production by urease-positive bacteria.

Microbe-targeted therapies: Increase potentially beneficial taxa Prebiotics

a. Prebiotics- Are compounds in food that enhance the growth or activity of beneficial

microorganism such as bacteria and fungi. Example is lactulose, fructo-oligosaccharides (FOS), soybean oligosaccharides, galacto-oligosaccharides (GOS), isomalto-oligosaccharides, xylo-oligosaccharides, and transgalacto-oligosaccharides (TGOS). The fructan inulin, cellulose, hemicellulose, reflux starch, and pectin are polysaccharide prebiotics.

b. Probiotics- Probiotics are living microorganisms which commonly comprises gut-friendly bacteria and sometimes also yeast are ingested in the form of foodstuffs and supplements. Bacteria which are helpful as probiotics are combinations of strains Bifidobacterium, Lactobacillus, and Clostridium butyricum and Bifidobacterium infantis the yeast Saccharomyces boulardii.

A disadvantageous feature of probiotics is that most have a short lifespan and thus repeated doses are necessary.

c. Synbiotics- Synbiotics refer to the combination of probiotics and prebiotics in food ingredients or supplements in a form of synergism. Synbiotics decrease blood ammonia and endotoxins by modifying gut microbiota, specifically increasing non-urease-producing Lactobacillus species while reducing pathogenic E. coli and Staphylococcal species in HE. Commonly used combination is Bifidobacterium longum (lactobacillus) and fructo-oligosaccharide.

VII. Chronic Constipation: Costipation can be cured by modifying the gut microbiota with symptoms of slow-transit constipation using probiotics, prebiotics, and synbiotics.

a. Prebiotics-Help to relieve constipatrion as they retain water in the feces, creating softer, heavier stools that are easier to pass. Prebiotics breakdown into chort-chain fatty acids act which increase bowel movements also add up the beneficial bacteria, which can be low in patients with chronic constipation.

b. Probiotics- Bifidobacterium lactis and Lactobacillus acidophilus strains are used as probiotics in constipation.

c. Synbiotics- Are combination of probiotics and prebiotics are effective for managing constipation by reducing colonic transit time, increasing defecation frequency, and improving stool consistency. They often include mixtures of Bifidobacterium and Lactobacillus strains combined with prebiotics like fructooligosaccharides (FOS) or inulin,

Advantages over conventional/ allopathy treatments

1. Engineered Microbes are live biotherapeutics that can produce personalized, on-demand, targeted drugs which replaces systemic, inflexible drugs with localized, responsive agents.
2. Engineered microbes can be designed to deliver drugs directly to the site of disease (e.g.in the case of Crohn's disease), enhancing bioavailability and reducing systemic toxicity (oral steroids).
3. Conventional drugs have to follow dosage regimen i.e. frequent dosing but engineered microbes can be engineered for long-term implantation in the gut, providing continuous, "autonomous" production of therapeutic molecules.
4. Microbiome treatments can be tailored to the specific microbial profile of an individual patient, allowing for personalized, precise, and highly effective interventions which indicates high Precision and Individualization
5. In Microbiome treatments "Smart" bacteria can be programmed with genetic circuits to sense specific disease biomarkers and release therapeutic drugs only when necessary thus contributing to less or no side effects.
6. This approach is promising for treatment of Complex & chronic diseases that have limited success with standard methods, such as metabolic disorders, IBD, and infections, by targeting the root cause (dysbiosis) rather than just the symptoms.

7. Fecal Microbiota Transplantation) restores complex ecosystem diversity, treats the whole system, not just a single molecular pathway technique has a success rate of over 90% for *C. difficile*, which is often superior to antibiotic therapies that can worsen long-term diversity.
8. Pre/Probiotics Improve microbiome composition and health safely. Often safer with fewer side effects than many pharmacological interventions.

Limitations

1. Poor Engraftment and Colonization Challenges & risks of altering the human microbiome. Due to poor colonization, these therapies frequently produce inconsistent effects or no effect at all, requiring frequent re-administration, unlike conventional medication regimes.
2. Conventional drugs typically provide a predictable pharmacological effect across large populations, whereas microbiome therapies are highly personalized.
3. Lifestyle factors like diet, age, and genetics heavily influence the efficacy of the treatment, making it harder to establish a standardized, uniform treatment plan.
4. Stability- Engineered microbes may undergo mutations or evolve, potentially becoming virulent or carcinogenic, creating severe safety concerns and Technical Limitations
5. Horizontal Gene Transfer (HGT): There is a risk that engineered microorganisms could transfer their genetic modifications to native, beneficial microbes, leading to unpredictable long-term health consequences.
6. Limited Regulatory and Safety Frameworks

Applications

Microbes are nonseparable part of human life and environment thus Microbiome engineering techniques have a large scope around human life like:

1. Human Health & Therapeutics- Modifying bacteria to target pathogens or deliver therapeutic molecules directly to the gut.
2. Engineering microbes to treat phenylketonuria (PKU) by metabolizing harmful compounds or treating inflammatory bowel diseases (IBD) through secretion of anti-inflammatory cytokines in Metabolic Disease Management.
3. Enhancing natural flora to prevent colonization by harmful pathogens leads to resistance against various infections.
4. Agriculture & Food Production- Engineering soil- or plant-associated microbiomes to improve nutrient cycling, plant resilience to salinity/drought and disease resistance i.e. plant growth & stress management.
5. Replacing chemical pesticides and fertilizers with beneficial microbial consortia.
6. Modulating food fermentation processes with engineered microbes to improve nutrition, flavor, and prevent spoilage adding to food safety & quality.
7. Environmental & Industrial Applications- Designing specialized microbial communities to break down pollutants and toxic chemicals in the environment.
8. Engineered microorganisms can be used to improve the conversion of waste into biofuel or other value-added products.

Future Trends

Microbiome engineering is shifting toward precise, personalized, and functional modulation, focusing on "designer" probiotics to treat chronic diseases and "synthetic consortia" for key trends include

1. Using CRISPR to tailor bacteria for targeting pathogens or metabolic disorders and implementing AI to model complex microbial interactions.
2. Genetically Engineered Probiotics & Living Medicines that can colonize the gut, detect environmental signals (e.g., inflammation), and release therapeutic compounds, such as in the treatment of metabolic disorders and kidney stones.
3. Synthetic Microbial Communities (SynComs): Rather than using single strains, a major trend is creating tailored consortia of microbes (SynComs) to ensure stable, robust, and functional colonization of the gut or plant rhizosphere.

4. CRISPR-Based Gene Editing: Advanced genetic tools allow for precise modification of microbes *in situ*, allowing scientists to edit bacteria directly within a complex, natural community.
5. AI and Computational Modeling: Machine learning is increasingly used to predict microbial behavior, interactions, and the long-term impact of therapeutic interventions on the ecosystem.
6. Agricultural Microbiome Optimization: Engineering the plant rhizosphere microbiome is being used to increase agricultural resilience against drought, reduce the need for fertilizers, and protect crops from pathogens.
7. Next-Generation Sequencing (NGS) and Diagnostics: High-throughput sequencing (metagenomics and metabolomics) is being used to identify and quantify the functional output (metabolites) of the microbiome in real-time.

Conclusion

The scientific term "microbiome" refers to the set of genes of all microorganisms that inhabit almost all human body parts. The microbiome is considered as a second genome that has a symbiotic relationship with the host. This relationship may be positive or beneficial, negative or pathogenic, or neutral; hence, microbiome interactions play a key role in human health. Gut microbiome are now recognized as dynamic contributors to host physiology, immune regulation, metabolic homeostasis, and neurobehavioral health. Medicines like antibiotics, NSAIDs, Anticholinergics, antipsychotics, Proton Pump Inhibitors (PPIs) upon frequent administration can cause dysbiosis. Dysbiosis may be responsible for illnesses like Neurological, Gastrointestinal diseases, Liver diseases, Allergies, Metabolic disorders, Skin disorders etc. Personalized microbiome profiling, biomarker discovery, and intervention strategies such as probiotics, prebiotics, synbiotics, fecal microbiota transplantation (FMT), and even genetically engineered bacteria are reshaping the clinical landscape. Microbiome engineering techniques hold great therapeutic potential not only for treatment but also for prevention and risk assessment in various GI disorders. Engineering methods like FMT, Genetically engineered bacteria, prebiotic, probiotics are gaining success. Products like **Rebyota,** *S*ER-109 / Vowst are successful although limitations like lack of standardization in sampling and analysis, regulatory concerns, and ethical considerations present challenge to scientific advances into routine clinical applications. Combining microbiome research into precision medicine might open the door to individualized therapies tailored to a persons unique microbial flora. To understand and apply the full potential scope of ME more research and optimization is required. But applications in engineering microbes using CRISPER with gene editing & disease curing by correcting the root cause, nurturing healthy flora makes it superior/ useful technique in health care sector. Thus on concluding remark ME is effective, safe & has clinical applications in treating GI disorders. Newer trends or research has large scope in development of this techniques also combining with smart tools like AI, ME is progressive and will be promising method in upcoming era.

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