

## INTRODUCTION OF THE NEW TERM “MICROBIOME” IN THE COMMUNITIES OF BIOSPHERE

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### **Abstract:**

*Macrobiomes are the living organisms in a microbial world with microbiotas filling discrete ecosystems in biospheric environments. Microbiomes are the total microbial forms associated with the macrobiomes e.g., human body include eukaryotes, archaea, bacteria and viruses. Microbiome studies are moving beyond mere inventories of specific ecosystems to quantifications of community diversity and descriptions of their ecological function. The human body is inhabited by billions of microbial forms and these microsymbionts play critical roles in human and plant health. The human body is colonized by a vast array of microbes. The microbes that exist in the human or plant body are collectively known as the human or plant microbiome. The ultimate objective of microbiome studies is to build complete, predictive models of how microbiomes interact and respond to stimuli such as climate change, agricultural practices and disease. Information visualization and visual analytics will become standard parts of microbiome research workflows.*

### **Keywords:**

*Biosphere, ecological interactions, macrobiome, microbiome, projects*

## **1. DEFINITIONS**

It can be useful to think about biology of microbiome as a branch of community ecology with different words and meaning e.g., microbiomes of digestive tract are shaped by priority effects (vertical transmission), resources (diets), immigration (bacterial transplantation) and disturbance (antibiotics). However, there is also a profound difference with ecology of microbiome community; the environment is itself and a host species that evolves, potentially in ways that affect the composition of the microbiome. Not surprisingly, there is evidence that gut microbiome composition is governed by host species. There is a great interest in explaining how useful microbiomes are assembled in the biosphere. Antibiotic-producing microbiomes are arguably the most abundant class of beneficial microbiome in nature, having been found on corals, arthropods, molluscs, vertebrates and plant rhizospheres. Microorganisms are involved in many biogeochemical and elemental cycles of the biosphere as well as in drug production, plant growth promotion, biodegradation, and in association with the human body. Human beings are ecosystems on two legs, each of us carrying enough microbes to outnumber our human cells by 10 to 1 and our genes by even more. Identifying the dizzying numbers of bacteria and other microorganisms that live in and on our bodies is like exploring a new planet.

Biome is defined as a major regional or global biotic community, such as a grassland or desert, characterized chiefly by the dominant forms of plant life and the prevailing climate, or it may any major regional biological community such as that of forest or desert. Also, it is a specific type of complex ecological community characterized by specific environmental conditions and a distinctive group of plants and animals, maintained in a relatively stable equilibrium, such as a rain forest biome or prairie biome. A biome is a distinctive ecosystem well-adapted for a specific geographic region and climate e.g., tundra, taiga, savannas, our mouth. So, a biome can consider as a large geographical area that is inhabited by particular plant and animal groups.

Noun:

- the microorganisms in a particular environment (including the our bodies or a part of the body): *we depend on a vast army of microbes to stay alive: a microbiome that protects us against pathogens, breaks down food to release energy, and produces vitamins for example.*
- the combined genetic material of the microorganisms in a particular environment: *understanding the microbiome e.g., for human, animal, and environmental is as important as the human genome*

The microbiome is the ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body space. This microbial community is complex and abundant, with estimates of the total species that inhabit an individual ranging well into the thousands. Although it is evident that we understand only a small component of the human microbiome, there is growing recognition that resident microbial communities influence human nutrition, development and disease.

So, microbiome can be defined as an aggregate of all digestive tract species and microbiota is the individual bacterial species in the biome. In general microbiome is defined as the total microbial community and biomolecules within a defined environment; microbiota is the total collection of microbial organisms within a community, typically used in reference to an animal host. While, microflora: an older term used synonymously with microbiota. A soil microbiome can be defined as the total microbial content, their genetic elements, and environmental interactions in a particular soil environment. Plants associate with a root microbiota distinct from the complex microbial community present in surrounding soil. In comparison, biofilm is a physically (and often temporally) structured aggregate of microorganisms, often containing multiple taxa, and often adhered to each other and/or to a defined substrate.

The concept of the human microbiome was first suggested by LEDERBERG & MCCRAY [1], who coined the term “microbiome, to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space”. The term “microbiome,” is the totality of microbes (commensals and pathogenic), their genomes (metagenome), and environmental interactions in a defined community or biological niche. The human body contains over 10 times more microbial cells than human cells, although the entire microbiome only weighs about 200 grams [2]. Because bacteria are 10-100 times smaller than human cells, the entire microbiome weighs about 200 grams.

All plants and animals, from protists to humans, live in close association with microbial organisms. These interactions of plants and animals with the microbial world have been defined mostly in one of the three terms positive, negative or neutral. An organism's complement of microbial inhabitants has been called a "forgotten organ" [3].

Humans are essentially sterile during gestation, but during and after birth, every body surface, including the skin, mouth, and gut, becomes host to an enormous variety of microorganisms. Under normal circumstances, these microbes help us to digest our food and to maintain our immune systems, but dysfunction of the human microbiota has been linked to conditions ranging from inflammatory bowel disease to antibiotic resistant infections. Modern high through put sequencing and bioinformatic tools provide a powerful means of understanding the contribution of the human microbiome to health and its potential as a target for therapeutic interventions [4]. The community formed by this complement of cells is called the human microbiome; it contains almost ten times as many cells as are in the rest of our bodies and accounts for several pounds of body weight and orders of magnitude more genes than are contained in the human genome [5,6].

## 2. MICROBIOME STUDIES

Much of our understanding of the human microbiome comes from culture-based approaches. Unfortunately, as much as 20% to 60% of the human-associated microbiome is presently uncultivable. Profiling bacterial communities by the culture-free, 16S rRNA gene sequencing approach has been enormously important in helping scientists identify the species present and quantify their abundance. 16S rRNA profiles can establish evolutionary relationships among bacteria but do not address gene content or gene expression. These limitations to the 16S rRNA gene sequencing have prompted efforts to examine the complexity of environmental samples by sequencing genomic libraries made from DNA extracted directly from the mixed sample, “metagenomics”, and from RNA, “transcriptomics”.

Members of a microbial community were identified by stains that targeted their physiological characteristics. Specific microbial species were detected by plating samples on specialized media selective for the growth of that organism, or they were identified by features such as the morphological characteristics of colonies, their growth on different media, and metabolic production or consumption. This approach limited the range of microorganisms that could be detected to those that would actively grow in laboratory culture, and it led the close study of easily-grown, now familiar model microbe such as *Escherichia coli* or *Bacillus subtilis*. However, *E. coli* as a taxonomic unit accounts for at most 5% of the microorganisms occupying the typical human digestive tract. The vast majority of microbial species have never been grown in the laboratory, and options for studying and quantifying the uncultured were severely limited until the development of DNA based culture-independent methods in the 1980s [7]. Culture-independent techniques, which analyze the DNA extracted directly from a sample rather than from individually cultured microbes, allow us to investigate several aspects of microbial communities. These include taxonomic diversity, such as how many of and which microbes are present in a community, and functional metagenomics, which attempts to describe which biological tasks of the members of a community can or do carry out. The earliest DNA-based methods probed extracted community DNA for genes of interest by hybridization, or amplified specifically-targeted genes by polymerase chain reaction (PCR) prior to sequencing. These studies were typically able to describe diversity at a broad level, or detect the presence or absence of individual biochemical functions, but with few details in either case.

One of the earliest targeted metagenomic assays for studying uncultured communities without prior DNA extraction was fluorescent *in situ* hybridization (FISH), in which fluorescently-labelled, specific oligonucleotide probes for marker genes are hybridized to a microbial community. The FISH probes can be targeted to almost any level of taxonomy from species to phylum. Although FISH was initially limited to the 16S rRNA marker gene and thus to diversity studies, it has since been expanded to functional gene probes that can be used to identify specific enzymes in communities [8]. However, it remains a primarily low through put, imaging-based technology.

## 3. TAXONOMIC DIVERSITY

**The 16S rRNA Marker Gene:** microbial community consists fundamentally of a collection of individual cells, each carrying a distinct complement of genomic DNA. As it is impractical to fully sequence every genome in every cell, microbial ecology has defined a number of molecular markers that more or less uniquely tag distinct genomes. A marker is a DNA sequence that identifies the genome that contains it, without the need to sequence the entire genome. Although different markers can be chosen for analyzing different populations, several properties are desirable for a good marker. A marker should be present in every member of a population, should differ only and always between individuals with distinct genomes, and, ideally, should differ proportionally to the evolutionary distance between distinct genomes. Several markers have been defined, including ribosomal protein subunits, elongation factors, and RNA polymerase subunits [9], but by far the most ubiquitous is the

small or 16S ribosomal RNA subunit gene [10]. This 1.5 Kbp gene is commonly referred to as the 16S rRNA (after transcription) or sometimes rDNA; it satisfies the criteria of a marker by containing both highly conserved, ubiquitous sequences and regions that vary with greater or lesser frequency over evolutionary time. It is relatively cheap and simple to sequence only the 16S sequences from a microbiome [11], thus describing the population as a set of 16S sequences and the number of times each was detected. Sequences assayed in this manner have been characterized for a wide range of cultured species and environmental isolates; these are stored and can be automatically matched against several databases including GreenGenes, the Ribosomal Database Project, and Silva.

**Binning 16S rRNA Sequences into Operational Taxonomic Unit:** A bioinformatic challenge that arises immediately in the analysis of rRNA genes is the precise definition of a “unique” sequence. Although much of the 16S rRNA gene is highly conserved, several of the sequenced regions are variable or hypervariable, so small numbers of base pairs can change in a very short period of evolutionary time. Horizontal transfer, multicopy or ambiguous rDNA markers, and other confounding factors do, however, blur the biological meaning of “species” as well as our ability to resolve them technically [12]. Finally, because 16S regions are typically sequenced using only a single pass, there is a fair chance that they will thus contain at least one sequencing error. This means that requiring tags to be 100% identical will be extremely conservative and treat essentially clonal genomes as different organisms. Some degree of sequence divergence is typically allowed 95%, 97%, or 99% are sequence similarity cut offs often used in practice and the resulting cluster of nearly-identical tags is referred to as an Operational Taxonomic Unit (OTU) or sometimes phylotype. OTUs take the place of “species” in many microbiome diversity analyses because named species genomes are often unavailable for particular marker sequences (Figure 1).

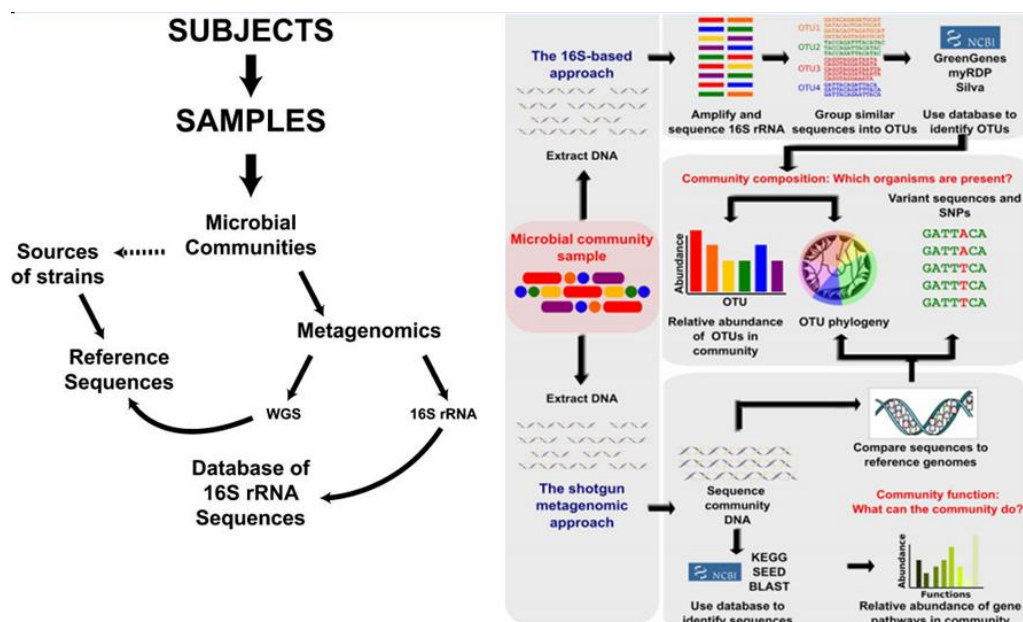


Figure 1 Bioinformatic methods for functional metagenomics (Source: [4])

Studies that aim to define the composition and function of uncultured microbial communities are often referred to collectively as “metagenomic” although this refers more specifically to particular sequencing-based assays. First, community DNA is extracted from a sample, typically uncultured, containing multiple microbial members. The bacterial taxa present in the community are most frequently defined by amplifying the 16S rRNA gene and sequencing it. Highly similar sequences are



grouped into OTUs, which can be compared to 16S databases such as Silva, GreenGenes, and RDP to identify them as precisely as possible.

Bioinformaticians studying 16S sequences must choose whether to analyze a collection of taxonomically-binned microbiomes as a set of abundance histograms, or as a set of binary presence / absence vectors. However, either representation can be used as input to decomposition methods such as Principle Components Analysis or Canonical Correlation Analysis to determine which OTUs represent the most significant sources of population variance and/or correlate with community metadata such as temperature, pH, or clinical features.

#### 4. STUDIED CASES

Between July 18<sup>th</sup> and 24<sup>th</sup> 2010, 26 leading microbial ecology, computation, bioinformatics and statistics researchers came together in Snowbird, Utah (USA) to discuss the challenge of how to best characterize the microbial world using next-generation sequencing technologies. There is a strengthening consensus among evolutionary biologists that one should not separate an organism's genes from the context of its resident microbes. The aim of the workshop was to explore the fundamental questions relating to microbial ecology that could be addressed using advances in sequencing potential. The main outcome from this meeting was the birth of a concept and practical approach to exploring microbial life on earth, the Earth Microbiome Project (EMP).

There are approximately  $1 \times 10^{30}$  microbial cells on earth. To date, the total global environmental DNA sequencing effort has produced less than 1% of the total DNA found in a litre of seawater or a gram of soil. Hence, we have vastly under-sampled the complexity and diversity of microbial life on Earth. Recent advances in high-throughput sequencing technologies have provided an unprecedented opportunity to explore the microbial universe. Scientists wish to sequence microorganisms and microbial communities from a broad range of biomes to achieve three main goals to:

1. define microbial community structure, and to explore the factors that affect community structure at different scales.
2. explore the universal protein and attempt to produce a complete inventory of protein family diversity.
3. create a global database of samples, genes and proteins the can be used to answer fundamental questions about the ecology of life on and off the earth.

As envisioned, the EMP would be a massively multidisciplinary effort to analyze microbial communities across the globe. Hence we propose to characterize the Earth by environmental parameter space relevant to microbes, and then to explore these different biomes using samples currently available from researchers across the globe. To achieve these general aims, the EMP will focus on 10 core questions which can be grouped into different sections:

- **Section 1 Community Structure:**

1. Are microbial communities structured primarily by environmental conditions or trophic/metabolic interactions?
2. If microbes are structured by environmental conditions, how do we define the Environmental Parameter Space (EPS) to characterize microbiomes?
3. What are the primary mechanisms of cross-kingdom interaction, metabolic or genetic?

- **Section 2 Defining Physiology and Metabolic Capability:**

1. Is ecosystem function defined by community taxonomy or by the trophic/metabolic dynamics in that ecosystem, i.e. who is doing what, how fast and by what mechanisms?
  2. What is the role of rare microorganisms in an ecosystem, e.g. functional plasticity or specific biochemical function?
- **Section 3 Practical Considerations:**
    1. How do we sample microbiomes to best explore global structure, e.g. temporal studies, experimentally controlled perturbations, biogeographic studies, and at what density?
    2. How do we best use metagenomic data to re-assemble genomes, and what can we learn from this study to improve the yield of novel microbial genomes from metagenomic studies?
  - **Section 4 Models and Visualization:**
    1. What aspects/metrics of microbial community structure is it necessary to measure to enable parameterization of predictive ecological models?
    2. At what taxonomic level does the pan-genome operate, and what controls this?
    3. How do we most accurately visualize global microbial space, and what can this tell us about extraterrestrial microbial communities and fundamental ecology?
    4. The EMP will cover many diverse environments, including marine, freshwater, terrestrial, air, extreme environments and man-made locations. However, environmental samples will not be the sole aim. We will also explore lab-based mesocosm and microcosm studies in which environmental manipulation will enable us to identify microbial community dynamics (e.g. Winogradsky columns).

The EMP will have many deliverables. The following key deliverables will be of considerable benefit to a wide number of communities:

- **Gene Atlas:** a centralized repository and database for all sequencing and metadata information acquired during this study.
- **Earth Microbiome Assembled Genomes:** all metagenome-derived assembled microbial genomes will be deposited in public repositories.
- **Earth Microbiome Visualisation Portal:** we want to view the Earth from the perspective of microbes, describing environmental parameter space and genomic functional space.
- **Earth Microbiome Metabolic Reconstruction:** based on metagenomic metabolome description and prediction (e.g. modelSEED and Relative Metabolic Flux) we will describe changes in metabolite profiles between all samples.

The EMP must be a cross-discipline effort, involving microbiologists, microbial ecologists, genomicists, physicists, computer scientists, mathematicians, and ecosystem modellers, to provide the most comprehensive global assessment of microbial life ever seen. Additionally, similar to the Human Genome Project, which has revolutionized biomedicine, the proposed EMP will revolutionize the way that can assess and model the health of our changing planet.

#### 4.1 Studies in humans

Looking at human beings as ecosystems that contain many collaborating and competing species could change the practice of medicine. A human being is an individual who has grown from a fertilized egg which contained genes from both father and mother. A growing band of biologists, however, think this definition incomplete. They see people not just as individuals, but also as ecosystems. In their view,

the descendant of the fertilized egg is merely one component of the system. The others are trillions of bacteria, each equally an individual, which are found in a person's gut, his mouth, his scalp, his skin and all of the crevices and orifices that subtend from his body's surface (Figure 2).

Total population sequences were analyzed to determine the enzymes levels involved in carbohydrate, lipid, and amino acid metabolism. Obesity is associated with phylum-level differences in the microbiota, a significantly reduced bacterial diversity, and an increase in the population expression of enzymes which result in an increased efficiency of calorie harvest in the diets of the obese twins [16].

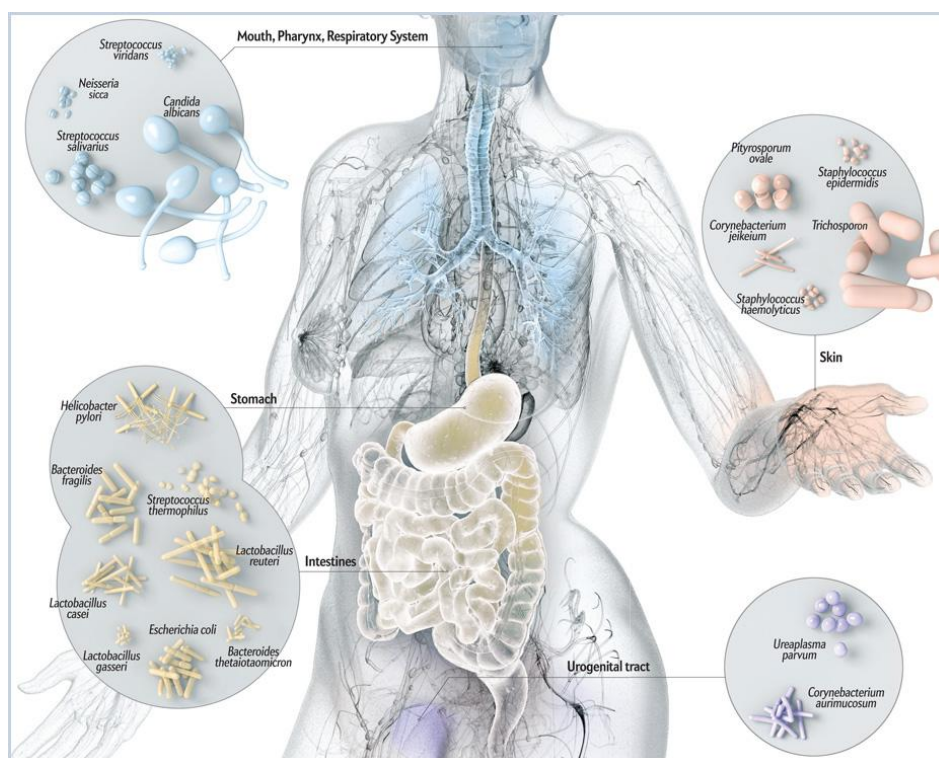
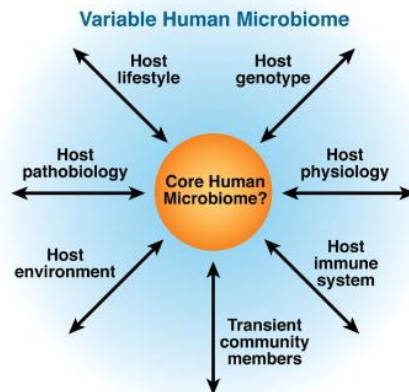


Figure 2 Human body and microbiomes. Source: *The Human Microbiome Project Consortium (2012): Structure, function and diversity of the healthy human microbiome. Nature, 486: 207-214. doi: 10.1038/nature11234*

Human skin represents the most extensive organ of the human body, whose functions include protecting the body from pathogens, preventing loss of moisture, and participating in the regulation of body temperature. Considered as an ecosystem, the skin supports a range of microbial communities that live in distinct niches. Hair-covered scalp lies but a few inches from exposed neck, which in turn lies inches away from moist hairy underarms, but these niches are, at a microbial level, as distinct as a temperate forest would be compared with savannas and tropical rain forest. Studies characterizing the microbiota that inhabit these different niches are beginning to provide insights into the balance between skin health and disease [17]. The human microbiome consists of about 100 trillion microbial cells, outnumbering human cells 10 to 1 [18]. Thus it can significantly affect human physiology. For example, in healthy individuals the microbiotas provide a wide range of metabolic functions that human's lack [19].

The ideas of a core and variable human microbiome have been proposed and provide a conceptual framework for considering early development and relationships of the human microbiome with multiple physiologic functions (Figure 3) [5]. Microbial colonization of the human intestine within the first few days of life is an intricate process that results in a symbiotic relationship with the

manipulation of microbial communities has the potential to ameliorate different gastrointestinal conditions [20].



*Figure 3 The concept of a core human microbiome. The core human microbiome (orange) is the set of genes present in a given habitat in all or the vast majority of humans. Habitat can be defined over a range of scales, from the entire body to a specific surface area, such as the gut or a region within the gut. The variable human microbiome (blue) is the set of genes present in a given habitat in a smaller subset of humans. Source: [21].*

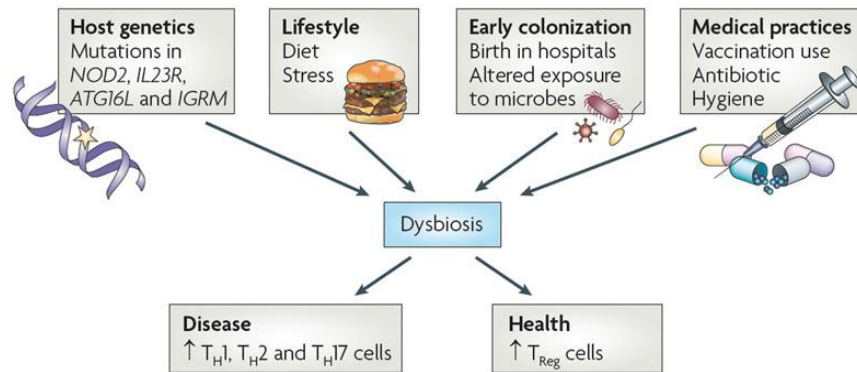
As the microbiome affects multiple aspects of human health, host biology influences the composition and function of the commensal microbiota. A subset of microbial genes may be found in most healthy human beings (core microbiome), whereas variable components are present only in specific ethnic groups, age groups, geographic locations, or associated with specific dietary patterns or disease states. Manipulation of either the core or the variable parts of the human microbiome can affect human physiology, overall health status, and disease susceptibilities. Combinations of these approaches might provide synergistic and effective therapies for specific disorders. The human microbiome could be manipulated by such “smart” strategies to prevent and treat acute gastroenteritis, antibiotic-associated diarrhea and colitis, inflammatory bowel disease, irritable bowel syndrome, necrotizing enterocolitis, and a variety of other disorders.

This variation could result from a combination of factors such as host genotype, host physiological status (including the properties of the innate and adaptive immune systems), host pathobiology (disease status), host lifestyle (including diet), host environment (at home and/or work) and the presence of transient populations of microorganisms that cannot persistently colonize a habitat. The gradation in colour of the core indicates the possibility that, during human micro-evolution, new genes might be included in the core microbiome, whereas other genes might be excluded.

When the relative phylogenetic composition of the microbiome is altered, a state of dysbiosis can ensue, and the local immune system can shift towards a proinflammatory milieu (Figure 4). The composition of the microbiota can shape a healthy immune response or predispose to disease. Many factors can contribute to dysbiosis, including host genetics, lifestyle, exposure to microorganisms, and medical practices. Host genetics can potentially influence dysbiosis in many ways. An individual with mutations in genes involved in immune regulatory mechanisms or proinflammatory pathways could lead to unrestrained inflammation in the intestine. It is possible that inflammation alone influences the composition of the microbiota, skewing it in favor of pathobionts. Alternatively, a host could “select” or exclude the colonization of particular organisms. This selection can be either active or passive. Selection of pathobionts by the host could tip the balance in favor of inflammation. Diet and stress also have the potential to influence the microbiota. Birth in the sterile environment of hospitals can



protect from exposure to dangerous pathogens, but can also prevent early exposure to health-promoting bacteria. Overuse of vaccination and antibiotics, which do not distinguish between pathogenic or symbiotic microorganisms, could adversely alter the microbiota.



*Figure 4 Proposed causes of dysbiosis of the microbiota. Abbreviations: ATG16L: autophagy-related gene 16-like; IGRM: immunity-related GTPase family, M; IL23R: interleukin-23 receptor; NOD2: nucleotide-binding oligomerization domain 2; Th: T helper; TReg: regulatory T. Source: [22]*

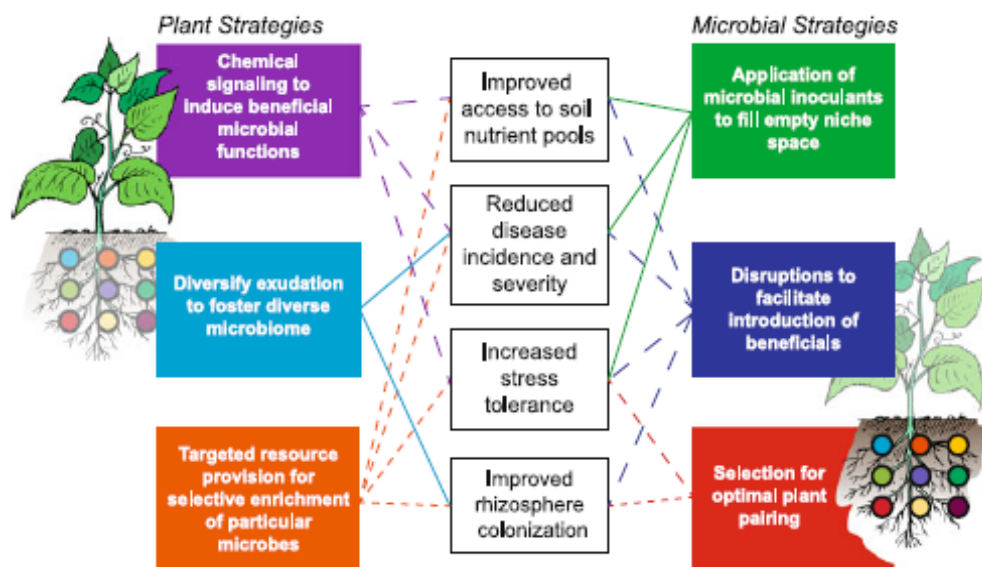
## 4.2 Animal studies

The bovine rumen harbours a complex microbiome that converts plant cell wall biomass into proteins, short chain fatty acids, and gases. Multiple species are involved in this conversion. Traditional methods of characterizing the microbial population, based on culture analysis, missed many of the participants in this process. Comparative metagenomic studies yielded the surprising result that individual steer had markedly different community structures, predicted phenotype, and metabolic potentials [23], even though they were fed identical diets, were housed together, and were apparently functionally identical in their utilization of plant cell wall resources.

## 4.3 Plant studies

The microbial diversity associated with plant roots is highly enormous, in the order of tens of thousands of species. This complex plant-associated microbial community is referred to the second genome of the plant, and is crucial for plant health. Recently, plant–microbe interactions research revealed that plants are able to shape their rhizosphere microbiome, as evidenced by the fact that different plant species host specific microbial communities when grown on the same soil.

Plant-microbiome strategies used to promote beneficial services and reducing chemical inputs for improving crop yields are presented in Figure 5.



*Figure 5 Plant – microbiomes interactions. Manipulating plant traits that are related to interactions with microbiome (left side), or manipulating soil microbiome directly (right side), could improve conditions for plant productivity (center mechanisms). Source [24]*

Soil physicochemical characteristics impose limiting factors on element availability for plants, excess of non-essential metals and metalloids pose a threat for plant health and the environment.

Nutrient uptake by plants is essential for their development and for the passage of minerals into the food chain, but it also faces several limitations.

- To improve nutrient uptake, the plant possesses several mechanisms to explore the soil for minerals such as root development, but the symbiosis with microorganisms clearly improves the ability of plants to overcome these limitations.
- Plants exhibit a broad range of relationships with symbiotic microorganisms, ranging from parasitism, in which the association is disadvantageous to the host organism, to mutualism, in which the association is beneficial to both, to commensalism, in which the symbiont benefits while the host is not affected. Exchange of nutrients between symbiotic partners is an important part of the relationship: it may be bidirectional or unidirectional, and it may be context dependent. The strategies for nutrient exchange are highly diverse. Oomycetes and fungi have, through convergent evolution, developed similar morphology and occupy similar ecological niches. They develop hyphae, filamentous structures that penetrate the host cell. In those cases where the association is mutualistic, the plant exchanges hexose sugars for inorganic phosphate from the fungal symbiont. It is speculated that such associations, which are very ancient, may have aided plants when they first colonized land [25].
- A huge range of bacterial symbionts colonize plants. Many of these are pathogenic, but others known as plant-growth promoting bacteria (PGPB) provide the host with essential services such as nitrogen fixation, solubilization of minerals such as phosphorus, synthesis of plant hormones, direct enhancement of mineral uptake, and protection from pathogens [26, 27]. PGPB may protect plants from pathogens by competing with the pathogen for an ecological niche or a substrate, producing inhibitory allelochemicals, or inducing systemic resistance in host plants to the pathogen [28].

## 5. PROJECTS

- The EMP is an initiative to collect natural samples and analyze the microbial community around the globe. Microbes are highly abundant, diverse and have an important role in the ecological system. Yet as of 2010, it was estimated that the total global environmental DNA sequencing effort had produced less than 1% of the total DNA found in a litre of seawater or a gram of soil [29], and the specific interactions between microbes are largely unknown. The EMP aims to process as many as 200,000 samples in different biomes, generating a complete database of microbes on earth to characterize environments and ecosystems by microbial composition and interaction. Using these data, new ecological and evolutionary theories can be proposed and tested [30].
- The Human Microbiome Project (HMP) is a United States National Institute of Health initiative with the goal of identifying and characterizing the microorganisms which are found in association with both healthy and diseased humans (their microbial flora). Important components of the HMP will be culture-independent methods of microbial community characterization, such as metagenomics, as well as extensive whole genome sequencing. The microbiology of five body sites will be emphasized: oral, skin, vaginal, gut, and nasal/lung. The project also is financing deep sequencing of bacterial 16S rRNA sequences amplified by PCR from human subjects. The HMP includes the following goals to:
  - develop a reference set of microbial genome sequences and to perform preliminary characterization of the human microbiome,
  - explore the relationship between disease and changes in the human microbiome,
  - develop new technologies and tools for computational analysis,
  - establish a resource repository, and
  - study the ethical, legal, and social implications of human microbiome research.

In addition to establishing the human microbiome reference database, the HMP project also discovered several surprises, which include:

- Microbes contribute more genes responsible for human survival than human's own genes. It is estimated that bacterial protein-coding genes are 360 times more abundant than human genes.
- Microbial metabolic activities; e.g., digestion of fats; are not always provided by the same bacterial species. The presence of the activities seems to matter more.
- Components of the human microbiome change over time, affected by a patient disease state and medication. However, the microbiome eventually returns to a state of equilibrium, even the composition of bacterial types has changed.

Among the first clinical applications utilizing the HMP data, as reported in several PLoS papers, the researchers found a shift to less species diversity in vaginal microbiome of pregnant women in preparation for birth, and high viral DNA load in the nasal microbiome of children with unexplained fevers. Other studies using the HMP data and techniques include role of microbiome in various diseases in the digestive tract, skin, reproductive organs and childhood disorders

## 6. STUDYING THE HUMAN MICROBIOME

The problem of elucidating the human microbiome is essentially identifying the members of a microbial community which includes bacteria, eukaryotes, and viruses. This is done primarily using

DNA-based studies; though RNA, protein and metabolite based studies have also been performed [31]. The human microbiome is defined as the aggregate of microorganisms that reside on the surface and in deep layers of skin, in the saliva and oral mucosa, in the conjunctive, and in the gastrointestinal tracts. They include bacteria, fungi, and archaea. Some of these organisms perform tasks that are useful for the human host. However, the majority have been too poorly researched to understand the role they play. Those that are expected to be present, and that under normal circumstances do not cause disease, but instead participate in maintaining health, are deemed members of the normal flora. Though widely known as "microflora", this is, in technical terms, a misnomer, since the word root "flora" pertains to plants, and biota refers to the total collection of organisms in a particular ecosystem. Recently, the more appropriate term "microbiota" is applied, though its use has not eclipsed the entrenched use and recognition of "flora" with regard to bacteria and other microorganisms. Both terms are being used in different literature. Studies in 2009 questioned whether the decline in biota (including microfauna) as a result of human intervention might impede human health [2].

In recent years, scientists have started to survey the microbiome in a new way: by gathering DNA. They scrape the skin or take a cheek swab and pull out the genetic material. Getting the DNA is fairly easy. Sequencing and making sense of it is hard, however, because a single sample may yield millions of fragments of DNA from hundreds of different species. To make sense of the genes that they're gathering, they are sequencing the entire genomes of some 900 species that have been cultivated in the lab. Before the project, scientists had only sequenced about 20 species in the microbiome. In May, the scientists published details on the first 178 genomes. They discovered 29,693 genes that are unlike any known genes. (The entire human genome contains only around 20,000 protein-coding genes.)

Scientists are even discovering ecosystems in our bodies where they weren't supposed to exist. Lungs have traditionally been considered to be sterile because microbiologists have never been able to rear microbes from them. Analyzing lung samples from healthy volunteers, scientists discovered 128 species of bacteria. Every cm<sup>2</sup> of our lungs is home to 2,000 microbes.

Some microbes can only survive in one part of the body, while others are more cosmopolitan. And the species found in one person's body may be missing from another's. Out of the 500 to 1,000 species of microbes identified in people's mouths, for example, only about 100 to 200 live in any one person's mouth at any given moment. Only 13% of the species on two people's hands are the same. Only 17% of the species living on one person's left hand also live on the right one.

This variation means that the total number of genes in the human microbiome must be colossal. European and Chinese researchers recently catalogued all the microbial genes in stool samples they collected from 124 individuals. In March, they published a list of 3.3 million genes.

The variation in human microbiomes emerges the moment we are born. "You have a sterile baby coming from a germ-free environment into the world". Most of the microbes associated with humans appear to be not harmful at all, but rather assist in maintaining processes necessary for a healthy body. A surprising finding was that at specific sites on the body, a different set of microbes may perform the same function for different people. For example, on the tongues of two people two entirely different sets of organisms will break down sugars in the same way. This suggests that medical science may be forced to abandon the one-microbe model of disease, and rather pay attention to the function of a group of microbes that has somehow gone awry [6].

In addition to helping us digest, the microbiome helps us in many other ways. The microbes in our nose, for example, make antibiotics that can kill the dangerous pathogens we sniff. Our bodies wait for signals from microbes in order to fully develop. When scientists rear mice without any germ in their bodies, the mice end up with stunted intestines. In order to co-exist with our microbiome, our immune system has to be able to tolerate thousands of harmless species, while attacking pathogens. Scientists are finding that the microbiome itself guides the immune system to the proper balance. One way the



immune system fights pathogens is with inflammation. Too much inflammation can be harmful, so we have immune cells that produce inflammation-reducing signals.

Scientists are not just finding new links between the microbiome and our health. They're also finding that many diseases are accompanied by dramatic changes in the makeup of our inner ecosystems. They discovered microbes in the lungs, e.g., also discovered that people with asthma have a different collection of microbes than healthy people. Obese people also have a different set of species in their guts than people of normal weight.

In some cases, new microbes may simply move into our bodies when disease alters the landscape. In other cases, however, the microbes may help give rise to the disease. Some surveys suggest that babies delivered by Caesarean section are more likely to get skin infections from methicillin-resistant *Staphylococcus aureus*. It's possible that they lack the defensive shield of microbes from their mother's birth canal. Caesarean sections have also been linked to an increase in asthma and allergies in children. So have the increased use of antibiotics in the US and other developed countries. Children who live on farms are less prone to getting autoimmune disorders than children who grow up in cities.

Some scientists argue that these studies all point to the same conclusion: when children are deprived of their normal supply of microbes, their immune systems get a poor education. In some people, untutored immune cells become too eager to unleash a storm of inflammation. Instead of killing off invaders, they only damage the host's own body.

A better understanding of the microbiome might give doctors a new way to fight some of these diseases. For more than a century, scientists have been investigating how to treat patients with beneficial bacteria. But probiotics, as they're sometimes called, have only had limited success. The problem may lie in our ignorance of precisely how most microbes in our bodies affect our health.

## 7. INTESTINAL FLORA

Trillions of commensal bacteria live in the human gastrointestinal tract, so pathogenic bacteria experience extreme competition for resources. PACHECO et al. [32] found that the commensal bacterium *Bacteroides thetaiotaomicron*, which grows in intestinal mucin, produced fucose that the pathogenic bacterium enterohaemorrhagic *E. coli* (EHEC) used to regulate its metabolism and effectively compete for nutrients in order to successfully colonize the intestinal epithelium. The gut flora is the human flora of microorganisms that normally live in the digestive tract and can perform a number of useful functions for their hosts. The average human body, consisting of about  $10^{13}$  (ten trillion) cells, has about ten times that number of microorganisms in the gut [20, 33, 34] The metabolic activity performed by these bacteria is equal to that of a virtual organ, leading to gut bacteria being termed a "forgotten" organ [31].

Bacteria make up most of the flora in the colon and 60% of the dry mass of faeces [20]. This fact makes faeces an ideal source to test for gut flora for any tests and experiments by extracting the nucleic acid from faecal specimens, and bacterial 16S rRNA gene sequences are generated with bacterial primers. This form of testing is often preferable to more invasive techniques, e.g., biopsies. Somewhere between 300 [20] and 1000 different species live in the gut [35], with most estimates at about 500 [34]. However, it is probable that 99% of the bacteria come from about 30 or 40 species [36]. Fungi and protozoa also make up a part of the gut flora, but little is known about their activities. Research suggests that the relationship between gut flora and humans is not merely commensal, but rather is a mutualistic, symbiotic relationship [35]. Though people can survive with no gut flora [34], the microorganisms perform a host of useful functions, such as fermenting unused energy substrates, training the immune system, preventing growth of harmful species [20], regulating the development of the gut, producing vitamins for the host (such as biotin and vitamin K), and producing hormones to

direct the host to store fats. However, in certain conditions, some species are thought to be capable of causing disease by causing infection or increasing cancer risk for the host [20].

## Summary

Total microbial cells found in association with humans may exceed the total number of cells making up the human body by a factor of 10 to 1. The total number of genes associated with the human microbiome could exceed the total number of human genes by a factor of 100 to 1. Many of these organisms have not been successfully cultured, identified, or otherwise characterized. Organisms expected to be found in the human microbiome, however, may generally be categorized as bacteria (the majority), members of domain Archaea, yeasts, and single-celled eukaryotes as well as various helminth parasites and viruses, the latter including viruses that infect the cellular microbiome organisms (e.g., bacteriophages, the viruses of bacteria).

The HMP will address some of the most inspiring, vexing and fundamental scientific questions today. Importantly, it also has the potential to break down the artificial barriers between medical microbiology and environmental microbiology. It is hoped that the HMP will not only identify new ways to determine health and predisposition to diseases but also define the parameters needed to design, implement and monitor strategies for intentionally manipulating the human microbiota, to optimize its performance in the context of an individual's physiology. In addition to establishing the human microbiome reference database, the HMP project also discovered several surprises.

Most current technologies rely on DNA sequencing to examine either individual taxonomic markers in a microbial community, typically the 16S ribosomal subunit gene, or the composite metagenome of the entire community. The role of microbiome in autoimmunity appears to be far-reaching and important. Indeed, it is now possible to find and identify thousands of bacteria and their function. Identification of pathogenic commensal organisms could provide insights into the environmental triggers of diseases lead to a new understanding of disease pathogenesis, perhaps leading to novel approaches for thereby.

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