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Biochemical Mechanisms of Beneficial Bacteria in Maintaining Human Health and Preventing Disease

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Abstract

This article discusses the importance of the trillions of bacteria that inhabit the human body, especially in the digestive tract, make up the human microbiome, a complex ecosystem that is essential to preserving health. The article highlights the role of beneficial bacteria (probiotics) as a key component that contributes to regulating microbial balance, improving digestion, supporting the immune system, and producing beneficial bioactive compounds. The article explains that the microbiome consists of various types of bacteria, most notably major groups such as Firmicutes and Bacteroidetes, and that this system develops from birth and gradually stabilizes during the first year of life. Maintaining the balance of this microbial community is essential for health, while its imbalance (dysbiosis) leads to various diseases, including obesity, immune disorders, and infectious diseases. The article also explains the main mechanisms of action of beneficial bacteria, which include: (1) It inhibits the growth of harmful microbes by competing for food and space and secreting inhibitory substances. (2) It regulates the immune system by stimulating immune responses and maintaining their balance. (3) By generating vitamins, short-chain fatty acids, and other bioactive molecules, it aids in metabolism. Since dietary habits have a big impact on microbiome diversity, the article also emphasizes the importance of nutrition. Beneficial bacteria are encouraged by diets high in fiber and plant-based foods, but this diversity is decreased by diets high in fats and carbohydrates. The ideas of probiotics, prebiotics, and synbiotics as well as their function in promoting gut health are also covered. Finally, the article highlights the importance of beneficial bacteria in preventing infectious diseases, both in the digestive system and beyond, by boosting immunity and preventing pathogen colonization. It also explains their role in reducing the effects of antibiotics and limiting serious infections such as *Clostridium difficile*. In general, the article emphasizes that understanding the microbiome and beneficial bacteria opens up broad prospects for improving health and preventing diseases through nutrition and modern therapeutic interventions.

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1. Introduction

The human body constitutes a distinct ecological microcosm that is inhabited by billions of living organisms. Humans are home to trillions of bacteria, viruses, fungus, protozoa, and other microbes ^[1]. The Consortium named the Human Microbiome Project has conducted extensive research on the composition and functions of these organisms, which have critical implications for maintaining human health and preventing disease ^[2]. Among these organisms, beneficial bacteria, colloquially known as probiotics, represent an important component. This category of microbes primarily improves the host's health by altering the human microbiome, which is essential for metabolism, digestion, the production of bioactive compounds, and the growth and

upkeep of the immune system^[3]. Probiotics are a promising supplementary strategy for treating and preventing a number of illnesses, including obesity and psychiatric problems, that are brought on by imbalance of the microbiome. Thanks to developments in systems biology, sequencing technologies, clinical validations, and the creation of high-throughput screening tools, probiotic knowledge and applications have grown significantly in recent years^[4].

2. The human microbiome: composition and habitats

Numerous microbial organisms inhabit the human body. This community, referred to as the human microbiome, consists of different microbes, including bacteria and viruses^[5]. Several sites in the human body host unique and diverse microbiomes, including the mouth, colon, skin, and vagina. About 99% of all microorganisms are found in the gastrointestinal system, which is home to the majority of the human microbiota^[2]. Among the microorganisms inhabiting the human body, bacteria predominate. Bacteria are classified in many ways: according to their size, structure, shape, motility, metabolism, etc. The human microbiome is classified into several phyla according to the ribosomal ribonucleic acid (rRNA) present in the bacterial species^[6]. Over 99% of the microbiota in the human gut is made up of Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Fusobacteria. After solid food is introduced at roughly one year of age, the diversity of the human gut microbiota stabilizes and continues to grow across the postnatal period^[7]. The early months of life are crucial for the growth and stability of the gut microbiota since these are when colonization occurs. Bacteria behave in a commensal manner, giving the human host advantageous nutrients in exchange for an environment that is conducive to growth. Each bacterium is useful and vital in its own way. A good balance of these microbiota in the human body significantly promotes human health; once disrupted by internal or external factors, the microbiota may lead to serious diseases^[8,9].

3. Mechanisms of action of beneficial bacteria

The lifespan of human beings is significantly influenced by the balance between health, disease, and aging^[10]. By improving diet, sanitation, and cleanliness, as well as by preventing and treating illnesses and diseases, modern science and technology have increased the average life span. However, aging leads to decreased physical and mental performance, declined immunity, and an increased risk of various diseases (e.g., degenerative, infectious, cancer, allergies)^[11]. Wellness (not only the absence of illness), national health, healthy longevity, and the quality of life across all age groups are increasingly highly valued by society and the government. Exploring the means for optimizing the maintenance of health and restoring health after injuries or adverse health effects incurred by external reasons is high on the agenda, especially to achieve a healthier life without an increase in health expenditure^[12]. Beneficial bacteria are widely acknowledged to help restore and maintain health and wellness^[13].

It is well established that beneficial bacteria exert positive effects on health through three major actions: (1) attachment to the mucous layers and colonization of different organs, followed by competitive exclusion of pathogens through nutrient competition, intestinal surface occupation, and secretion of inhibitory substances; (2) modulation of the immune system, which involves promotion of IgA secretion,

stimulation of dendritic cell functions, induction of infection-level I-type immune responses, enhancement of both cellular and humoral immune responses to the co-administered antigens, and the regulation of balance of different T-helper cell subtypes; and (3) metabolic contributions and production of bioactive compounds that include vitamins, anti-cancer agents, anti-cholesterol agents, anti-atherosclerosis agents, anti-allergic agents, anti-oxidants, and detoxification substrates^[14].

3.1. Colonization and competitive exclusion

After birth, bacteria can swiftly colonize the mucosal surfaces of mammals. Human milk oligosaccharides (HMOs) are prebiotics that promote the formation of short-chain fatty acids by stimulating the growth of Bifidobacteria in newborns and exerting strong metabolic control over the gut microbiome. Commensals that produce bacteriocin strengthen colonization resistance to foodborne infections.^[15] In order to create bile acid derivatives that ward off non-resident bacteria like Salmonella 4, gut microorganisms import dietary cholesteryl esters. By preventing the production of virulent factor, bile salts limit the growth of numerous intestinal infections. Find other references in the insights offered, in addition to its involvement in intestinal inflammation and dysbiosis (induced by diets rich in fat and low in fiber)^[16].

3.2. Modulation of the immune system

The body must maintain vigilance against its own cells and non-pathogens in the environment that pose no threat, even while its defensive armament protects against pathogens. The complex process of immune system equilibrium or homeostasis is controlled by enteroendocrine hormones, regulatory cells, probiotic-secreted bioactive substances, and possibly commensal symbionts that stimulate the immune system through conjugates or metabolites. The development of mucosal and systemic immunity is probably greatly influenced by the microbiome, which also continues to have an impact on both systems throughout adulthood. While significant post-antibiotic changes are linked to dysregulated development, some high-abundance taxa in early life are linked to the formation of a robust immunological repertoire. In general, an early-life high burdensome microbiome raises the risk of immune-related disorders.^[17; 18 ; 19]

3.3. Metabolic contributions and production of bioactive compounds

The comprehensive review of the human gut microbiota published in 2019 in the journal Nature Microbiology points out that host health is sustained through microbiota-mediated nutrient exchange, immune modulation, pathogen exclusion, and bioactive compound production^[20]. Fermentation of substrates that escape digestion produces short-chain fatty acids, protein fermentation generates branched-chain fatty acids, and the degradation of polysaccharides leads to bioactive compounds such as Vitamin-K2, polysaccharides, and anthocyanins^[21]. Lactic acid, acetic acid, and propionic acid of probiotic bacteria introduce low pH to hosts, supporting good health^[22]. Global nutrient depletion intensifies the microbiota–host interaction which accentuates the decision by lactose probiotics (Bifidobacterium adolescentis, Lactobacillus rhamnosus, etc.) on whether to flourish in intestine or subside in intestinal tract. Probiotic-rich fermented foods like yogurt, cheese, milk, etc. improve

the absorption of lactose and macronutrients while lowering the risk of lactose intolerance ^[23].

4. Beneficial bacteria in food and nutrition

The colony of good bacteria that live in the digestive system, known as the gut microbiota, is intimately related to human health. The processes by which these microbes work and the possibility of using them for medicinal purposes are being discovered by science. This information is particularly pertinent to food and nutrition, which are important venues for consuming beneficial microorganisms ^[13]. As a result, researchers are investigating strategies like probiotics, prebiotics, and synbiotics to see how they affect gut microbiota and associated health outcomes. ^[24].

4.1. Probiotics: definitions, strains, and applications

When ingested in sufficient amounts, probiotics—live microbes, typically bacteria—benefit human health. They are included in fermented foods, food supplements, and pharmaceuticals. Probiotics must reach the intestines alive and be able to attach to intestinal surfaces; they should also survive in substrates such as milk, yogurt, or cereal ^[25]. The genera *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Saccharomyces*, and *Streptococcus* contain common probiotic bacteria ^[26].

4.2. Prebiotics and synbiotics

Prebiotics are indigestible food components that promote the development or activity of advantageous microbes. Oligosaccharides including lactulose, inulin, and galactooligosaccharides are the most prevalent prebiotics. Probiotics and prebiotics together to improve human health are known as synbiotics ^[27].

4.3. Dietary patterns and microbiota diversity

Food consumption impacts gut microbiota. Greater microbiota diversity is associated with higher intake of fibre, legumes, nuts, fruits, vegetables, and fish, and lower intake of total fat, saturated fat, red and processed meat, sweetened beverages, and refined grain ^[2].

4.1. Probiotics: definitions, strains, and applications

Probiotics are live microorganisms that confer health benefits when administered in adequate amounts ^[28]. The majority of them are lactic acid bacteria (LAB), which the US FDA deems harmless. With more than 300 species, LAB comprises more than 50 genera in six families, including *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, and others. The creation of effective probiotic strains is a crucial business scenario, and the worldwide market is expanding quickly, particularly in Europe ^[29]. Growing consumer awareness and the health advantages of probiotics are anticipated to fuel industry expansion. Microorganisms must fulfill three essential requirements in order to be categorized as probiotics: they must be alive at the time of administration, be given in an adequate dosage, and benefit the host. They must be safe for human use and identifiable at the strain level ^[30].

4.2. Prebiotics and synbiotics

Prebiotics are non-digestible carbohydrates, typically polysaccharides or oligosaccharides. Because they resemble the natural substrates for saccharolytic organisms, they encourage the growth of certain beneficial microbes in the digestive tract, such as *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, and *Eubacterium* ^[13].

Synbiotics were defined as food ingredients, additives, or dietary supplements containing live microorganisms and prebiotic substrates, selectively stimulating the growth or activity of beneficial autochthonous microorganisms in the host gastrointestinal tract ^[31]. The health effects of synbiotics likely depend on the choice of probiotic and prebiotic organisms. Synbiotic formulations commonly pair *Bifidobacterium* or *Lactobacillus* strains with fructooligosaccharides. By allowing necessary preconditioning of diet and environmental factors before their arrival in the colon, such combinations may facilitate effective probiotic action ^[32]. Because probiotics predominantly affect the small and large intestines whereas prebiotics primarily affect the large intestine, encouraging probiotic development, fermentation, and transit, synbiotics may also work in concert ^[33].

While synbiotics enhance the probiotic population, prebiotics promote the growth of naturally occurring beneficial bacterial strains. Synbiotics facilitate the transfer of living microorganisms to the gastrointestinal system by improving probiotic viability and extending the potential therapeutic effects of probiotics during storage ^[34]. Probiotic tolerance to environmental elements including oxygen, pH, and temperature may be increased when a proper probiotic strain and prebiotic ingredient are combined ^[35].

4.3. Dietary patterns and microbiota diversity

Dietary patterns influence microbiota diversity and functionality. Highly processed diets and high sugar intake are typically associated with low microbiota diversity and low levels of beneficial species involved in maintaining gut integrity and regulating metabolism ^[36]. Animal-based diets are also associated with decreased microbial diversity, while plant-based diets are positively correlated with provisioning of pre-biotics and microbial community structure that production key metabolites such as short-chain fatty acids with beneficial effects on health ^[37]. Beneficial genera like *Oscillospira* and *Butyrivibrio* are inversely correlated with Western diets that are high in animal protein, saturated fats, and simple carbohydrates ^[38]. It seems especially advantageous to follow a Mediterranean diet and other plant-based diets centered on high-fiber plant foods including whole grains, legumes, vegetables, oats, and fruits. Higher bifidobacterial counts, higher fermentation of complex plant polysaccharides, and increased synthesis of bioactive metabolites, such as short-chain fatty acids with important roles in health promotion, are all consequences of such dietary consumption patterns ^[39].

5. Protection against infectious diseases

Beneficial bacteria play a critical role in the defense against infectious diseases, improving resilience to both gastrointestinal and extraintestinal ailments. Efficacy arises not only from the direct inhibition of pathogens but also through the modulation of immune responses. Certain bacterial strains can protect against seasonal and pandemic influenza and attenuate the severity of respiratory syncytial virus (RSV) infections ^[40].

Gastrointestinal infections remain a significant global public health concern, with children being particularly susceptible. Asymptomatic viral shedding can last up to two weeks after recovery, raising the risk of secondary infections. Non-enveloped viruses such as rotavirus are estimated to cause

over 250,000 deaths annually in children aged under five. The prior colonization of pathogen-inhibiting bacterial strains can block the entry of invading pathogens and limit their systemic spread ^[41].

Correlations have emerged between dysbiosis and the rise of extraintestinal infections—occurring outside the gastrointestinal tract—and associated immune-mediated disorders such as allergies, asthma, and autoimmune conditions ^[42]. These excess condition patterns raise the demand for remedies, food supplements, and pharmaceutical products capable of rapidly reinstating intestinal homeostasis ^[43]. The opportunistic pathogen *Clostridium difficile* has gained significance as a cause of severe diarrhea, particularly for hospitalized patients receiving broad-spectrum antibiotics that disrupt gut microbiota. Prevention studies confirmed the potential of certain strains to minimize risk of *C. difficile* infection ^[44].

5.1. Gastrointestinal infections

During the last two decades, understanding of the human microbiota has gained substantial attention. The gastrointestinal (GI) tract is a complex ecosystem that is one of the largest interfaces connecting the host with the environment and antigens. A dynamic population of 10^{14} microorganisms, mainly bacteria, co-exists, providing benefits such as maintaining gut integrity, harvesting energy, modulating immunity, and protecting against pathogens. Disruption of these protective functions—termed dysbiosis—may contribute to GI disorders and other diseases. This knowledge has stimulated interest in the population of beneficial commensal organisms, described as extraintestinal and gastrointestinal pathogens, and in ways to promote and protect the beneficial biota ^[4].

Antibiotic treatment can cause the transient overgrowth of *Clostridium* species in the colon, resulting in diarrhea or pseudomembranous colitis. Antibiotics also inhibit the ability of the microbiota to control infection by restricting the activity of protective commensals such as *Lactobacillus rhamnosus* GG ^[45]. Following a broad-spectrum antibiotic treatment, hospitalized patients are at risk of *Clostridium difficile* infection, which can be life-threatening due to disruption of the microbiota ^[46]. Disease can be prevented by administering preparations of *Lactobacillus* and other protective strains capable of suppressing early *Clostridium* outgrowth. Reducing the number of *C. difficile* spores in the intestinal reservoir would thus promote restoration of the antibiotic-disrupted microbiota ^[47]. *Clostridia* genus continues to be the leading epidemiological cause of hospital-acquired infection and diarrhoeal disease in the world, thereby the number of *C. difficile* cases is expected to rise ^[48].

5.2. Extraintestinal infections and immune-mediated conditions

Despite benefits provided by the gut microbiota in protecting against intestinal infections, commensals still play a protective role in extraintestinal infections or immune-mediated conditions initiated following intestinal dysbiosis or gastrointestinal infections ^[49]. *Escherichia coli* producing Shiga toxin 2 (Stx2) is responsible for lethal disease but the concurrent presence of enterotoxigenic *E. coli* strain E2432A capable of inducing protective immunity renders Stx2-expressing *E. coli* unable to cause disease. Separate strains of *E. coli*, that are harmless to the host and involve highly conserved interaction mechanism, could both prevent

systemic dissemination and tissue-colonization of Stx2 *E. coli* 4. Probiotic *Lactobacillus plantarum* prevents severely compromised innate immunity of mice caused by antibiotics and maintains low level viremia and delays mortality upon challenges with enterovirus type 71. Protective immunity is elicited through TLR-2 and TLR-9 stimulation recognising peptidoglycan and CpG contained in *L. plantarum* ^[50].

Chronic colonization of the gut by pathogenic bacteria is capable of increasing the risk of non-intestinal diseases ^[51]. *Bacillus Calmette-Guérin*, a bacterial vaccine against tuberculosis, protects preneoplastic lesions induced by azoxymethane and immune stimulation by *Bacillus Calmette-Guérin* prevents lupus-like syndromes. Such systemic protection requires commensal and *Lactobacillus*, which promote endogenous production of IFN- α and drive the migration of dendritic cells to the spleen ^[52].

6. Role in chronic diseases and systems biology

Obesity-related metabolic problems are impacted by the extremely varied composition and functional capacity of metabolic pathways in the human gut microbiota, which also affect host energy homeostasis and fat deposition. Human metabolic problems like BMI, obesity, diabetes, and cardiovascular disease have been linked to the composition of the gut microbiota ^[53]. Studies have shown that high-fat diets can alter gut microbiota composition in animals and promote obesity and metabolic disorders. Excess calories from lipid diets preferentially contribute to the replenishment of nonesterified fatty acids in adipose tissue and exacerbate obesity and insulin resistance ^[54]. Therefore, microbial control of lipid absorption may provide additional strategies for the treatment of obesity. Obese individuals typically have increased abundance of Firmicutes and decreased Bacteroidetes compared with lean individuals. There is a favorable correlation between body fat and obesity status and the Firmicutes/Bacteroidetes ratio. ^[55] By increasing satiety and glucose homeostasis, decreasing hepatic gluconeogenesis and insulin resistance, and encouraging browning and thermogenesis of white adipose tissue, the SCFAs acetate and butyrate, which are produced when gut bacteria ferment food fiber, also boost metabolism. Metabolically sick people typically have fewer SCFA-producing bacteria in their guts. ^[2]

A few bacteria can influence metabolic health by regulating immunological and inflammatory activity through the synthesis of bioactive compounds ^[56]. Gut bacteria-mediated perturbation of the gut barrier is associated with metabolic disease through the induction of chronic low-grade inflammation and inter-organ insulin resistance in a mouse model, and enhanced permeability is characteristic of metabolic diseases such as obesity and type 2 diabetes ^[57]. Autoantibodies targeting the glial-derived neurotrophic factor (GDNF) are associated with psychiatric and neurodegenerative disorders. GDNF-interfering bacteria such as *B. fragilis*, *Clostridium bosniense*, *Enterococcus faecium*, and *Lactobacillus plantarum* are abundant in healthy human gut microbiota and *Scd1*^{-/-} mice, which are resistant to obesity and insulin resistance ^[58]. The gut microbiota is widely recognized to exert a dramatic effect on food intake, metabolism, and energy homeostasis, while microbiota-induced modulation of the metabolism of chemical food additives, such as artificial sweeteners, may have an adverse impact on these essential qualities of health ^[59]. Exposure to *Toxoplasma gondii* affects energy homeostasis and increases

food consumption and energy expenditure. *T. gondii* infection reshapes the gut microbiota through the activation of immune responses, and gut-microbiota-fed germ-free mice display increased energy expenditure and reduced tissue lipid accumulation. A reduced capacity of the gut microbiota to metabolically transform *T. Gondii* has been found in diseased animals, indicating that microbiota plays a crucial role in the communication between the stomach and brain [60].

Dysregulation of immune and inflammatory responses is closely linked to a number of chronic diseases, including inflammatory bowel disease, obesity, cardiovascular disease, type 2 diabetes, liver disease, and cancer. Early-life establishment of the gut microbiota is crucial in preventing these diseases [61]. For example, the gut microbiota may influence the health of the offspring based on the nutritional state of the mother during pregnancy. High-fat diets, obesity, and antibiotics have all been shown to affect gut flora and worsen a number of metabolic diseases [62]. Obesity, insulin resistance, and early-onset type 1 diabetes have all been linked to gut microbial dysbiosis [63].

6.1. Metabolic health and obesity

Using mostly germ-free mice, the relationship between the gut microbiota and metabolic health was among the first to be discovered [64]. Chronic low-grade inflammation, abnormal lipid and glucose metabolism, and an increased risk of metabolic disorders like type 2 diabetes, dyslipidemia, and atherosclerosis are all linked to obesity, a complicated condition [65]. The trillions of microorganisms that make up the gut microbiota have an impact on the host's immunity, metabolism, energy harvesting, and defense against infections [66]. The ratio of two major phyla, Firmicutes and Bacteroidetes, in the gut microbiota emerges as a crucial factor in determining the propensity to accommodate excess nutrient intake without over-welcoming the host. Firmicutes, often found at a higher abundance in obese individuals, is typically associated with adipose deposition and obesity due to its capacity to enhance energy extraction from food, suggesting a concealed selective metabolic effect from contemporary dietary hypnosis [67]. Certain bacterial species, such as *Akkermansia muciniphila*, *Bifidobacteria*, and *Lactobacillus*, promote host metabolism and improve insulin sensitivity; their genotoxicity, virulence, infectivity and infectiousness appear nearly zero, rendering them highly promising alternatives to implement therapeutic strategies to block obesity development [68]. Short-chain fatty acids (SCFAs) and secondary bile acids are among the many metabolites secreted by the gut microbiota that reach the bloodstream and have a variety of effects on host metabolism and immunological systems, strengthening the interaction between gut bacteria and host metabolism [69]. Obesity and type 2 diabetes are still characterized by clear changes in the gut microbiota composition, the depletion of SCFA-producing bacteria and the reduction of SCFA-activity bacteria are marked signs in the development of obesity-related metabolic disorder [70]. Increasing the abundance of microbiota beneficial metabolites and the supply of other valuable metabolites, on the contrary, are anticipated to improve the health status, whereas the heightened quantity of certain microbiota harmful metabolites and the exacerbation of other noxious metabolites facilitate the continuity of disease progression [71].

Expanded availability of sequencing technologies has

spurred a growing interest in microbiome-related studies worldwide [72]. Various experimental models, including animal models and *in vitro* systems, are used to provide a better understanding of how the gut microbiota impacts the host parasite and advocates of a successful implementation of microbiome-based intervention within the fishing and agricultural sectors, nevertheless, the knowledge gained via these models remains imperfect and the translation process from bench to animal studies yet encounter multiple challenges [73]. Another critical component to take into consideration encompasses the ethical dimension, despite the relevance of microbiome data for the elaboration of individualised interventions addressing elsewhere in the dusky alleys of big data and genomic technology, yet the open/public nature of data released and the continuous advances in sequencing resolution allow tracing strategies and influences on health performances across the population; therefore data stewardship GDPR compliance, consent framework and data sharing policy ought to be meticulously and properly designed and enforced in accordance with standard practices and procedures [74, 75].

6.2. Inflammatory and autoimmune diseases

Chronic inflammatory Dysregulated immune responses that cause excessive damage to cells and tissues are the root cause of conditions including inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA). Mucosal epithelia and skin are both impacted [76]. The mammalian microbiome has emerged as an important environmental influence on the development of inflammatory and autoimmune diseases through non-infectious agents. Evidence links dysregulated immunity in susceptible individuals with altered microbiota composition, suggesting the potential of beneficial bacteria as a preventive strategy [77]. Autoimmune diseases are genetically determined, but the environmental trigger is today ascribed to the loss of self-tolerance to host molecules. Nonetheless, microorganisms and their metabolites are also recognized to modulate inflammation and regulate the intestinal epithelial barrier. Relevant microbial pathways involving short-chain fatty acids (SCFAs) are being examined to devise new preventive strategies [78]. The intestinal microbiota is involved in preventing autoimmune diseases through the induction of protective regulatory T cells, suppression of ileal dysbiosis, production of beneficial metabolites that mitigate local inflammation, and maintenance of IgA-producing B cells that sustain intestinal barrier function [79].

6.3. Mental health and the gut-brain axis

The pathophysiology of metabolic illnesses, such as obesity, insulin resistance, and type 2 diabetes, is increasingly linked to dysbiosis, or an imbalance of the gut microbiome. [80]. Modulating metabolic homeostasis is a critical function of the gut-brain axis, a sophisticated bidirectional communication system between the brain and the gastrointestinal tract [81]. According to recent research, dysbiosis may affect the gut-brain axis and cause metabolic problems. When compared to conventional animals, germ-free animals exhibit variations in brain activity and hormone production linked to glucose metabolism, suggesting a critical function for intestinal microbiota in controlling metabolism via the gut-brain axis [82]. Treatment with microbiota from obese mice significantly alters gene expression of metabolic-related hormones in the brain of conventional mice [83]. Stress, insulin resistance, and

neurodegenerative diseases also affect intestinal microbiota, highlighting the gut-brain axis as a potential pathway influencing physiology and/or behavior ^[84]. Chronic inflammation and peripheral signals stimulated by metabolic dysregulation can modulate the gut-brain axis, influencing metabolic homeostasis in various metabolic states ^[82].

7. Methodologies in microbiome research

Recent years have seen the evolution of the research emphasis on the gut microbiome, a paradigm shift from the earlier identification of species present, driven by the “Human Microbiome” initiative, towards a much stronger health- and disease-oriented perspective ^[85]. However, the intrinsic properties of microbiomes, technical challenges of the methodologies used to study them, and complex host-microbiome interactions continue to render the elucidation of the links between the gut microbiome and health/disease a daunting task. Current views advocate the possibility of tuning microbiomes through dietary (including fermentate) and/or live (probiotic) interventions against diverse diseases and conditions ^[28].

7.1. Sequencing technologies and data interpretation

Sequencing technologies have transformed the investigation of the human microbiome, facilitating cost-effective and efficient exploration of microbial communities. The large assemblages of bacteria, archaea, fungi, and viruses colonising the skin, oral cavity, and gut outnumber human cells and genes by an order of magnitude, yet analysis using sequence-based approaches has also proven tractable ^[86]. High-throughput sequencing has revealed links between the microbiome and human health, disease, and disease risk factors, including inflammatory bowel disease, diabetic mellitus, and colonisation by multiple antibiotic-resistant pathogens. These connections have prompted exploration of microbial dysfunctions as diagnostic biomarkers and therapeutic targets, making the microbiome a major area of contemporary research and discovery ^[87].

7.2. Experimental models and translational challenges

Establishing causality between the human microbiome and health remains a major challenge. The immense complexity of the gut microbiota, the extensive genetic, epigenetic, proteomic, and metabolomic variation among individuals, and the scope for lifestyle factors to contribute to health status complicate the interpretation of amplicon sequencing and shotgun metagenomic studies that are often observational and not designed to ascertain causation ^[88]. Most mechanistic knowledge about microbiota causality derives from experimental studies in model organisms. Studies in mice, rats, and gnotobiotic (germ-free) species have shown that interventions influencing host-microbe interactions—such as nutrient availability, prebiotics, paraprobiotics, probiotics, and faecal microbiota transplantation—can modify the onset and progression of metabolic disorders, gastrointestinal disease, ischaemic injury, atherosclerosis, multiple sclerosis, and other conditions ^[89]. The extensive provision of germ-free and gnotobiotic models allows controlled colonization with defined microbial communities and systematic investigation of the gut-brain axis, microbiota variation with diet, immunisation, and other interventions, and longitudinal analyses of microbiota engineering alongside host-response and health-changing experimental perturbations ^[90]. Such studies have identified knowledge gaps that underline the

need for screening in emerging models like *Caenorhabditis elegans*, *Drosophila melanogaster*, zebrafish, and pig and piglet. Mixtures of viable and non-viable microbes, microbial metabolites or other products (paraprobiotics), and microbiota-modifying supplements are now being assessed across species to define the minimal requirements for sustained and health-correlated microbial colonisation, further inform the design of microbiota-guided interventions, and assess microbiota-centric principles such as the dominance of bacterial phyla and selective transmission of genera within them ^[91].

7.3. Ethical considerations and data stewardship

The ethical exploration of the microbiome and human health is relatively recent given the scientific community’s heightened focus since the early 2000s. Large-scale, open-space databases for microbiome and metagenome studies pose ethical dilemmas regarding sampling procedures, informed consent regulations, and data sharing practices ^[92]. A crucial component of this discussion arises from the long-standing dilemma over whether microorganisms qualify as part of the human body, with implications for property rights and stewardship of microbiome data. Although the term ‘human microbiome’ is widely used, many practices related to microbiomes of other species have failed to find equivalent acceptance, making it relevant to examine the ethical discourse surrounding human initiatives. The infant stage of microbiome research leaves a substantial portion of databases uncharted, introducing challenging interpretive dimensions. Furthermore, key details concerning relationships between bacterial colonization and their impact on health remain obscure, complicating ethical considerations surrounding the sharing of findings without rigorous validation ^[93].

Protocols for obtaining informed consent have similarly come under scrutiny. Existing legal frameworks require disclosure of risks and benefits, yet when combined with considerations encompassing privacy, ownership, and restrictions on merchandise sales, microbiome data may present complex risks and advantages ^[94]. Given the interpretative demands of much data and the extensive unknowns within the field, drafting concise and readily comprehensible consent forms emerges as a major priority. Even in the case of pathogens, the vast majority of bacteria remain harmless or beneficial, contributing positively to both human health and environmental sustainability. Attitudes toward bacteria frequently emphasize efforts to purge organs of disease-associated taxa, thereby reinforcing notions of bacteria as malevolent agents when public health campaigns might illuminate the overall contribution of microbes to prevailing notions of ‘health’ ^[95].

Considerable attention has also focused on ethical dimensions for human microbiomes. The expansion of research initiatives targeting the microbiomes of other species demands broader ethical engagement. The rapid development of microbiome-based innovations has underscored the need for shared principles guiding research conduct involving human-associated microbiomes ^[72].

8. Therapeutic implications and future directions

The multidirectional influence of beneficial bacteria on human health opens several avenues for intervention. There are opportunities to manipulate the microbiome directly to prevent and treat diseases, and the possibility of harnessing factors secreted by the microbiome, which could substitute

for live microbes. Evidence suggests a rational basis for, and potential impact of, such approaches ^[96].

Among the most developed concepts in microbiome therapeutics are personalized microbiome interventions ^[97]. High variability in the gut microbiome between people introduces the challenge of proprietary, one-size-fits-all, formulations. Data gathered through extensive screening can instead be used to deliver tailored treatments targeting the specific composition and functions of the gut microbiome. Personalization also extends to dietary patterns for prebiotics and fermentable substrates ^[98]. Individualization is complemented by concepts emphasizing autochthonous or indigenous microbiome members. To minimize side effects and optimize therapeutic results, it may be preferable to exploit strains present in healthy asymptomatic donors rather than unrelated standard commercial strains. Emerging avenues for engineered probiotics, including synthetic and controlled-release, offer hope of refining these strategies. Widespread exploitation of a few probiotic strains is constrained by substantial market demand anticipated for non-redundant proprietary strain formulations emergent of the personal microbiome-analysis market ^[99].

Personalized microbiome interventions are conceptually straightforward, but practical dispersion and risk of exploitation remain. Quality assurance, biosafety, and means of establishing evidence or fitness of strains remain open practical questions before prokaryotes or active elements can safely and reliably enter human subjects outside controlled environments ^[100]. Clear distinction between therapy and dietary supplementation can further decelerate wide rollout even of mature formulations. Pharmaceutical regulatory frameworks ill-fit prokaryotic agents, and the lack of sterile guarantee and uncertain composition for many probiotic products widen options for safe concession precluding governmental review. Uncontrolled vagaries thus confront innumerable candidates promising clinical benefit from industry or academia in the gap between foundational assessment and commercial private-profit implementation ^[101].

8.1. Personalized microbiome interventions

Diet plays a pivotal role in determining the composition and function of human gut microbiota, as microbial metabolism of dietary components can significantly impact host physiology and health ^[102]. Numerous factors, including physiological and environmental aspects, influence the early formation and subsequent development of gut microbiota composition. The composition of the gut microbiota is significantly influenced by the environment rather than host genetics, suggesting that microbiota is a more manageable target for dietary modification ^[103]. The potential for microbiome-based interventions to operate at an individual level—the rapid technological advances and active research efforts in the large and fast-growing field of metagenomics—are factors driving increasing interest in personalized dietary prescriptions that match individual nutrient needs and modulate microbiota to avoid or alleviate metabolic disorders and diseases ^[104].

8.2. Safety, regulation, and public health considerations

Although food containing live microbial cultures has long been consumed without any significant adverse effect it remained uncertain whether the Gram-negative probiotic strains under consideration—*Lactobacillus rhamnosus* GR1,

Lactobacillus reuteri RC-14, and *Bifidobacterium longum* BL-05—would be considered safe under the new rules for foodstuffs. Furthermore, until recently, it was unclear whether the stipulated safety standards covered “natural foodstuffs,” as such food products are termed in Europe, which have not been subjected to specific studies because of their presumed inherent safety ^[105]. These concerns were resolved by the adoption of the former “active” and “passive” probiotic, “hygienic,” and safety-unqualified probiotic-strain-based probiotic dietary style, into the overall LSRS. When the worldwide situation became acute, it became evident that the pre-probiotic protophenomenon-based probiotic dietary style would also not satisfy any of the provisions, to which nonliving (dead) bacteria do not cause adverse effects, and so it was discarded from the LSRS ^[106].

9. Conclusion

The contribution of beneficial bacteria to human health and disease prevention is substantial and spans diverse health conditions. Beneficial bacteria exert positive health effects by maintaining intestinal and overall health across various life stages against both infectious and chronic diseases. These effects appear to be mediated through colonization and competitive exclusion mechanisms, immune system modulation, and the provision of bioactive metabolites. Beneficial bacteria are naturally acquired through breast milk, food, and the environment, but obtainment of new species and strains decreases with age. An increasing reliance on processed food, fatty and sugary dietary patterns, and limited or no fermented food intake reduces beneficial bacterial diversity in the gut and shifts the gut microbiome toward an unhealthy state. These alterations in the gut microbiome make people more vulnerable to infections and encourage the emergence of chronic diseases. Corrective interventions should aim to restore the diversity of beneficial bacteria, including customised food interventions, nutritional formulations, or supplementation of foods with beneficial microbial strains or species. External intervention methods offer opportunities to promote health maintenance and establish resilient microbiome systems that can better handle diverse stressors.

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