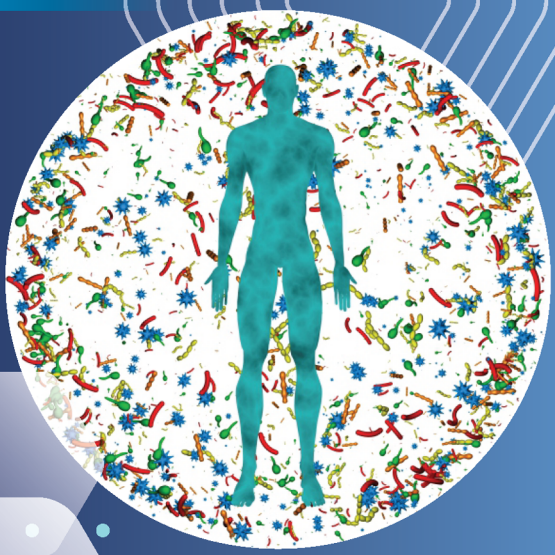
Microbial Ecology

Microbiomes, Viromes, and Biofilms



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Preface

The study of microbial ecology is a rapidly advancing field shedding new light on the complex relationships between microorganisms and their environments. At the heart of this field are three key concepts: Microbiomes, viromes and biofilms. Microbiomes refer to the communities of microorganisms that live on or within a particular host or environment, while viromes represent the viruses that infect these microbial communities. Biofilms are complex communities of microorganisms that attach to surfaces and form intricate structures. Understanding the roles that these microbial communities play in shaping human health and disease is a major focus of this book. Microbial infections are a major cause of morbidity and mortality worldwide. Apart from basic/fundamental microbiological analysis; microbiomes, viromes and biofilms have been linked to various diseases, including diabetes mellitus and liver diseases. For example, the gut microbiota plays a crucial role in metabolic processes and immune function, and disruptions to this community have been linked to the development of diabetes mellitus. Similarly, changes in the gut microbiota have been implicated in the development of alcoholic and non-alcoholic fatty liver diseases. This book explores the latest research on the role of microbiomes and viromes in infections, providing insights into how we might prevent and treat these diseases. Microbiomes, viromes and biofilms are not only associated with infections but also with human traits and environmental conditions. For example, the gut microbiota has been linked to mood and behaviour, and changes in this community have been implicated in the development of depression and anxiety.

Additionally, microbiomes, viromes and biofilms play critical roles in environmental processes, including nutrient cycling and bioremediation. This book explores the many ways in which microbiomes and viromes influence human health and the environment. Probiotics and other interventions that manipulate the microbiome and virome have become increasingly popular in recent years. However, the effectiveness of these interventions remains unclear, and there is much debate about the appropriate use of such therapies. This book provides an in-depth

examination of the microbiome and probiotics, exploring the evidence for their efficacy and discussing their potential applications in various disease states. Hence, the use of next-generation sequencing and bioinformatics tools has revolutionised our ability to study microbiomes, viromes and biofilms. These tools allow us to identify and characterise microorganisms with unprecedented accuracy and speed, providing new insights into the roles that these communities play in shaping our world. This book provides a comprehensive overview of these technologies, offering a practical guide on their use in microbiome, virome and biofilm research.

Dr. Bhagwan Narayan Rekadwad

Contents

Chapter 1

Introduction of Microbiomes, Viromes and Biofilms

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Introduction

One of the hardest things about ecology is trying to figure out what controls the number and variety of species, how these things are affected by natural or human-caused changes, and how these changes manifest in the processes and features of an ecosystem. These are some of the most critical questions that ecology can address. Globalization and climate change facilitate species' mobility between locations, consequently leading to an increase in the prevalence of invasive species. However, significant uncertainties persist regarding the capacity of humans to detect and predict biological invasions (Van der Putten et al. 2007). The biological charges of

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exotic species into terrestrial ecosystems are among the most critical threats to the native biodiversity and ecological stability of these ecosystems. (Sousa et al. 2011). When discussing ecosystems, people often focus on invasive plants and other animals that dwell above the ground (Bardgett and Wardle 2010). However, the visible biota that inhabits ecosystems can significantly impact and move the unseen microbiological components of ecosystems and the processes that are driven by those components. In addition, there is an increasing realization that invasive microbes, such as those that cause diseases in humans, animals or plants, can change the structure and function of entire ecosystems. These diseases can affect humans, animals, or plants (Litchman 2010). The microbial communities found in the environment are immensely diverse and complicated. Some examples of these communities include microorganisms such as bacteria, archaea, fungi, and viruses. The intricate networks of connections created by these bacteria significantly impact the functioning of ecosystems and the biotic and abiotic components that make up an ecosystem (Shade et al. 2012).

This chapter will focus on three essential aspects of microbial ecology: microbiomes, viromes, and biofilms. Microbiomes - colonies of bacteria – are found in environments, and these microbiomes are diverse and dynamic. The billions of bacteria that inhabit the human body have co-evolved with us, resulting in highly specialized adaptive ecosystems that are precisely tuned to the continuously fluctuating physiology of the host. Because of this, the human body is home to various microbes (Jenkins 2019). Cancer, Asperger's syndrome, inflammatory bowel disease, multiple sclerosis, allergies, asthma, and type 1 and type 2 diabetes are just a few of the disorders linked to microbiome dysbiosis. Determining the pathogenicity of a single microbial taxon or the dysbiosis of an entire microbial community might be challenging. Dysbiosis may be understood as a deviation from a healthy ecology that serves to prolong, exacerbate, or generate an unfavorable health outcome (Decker et al. 2021). Finding new approaches to prevent diseases or improve prognosis may depend on uncovering the characteristics that distinguish healthy microbiomes from bad microbiomes, which could aid in diagnosing microbiome-related disorders. Healthy microbiomes are thought to share certain features, including common species and metabolic activity (Lloyd-Price et al. 2016). According to Willis and Gabaldón's 2020 research, the human microbiome, much like the earth's multiple terrestrial biomes, is composed of a varied collection of microbial communities, which changes depending on the surrounding environment. It is possible to think of different body sections as different biomes, each with a unique atmosphere and availability of various nutrients – both encouraging their

unique community of cells. The makeup of a microbiome might vary substantially (even at the level of a single body site) across persons whose health and lifestyles are drastically different from one another (Porras-Alfaro and Bayman 2011). The viruses included in a virome are numerous and diverse and come from various kingdoms of life. The human body is home to various eukaryotic and prokaryotic viruses, collectively comprising the human viromes. However, since every organ and tissue in the body has its particular microenvironment, the viromes might change depending on where they are situated. The makeup of these viromes can also be influenced by factors such as age, diet, and the presence or absence of specific microbiome components (Liang and Bushman 2021).

Biofilms are formations that are both dynamic and complex. They are produced by communities of microorganisms that live close to one another and adhere to surfaces. Communities of bacteria attached to surfaces can be referred to as biofilms. Cells that constitute a biofilm can be differentiated from their counterparts suspended in the medium by producing an extracellular polymeric substance (EPS) matrix, slowing their growth rates, and up-and-down-regulation of specific genes. The regulation of the attachment process is affected by various elements found on the cell surface, as well as those in the growth medium and the substratum (Kokare et al. 2009). A developed biofilm structure comprises microbial cells and extracellular polymeric substances (EPS), has a unique structure, and provides an excellent environment for cells to trade DNA with one another. Cell-to-cell communication in quorum sensing may regulate biofilm activities such as detachment (Dewasthale et al. 2018).

The advancements that have been made in next-generation sequencing over the last decade have led to a significant expansion of our understanding of the human microbiome and the role it plays in both health and disease (Malla et al. 2019). However, our knowledge of the human virome is still quite limited, particularly in how it interacts with essential microbes that affect human health. The human body is home to many prokaryotic and eukaryotic viruses, each contributing to maintaining a distinct niche and having various consequences on our health (Zárate et al. 2017). Viruses may be divided into two categories: those that are prokaryotic and those that are eukaryotic. Although phages and other prokaryotic viruses have been discovered in different regions of the body, the digestive tract of a human being provides an especially favorable environment for the growth of these viruses (Hidalgo-Cantabrana et al. 2018). Because of the matrix in which they are encased, bacteria that generate biofilms are protected from various stimuli, including antibiotics, ultraviolet light, chemical biocides, the immunological response of the host, and other irritants (Berhe et al. 2017). As biofilms provide a

protective coating, microorganisms can endure adverse conditions such as high salinity and pressure, extremes in temperature and pH, insufficient nourishment, antibiotics, etc. (Yao et al. 2022). Most antibiotic resistance may be traced back to structural barriers and the persistent cells found in biofilms. According to research, infections brought on by biofilms are notoriously difficult to treat (Abdelghafar et al. 2022). Antibiotics are no longer effective in treating conditions caused by biofilms due to the development of antibiotic resistance and genetic mutations. Biofilms, which have recently been identified as a significant factor in causing illnesses not cured by antibiotics, are frequently the source of antibiotic-resistant bacteria (Bowler 2018). It is common knowledge that biofilms are responsible for more than 80 percent of all chronic infectious disorders. It is also common knowledge that conventional antibiotic therapies cannot address infections mediated by biofilms and can not cure them (Li and Lee 2017). Consequently, the healthcare business faces a considerable risk from the presence of bacteria that create biofilms. This chapter aims to provide a brief summary of what is currently known about microbiomes, viromes and biofilms, along with their significant contributions to and interactions with microorganisms and their environment, as well as the challenges presented by this rapidly growing field and the opportunities it presents.

Microbiome Diversity

Throughout millions of years, microorganisms on Earth have co-evolved with other species to become highly specialized contributors to ecological communities. Microbiomes are communities of microorganisms that can live in and on a host organism for an extended period (Foo et al. 2017). The microbiome is a complex ecosystem of microorganisms found in or on the human body and includes microbes such as bacteria, viruses, fungi, and protozoa (Davis 2016). Owing to its vital role in maintaining human health and well-being, research on microbiomes has grown increasingly prevalent in recent years. One of the essential elements of a microbiome is its diversity, which can be defined as the wide range of microorganisms found in a specific habitat (Kim et al. 2017).

About ten percent of the cells that make up our bodies originate from the human host; the remaining cells come from the human microbiota. These commensal microbes help us resist diseases, train our immune systems, and impart some characteristics that humans did not initially evolve within their bodies (Byrd et al. 2018). For instance, we frequently ingest plant polysaccharides that are abundant in carbohydrate structures containing xylan, pectin, and arabinose. Even though human DNA

lacks most of the enzymes necessary for decomposing these chemicals, the microbiota in our distal gut allows us to do so (Agans et al. 2018). The human genetic landscape combines the human genome and the metagenomes of microbes that have colonized human bodies (Smith and Wissel 2019). Because of this, the genetic diversity of humans is not only found in the allele frequencies of shared *Homo sapiens* genes but also the genes included within our various microbial communities (Zilber-Rosenberg and Rosenberg 2021). To understand the genetic and physiological differences observed, it is necessary to characterize the composition and structure of human microbiota in significant regions of the body (such as the mouth, skin and gut), as well as the variables that influence them.

Oral Bacterial Microbiome

The oral cavity is home to about 700 unique species of bacteria, making it the second most varied microbiota after the digestive system. In addition to bacteria, fungi, viruses, and protozoa, it fosters the growth of many other microorganisms and is a favorable environment for their survival (Wiley et al. 2017). Tooth decay, also known as dental caries, and gum disease, sometimes periodontal disease, are the two most frequent bacterial diseases that affect humans (Mosaddad et al. 2019). The mouth is a breeding ground for a wide variety of bacterial species. It is consistently exposed to environmental factors, and research has revealed that it is susceptible to the effects of these influences. The human microbiome has a relatively stable core and a more fluid peripheral. The core microbiome of a body in good health comprises the most prevalent species at each location. Since it has grown in reaction to a person's distinctive genetic make-up and how they live their life, each individual's microbiome is unique (Balan et al. 2017). Approximately 700 unique types of prokaryotes have been discovered. Around 54% of these species have been given their proper names, 14% are not named but are cultivated, and 32% are only known as uncultivated phylotypes. These species are divided into 185 genera and 12 phyla (Zhao et al. 2017b). The following phyla and orders of oral bacteria are found most frequently in humans (Table 1).

There is a consistent microbial community in healthy mouths, down to the genus level in the oral microbiome. Despite the similarities, the variety of a person's microbiome is highly dependent on their geographical location. Microflora, some of which are anaerobes, can be found on the tongue, thanks to its many papillae, despite its relatively low number of anaerobic sites. A limited variety of microorganisms is present in the buccal and palatal mucosae (Sultan et al. 2018). The most prevalent bacteria

Table 1. Major phyla and class of human oral microbiota (Idris et al. 2017).

Phylum	**Class**
Proteobacteria	*Alphaproteobacteria* *Epsilonproteobacteria* *Deltaproteobacteria* *Gammaproteobacteria* *Betaproteobacteria*
Chloroflexi	*Caldilineae* *Anaerolineae*
Gracilibacteria (GN02)	*GN02 [C-2]* *GN02 [C-1]*
WPS-2	*WPS-2 [C-1]*
Saccharibacteria (TM7)	*TM7 [C-1]*
Actinobacteria	*Actinobacteria*
Firmicutes	*Erysipelotrichia* *Negativicutes* *Mollicutes* *Bacilli* *Clostridia*
SR1	*SR1 [C-1]* *SR1 [C-2]* *SR1 [C-3]*
Bacteroidetes	*Flavobacteriia* *Bacteroidia* *Sphingobacteriia* *Bacteroidetes [C-1]* *Bacteroidetes [C-2]*
Spirochaetes	*Spirochaetia*
Chlamydiae	*Chlamydiia*
Synergistetes	*Synergistia*
Fusobacteria	*Fusobacteriia*
Chlorobi	*Ignavibacteria* *Chlorobia*

that can be discovered in a healthy mouth are listed below (Amaroli et al. 2022).

Gram-positive:

1. **Rods:** *Bifidobacterium, Propionibacterium, Eubacterium, Lactobacillus, Pseudoramibacter, Actinomyces, Rothia, Corynebacterium.*
2. **Cocci:** *Abiotrophia, Peptostreptococcus, Streptococcus, Stomatococcus.*

Gram-negative:

1. **Rods:** *Campylobacter, Leptotrichia, Hemophilus, Desulfobacter, Capnocytophaga, Simonsiella, Desulfovibrio, Eikenella, Fusobacterium, Treponema, Prevotella, Selemonas, Wolinella.*
2. **Cocci:** *Neisseria, Moraxella, Veillonella.*

In addition to bacteria, the mouth is home to a wide variety of other organisms, including protozoa, fungi, and viruses; *Entamoeba gingivalis* and *Trichomonas tenax* are the protozoa that are most commonly encountered. Both species are predominantly saprophytic and pose no threat to human health (Deo and Deshmukh 2019). Fungi of many different kinds can be discovered in and around the oral cavity, but the *Candida* species is by far the most common. In culture-independent studies conducted on twenty more healthy hosts, researchers detected a total of 85 unique species of fungi. It has been concluded that the bulk of the detected species might be attributed to the genera C*andida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium,* and *Cryptococcus* (Sharma et al. 2018).

Skin Microbiota

The skin is the body's largest organ and its primary protection mechanism against external environments (McLoughlin et al. 2022). There are millions of different kinds of bacteria, fungi, and viruses in the skin's microbiome. Like the microorganisms in the digestive tract, those on the skin also perform essential roles, including defense against pathogens, immune system training, and waste disposal (Falcao and Inaturals 2021). Reduced diversity of gut microbiota compared to cutaneous microbiota is possible. According to Lima et al. (2020), the vulnerability of this microbial network lies in the multiple intrinsic and extrinsic components that affect it (Figure 1). The connections between these two areas highlight the value of skin homeostasis in treating wounds and preventing complications from infections or the effects of harsh environments. Recent research has found a correlation between alterations in these mutually beneficial populations and physiological changes, such as aging and other skin disorders, in animals and humans (Nguyen and Kalan 2022).

Gut Microbiota

The term 'gut microbiota' refers to the diverse population of bacteria, archaea, and eukaryotic organisms that inhabit the digestive tract. The

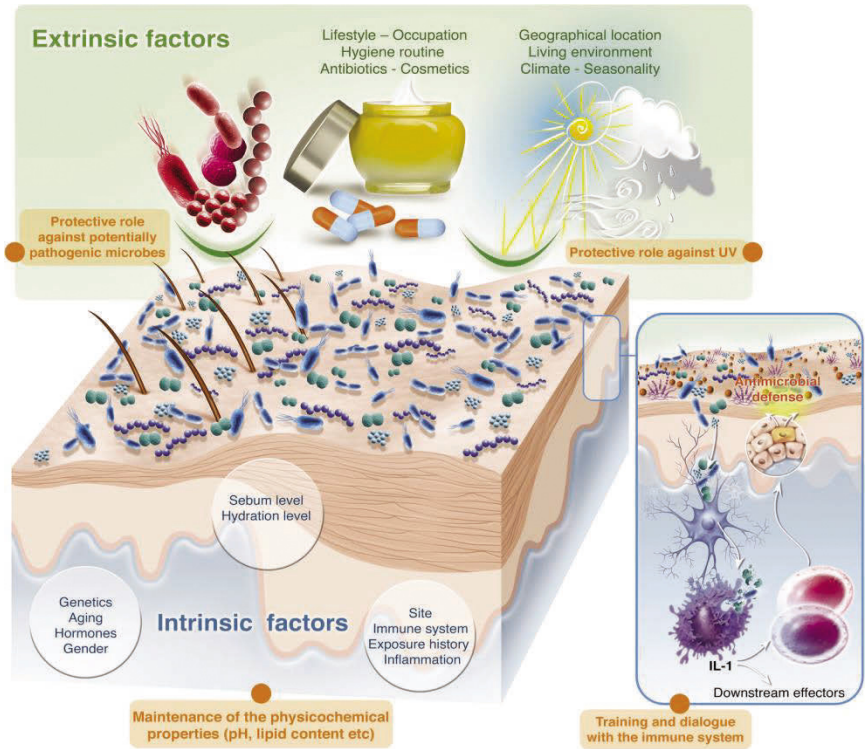

Figure 1. The elements that shape the microbiota that lives on or within the human skin. The microbiota that dwells on or in the skin is formed by both external (such as lifestyle, which includes work, hygiene practices, and drug and cosmetic usage) and internal (such as inheritance) influences (genetics, aging, sex, site of the body, etc.). Protecting the skin from potential infections or climatic changes and maintaining the skin's integrity are only two of the many ways these factors might affect the functioning of the skin's microbiota (Boxberger et al. 2021).

human host and its accompanying bacteria have co-evolved in a complex and mutually beneficial relationship over millions of years (UMAR et al. 2022). It has been estimated that more than 1,014 types of bacteria dwell in the human digestive system. There are around ten times as many bacterial cells as human cells, and the microbiome has over one hundred times the genomic information in the human genome. A recent study, however, indicates that the ratio of human to bacterial cells is closer to one-to-one. The combination of a host and its microorganisms is frequently called a 'superorganism.' This happens because the human body contains many bacterial cells (Thursby and Juge 2017).

The gut microbiota has been linked to numerous human diseases, including luminal disorders such as inflammatory bowel disease (IBD)

and irritable bowel syndrome (IBS), metabolic diseases such as obesity and diabetes, allergic diseases, and neurodevelopmental disorders. However, the evidence supporting many of these claims is scant. In recent years, scientists have focused much attention on gut microbiota (Gomaa 2020). It has been hypothesized that the microbiota in the gut plays a crucial role, both structurally and functionally, in the maintenance of the gut in healthy individuals and human health as a whole (Sinha et al. 2023, Makki et al. 2018).

The host's immune system can recognize infections and attempt to eliminate them. Immunology serves as the basis of the immune system, which explains why this is the case. Yet, most gut bacteria are harmless and connect positively with enterocytes (Kundu Smita and Rana 2021). In the digestive system, commensal bacteria are essential in metabolizing nutrients and medicines, preventing infections, and maintaining intestinal barrier function. In the interim, the immune system has co-evolved to collaborate with microbiota that is helpful to the host while protecting the host against pathogenic microorganisms. As a result, the immune system can perform both roles effectively (Kogut et al. 2020).

A person's genetics, nutrition, lifestyle, age, and environment influence the variety of the microbiota in their gut (Rinninella et al. 2019). The foods we eat affect the bacteria that inhabit our gastrointestinal system. Compared to a diet rich in fiber, fruit, and vegetables, a diet rich in fat and sugar results in an imbalanced gut flora. In contrast, a diet rich in fiber, fruit, and vegetables promotes the growth of beneficial microorganisms (Luo et al. 2021). This microbial population may experience a decline in variety due to the use of antibiotics and other drugs, which can alter the composition of the gut microbiota (Barko et al. 2018).

Virome

Infectious agents known as viruses can infect living things throughout all kingdoms of life. They frequently infect new hosts and occasionally produce diseases that incapacitate the host (Chevallereau et al. 2022). The virome contains the world's most abundant and rapidly changing genetic components. It is also known as the viral genome. The term 'mammalian virome' refers to the collection of viruses that infect host cells, chromosomal segments produced from viruses, and viruses that infect the wide variety of other organisms that live in and on mammals. Chromosomal components that are made from viruses are also included in this collection. Evidence shows these viruses have contributed to the mammalian virome (Santoro et al. 2020). The microbiome comprises all the bacteria that reside inside and on our skin, and the virome is a subset

of the microbiome. The collection of all of these bacteria is known as the microbiome. This group includes bacteria and archaea, fungi, protozoa, and any other meiofauna that can be present (Cadwell 2015).

The virome has only recently become 'visible' in large sequence datasets, thanks to developments in next-generation sequencing and bioinformatics that make it possible to detect links between viruses despite significant nucleotide sequence variation. This has been made possible by the fact that we can now see viral links even though nucleotide sequences can vary greatly. As a direct result, research on the virome is still in its infant stages. The virome has recently become 'visible' in big sequencing datasets (Esposito et al. 2022). The mammalian virome contains a variety of viruses, including eukaryotic viruses (eukaryotic virome), bacteriophages (bacterial virome), archaeal viruses (archaeal virome), and virus-derived genetic elements (VGEs) on host chromosomes. All of these viruses (or virus components) can alter the gene expression of their hosts, express proteins, and even produce infectious virus particles (pro-phages, endogenous retroviruses, endogenous viral elements). Currently, we have the least information on the archaeal virome, which refers to the viral population that can be detected in human cells (Zhao et al. 2019). Despite the considerable variations between eukaryotic and bacterial viruses, both utilize 'lytic' and 'latent' life cycles. The host cell is killed during viral replication, and the virus remains dormant within a living cell. The host cell is destroyed during viral replication in lytic life cycles; in latent life cycles, the virus remains dormant within a living cell. The viral genome can remain inactive in the host cell as an episome (as with herpesviruses) or be incorporated into the host's chromosome (as with prophages) until it becomes more infectious. This phenomenon is seen in the case of herpesviruses. Herpesviruses are an excellent example of this behavior in the natural world (Legoff et al. 2020). Because of the diverse range of lifestyles that can be exploited, the virome can persist, evade defenses, diversify, and form astonishingly complex and frequently mutualistic, symbiotic interactions with the host. This is possible because of the vast number of lifestyles that can be exploited. The virome can endure because of the interactions between its components. It is necessary to consider that these linkages frequently involve 'trans-kingdom' interactions between different species of viromes and microbiomes that originated in different kingdoms of life (for example, virome interactions with bacteria).

Diversity of Viromes

It is estimated that there are approximately 10^{31} to 10^{32} virus particles in the world at any given time, which is orders of magnitude higher than the number of cells (Mushegian 2020). The biome offers an incredible

variety of pathogens, which can infect even the healthiest individuals. The human virome contains antibodies that target bacteria, bacteriophages (phages) that infect archaea, viruses that are infrequently encountered in food, and those capable of infecting human cells (Muhammad et al. 2024c). The human virome consists of these components. Within a single host, many viral subpopulations can coexist. Most studies have been conducted on the microbiota of the human gut, where the most varied communities have been discovered. The human gut is a perfect place for viruses to reproduce, given the abundance of human gut cells and prokaryotic bacteria (Muhammad et al. 2024d). Even while most other parts of a healthy human body have less microbiota, new research shows large viral populations in numerous body parts. Like the reported variation in bacterial and fungal populations, the human virome exhibits substantial inter-individual variations (Table 2). This begs the question: "To what extent do variances in the virome account for observed phenotypic variations?" (Liang and Bushman 2021).

Different characteristics can be used to classify the viruses found in humans. The genetic material that makes up a virus is called its genome, which can be RNA or DNA and double-stranded or single-stranded. The size of a genome can range from just a few kilobases to hundreds of kilobases, depending on the organism (Chaitanya and Chaitanya 2019). The genetic content of every virus is protected inside a protein shell known as a capsid. Viruses may have more than one lipid membrane surrounding them. A virus particle's shape is one factor that can be considered while classifying viruses. Virus particles can take several possible forms, including spherical (typically icosahedral), filamentous, bullet-shaped, pleomorphic, or even tailed, similar to how phages are structured. Phages likely make up most of the human virome (Fermin 2018). In recent years, several studies have been conducted to define the human virome in various body locations, indicating the presence of diverse and prolific populations (Figure 2). Phage populations in different anatomical regions of the body may be very different. This is likely to be the case because the human body is home to a diverse collection of bacteria that serve as hosts. *Microviridae*, which includes phage X174, are icosahedral, non-tail-bearing phages, while the *Caudovirales* are icosahedral, tail-bearing phages. There are also differences in the distribution of eukaryotic viruses at various sites throughout the body. An overview of certain perilous attributes specific to each part is illustrated in Figure 2.

Major Realms of Viruses

The International Council on Viral Taxonomy has recently developed a complete hierarchical taxonomy of viruses; at its highest level, it has

Table 2. Examples of viral population alterations in human disorders.

Human disease	Sample	Major virome alteration	References
Severe acute undernourishment	Feces	Condensed viral variety	(Reyes et al. 2015)
Crohn's disease and ulcerative colitis	Feces	Enlarged *Caudovirales* fertility	(Norman et al. 2015)
Crohn's disease	Feces and biopsies	Reasonable variations	(Pérez-Brocal et al. 2015)
AIDS	Feces	Amplified enteric adenoviruses	(Monaco et al. 2016)
Type 1 diabetes	Feces	Condensed viral diversity	(Zhao et al. 2017a)
Hypertension	Feces	*Erwinia* phage ΦEaH2 and *Lactococcus* phage 1706 may be associated with hypertension.	(Han et al., 2018, Muhammad et al. 2021)
Type 2 diabetes	Feces	Increased putative phage scaffolds	(Ma et al. 2018)
DOCK8 deficiency	Skin swabs	Increased skin virome, especially human papillomavirus	(Tirosh et al. 2018)
Colorectal cancer	Feces	Increased viral diversity	(Nakatsu et al. 2018)
Crohn's disease and ulcerative colitis	Feces	Increased *Caudovirales* abundance	(Fernandes et al. 2019)
Type 1 diabetes during pregnancy	Feces	Amplified picobirnaviruses and tobamoviruses	(Wook Kim et al. 2019)
Bacterial vaginosis	Vaginal swabs	Viral population structures correlated with bacterial vaginosis	(Jakobsen et al. 2020)
Early-diagnosed Crohn's disease and ulcerative colitis	Gut biopsies	Amplified *Hepadnaviridae* and *Hepeviridae*; reduced *Polydnaviridae*, *Tymoviridae* and *Virgaviridae*	(Ungaro et al. 2019)
Coeliac disease autoimmunity	Feces	Increased enteroviruses	(Lindfors et al. 2020)

Crohn's disease	Feces	In their place, milder phages have been introduced.	(Clooney et al. 2019)
Ulcerative colitis	Gut biopsies	Increased virulence of *Caudovirales*, phages, and bacteria, but disappeared connections between viruses and bacteria.	(Zuo et al. 2019)
HIV viraemia	Seminal fluid	Amplified human cytomegalovirus	(Li et al. 2020)
Very early-onset inflammatory bowel disease	Feces	Amplified ratio of *Caudovirales* to *Microviridae*	(Liang et al. 2020)
Haematopoietic stem cell transplantation	Feces	Improved picobirnaviruses	(Legoff et al. 2017, Muhammad et al. 2024b)

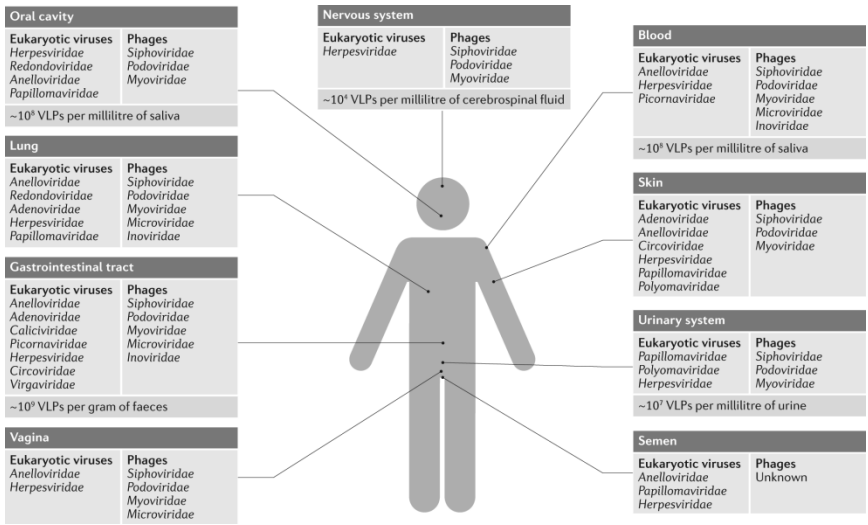

Figure 2. Viruses found at each human body site (Liang and Bushman 2021).

classified six taxa as putatively monophyletic groups (ICTV). The International Committee on Viral Taxonomy (ICTV) created this virus classification system (The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks 2020). Metaviromics studies on a grand scale have revealed a shocking expansion in the number of virus species present within each of the four major virus domains (Riboviria, Monodnaviria, Duplodnaviria, and Varidnaviria). Viruses are classified as members of the fourth domain, Varidnaviria (Koonin et al. 2020). The environment *Riboviria* includes RNA and DNA viruses capable of reverse transcription and positive-sense, negative-sense, and double-stranded (ds) RNA viruses. Viruses use homologous RNA-dependent RNA polymerases (RdRPs) and reverse transcriptases in this vast universe (RTs). The domain *Monodnaviria* is comprised of circular-genome viruses, most of which are single-stranded DNA (ssDNA) viruses and tiny double-stranded DNA (dsDNA) viruses (papillomaviruses and polyomaviruses). This universe would not hold together without the hallmark gene which encodes a special endonuclease (or a derivative that has been inactivated). This endonuclease is often engaged in the initial rolling circle of replication during genome replication. *Duplodnaviria* is home to dsDNA viruses, including the recently discovered *mirusviruses,* animal herpesviruses, and tailed bacterial and archaeal viruses (Gaïa et al. 2021). The HK97-fold main CP (MCP), the ATPase-nuclease (terminase) responsible for packaging the genome, the portal protein, and the capsid maturation protease are all proteins encoded by the structural gene module of these viruses.

Varidnaviria is the supergroup of viruses that can infect bacteria, archaea, and eukaryotes due to their dsDNA genomes. These pathogens share a common characteristic in their vertical jelly-roll MCPs. The *Bamfordvirae* kingdom contains most viruses because their main capsid proteins fold in a double jelly-roll (DJR) shape.

In contrast, each of the two MCPs seen in Helvetiavirae viruses is composed of a single vertical jelly-roll domain. The International Council for the Taxonomy of Viruses recently acknowledged two new, smaller virus worlds: Adnaviria and Ribozyviria (ICTV). Both rod-shaped and filamentous viruses can be found in the phylum Adnaviria. These viruses infect hyperthermophilic archaea belonging to the phylum Thermoproteota, and they encapsidate linear dsDNA in the A form (Krupovic et al. 2021). In addition to viruses that can infect humans, the domain Ribozyviria also contains viruses that can infect various other animals (Hepojoki et al. 2021). *Ribozyviruses*, like viroids, have nucleocapsid proteins encoded by circular RNA genomes (Delta antigen).

Monophyly is the notion that all viruses can be traced back to a common ancestor. Yet, this is not true for at least three of the four primary realms that exist at present. It remains valid even if we ignore that monophyly depends on almost no VHG. Even though the two kingdoms that make up the realm *Riboviria* have a replicative enzyme identical to the other, it is clear that the origins of these viruses, which emerged from the recruitment of different CPs, were completely separate from one another (Figure 3). It appears that the two groups of Pararnavirae – Ortervirales and Blubervirales – originated from independent retrotransposons, suggesting that *Riboviria* should be separated into at least two new domains to maintain the criterion that taxa should be strictly monophyletic (Gong and Han 2018, Krupovic et al. 2018). The viruses that make up the kingdom Monodnaviria evolved at least four times in a row, each taking a slightly different path. Little rolling-circle plasmids can capture the viral gene that encodes a CP, or cellular genes repurposed as CPs (Kazlauskas et al. 2019). The viruses that make up this region have evolved into what they are now (Figure 3). It was once thought that the two solitary jelly-roll vertical CPs seen in *helvetiaviruases* represented an early version of the MCP in the *Varidnaviria* universe. DJR MCP would have been the result of gene fusion in this scenario. Current research into the cellular origins of the vertical jelly-roll MCPs has revealed that Bamfordvirae and Helvetiavirae form their capsids independently. A recent study (Krupovic et al. 2022) has found that the current state of affairs guarantees the continued existence of the viral planets of *Duplodnaviria, Adnaviria,* and *Ribozyviria,* each with its monophyly. Yet, some viruses do not fit into the already recognized categories; instead, they appear to have evolved independently, making them good candidates for new and more limited types. Archaeal viruses,

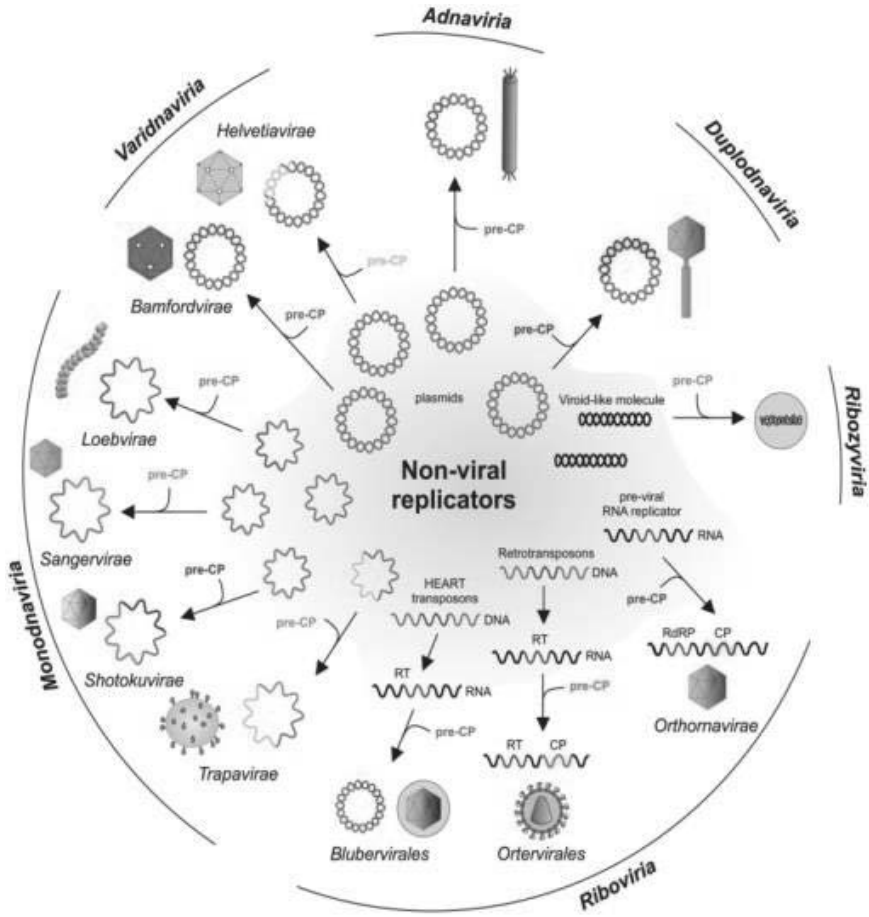

Figure 3. The possible division of virus families and their organization into individual domains would follow taxonomic principles and genetic relationships among these viruses. During the process of virogenesis, non-viral replicators like plasmids and transposons acquire cellular genes that are then repurposed to build viral capsids. This happens because these non-viral replicators have reached cellular DNA. The most monophyletic taxa are emphasized for three of the six categories to illustrate the potential for distinct paths of virogenesis from non-viral replicators. The DNA and RNA replicators, not viruses, are denoted in grey and black, respectively. There are several ancestry branches for capsid proteins, and a distinct color indicates each component. The capsid protein, RNA-dependent RNA polymerase, and reverse transcriptase are each represented by their initials as CP, RdRP, and RT, respectively (Koonin et al. 2023).

giant dsDNA viruses like those in the class Naldaviricetes (bacula-like viruses), and animal *anelloviruses* with small ssDNA genomes appear to be missing the signature rolling-circle endonuclease that binds the members of Monodnaviria together (Taylo et al. 2022). Many virus families fall