

The supraorganism

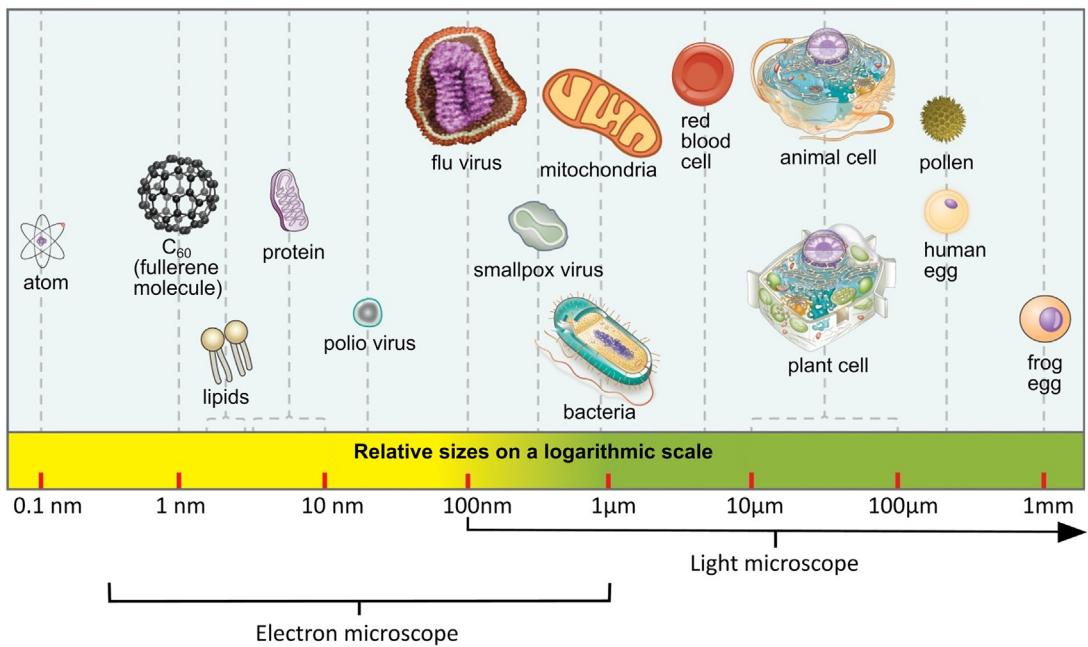
1.1 New discoveries with new technology— A historical account

Antoni van Leeuwenhoek looked at all kinds of things he could think of using his mysterious single-lens microscope, which had an impressive resolution of around $1\text{ }\mu\text{m}$ (10^{-6} m , Fig. 1.1) [1,2]. From the dental plaques of a few people, he described with astonishment several different bacteria, despite paying more attention to oral hygiene than his contemporaries (Box 1.1) [3]. For size comparison, Leeuwenhoek mentioned sandgrains (Box 1.1), the diameter of which are in the submillimeter range, and the finer ones are indeed at the resolution of the human eye (100–200 μm).

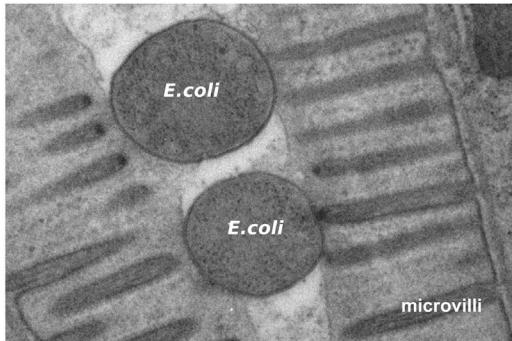
Acceptance of Leeuwenhoek's various works grew after Robert Hooke recapitulated one of Leeuwenhoek's pepper-water experiments with his not as high-resolution but more accessible two-lens microscope (compound microscope) [1]. Nonetheless, Carolus Linnaeus did not give microbes a slot in his 10th edition of *Systema Naturae* (1758). His tree of life was only about plants and animals. Ernest Haeckel added "Protista" (unicellular organisms) in 1866. The taxon "Monera" for unicellular organisms that lack a nucleus, such as bacteria, was proposed as a phylum, and later elevated among the kingdoms. Robert Whittaker added the fungi kingdom to the tree of life in 1969, until Carl Woese revamp of the tree with archaea, according to sequence comparisons in the 16S ribosomal RNA [4].

Developments in metagenomics in the past two decades represent another major leap in technology that has allowed us to better appreciate the microbial world (Fig. 1.2). Also with curiosity and excitement, we applied the technology to all kinds of samples. However, "messy" or "shitty" the host-associated (animals or plants are the hosts for the microbes) or environmental sample is, we can sequence all the DNA in the

A



B



C

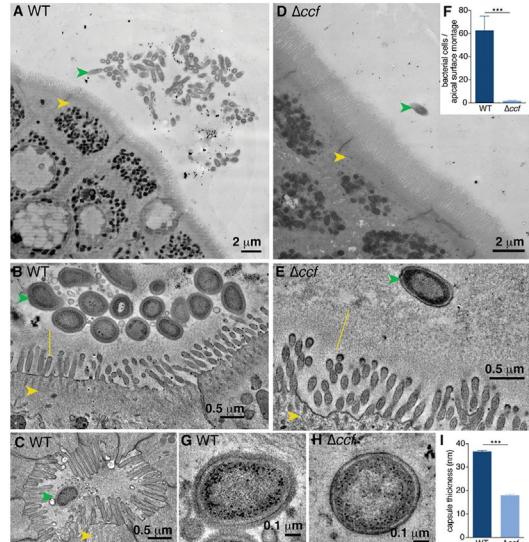


Fig. 1.1 Most bacteria are smaller than their host cells by 1 order of magnitude. (A) Size chart for biomolecules and cells. (B) *Escherichia coli* cells are like tapioca pearls in a bubble tea straw when in the intestine of *Caenorhabditis elegans*. The cross-section of *E. coli* is about 400 nm. (C) Wildtype and Δccf mutant *Bacteroides fragilis* in the colon of mice monocolonized with the bacterium. Note the thinner cell wall of the Δccf mutant. Credit: (A) <https://courses.lumenlearning.com/microbiology/chapter/types-of-microorganisms/>, (B) photo Shigeki Watanabe and Erik Jorgensen, and (C) Fig. 1 of <http://science.sciencemag.org/content/360/6390/795.long>.

Box 1.1 Dental bacteria observed by Leeuwenhoek in 1683

Although saliva is somehow free of ‘animalcules’, clearly mobile microorganisms referred to as little animals, Mr. Antoni van Leeuwenhoek’s study on the bacteria in dental plaques, as described in his September 17th, 1683 letter to the Royal Society of London [3], showcases his scientific rigor.

Oral hygiene, and Leeuwenhoek’s dental sample were examined multiple times:

“I am in the habit of rubbing my teeth with salt in the morning, and then rinsing my mouth with water. After eating I usually pick my molars with a tooth-pick and also rub them with a cloth quite vigorously. This keeps my teeth and grinders so clean and white that only few people of my age can compare with me. Also when I rub my gums with hard salt, they will not bleed. Yet all this does not make my teeth so clean but that I can see, looking at them in a magnifying glass that something will stick or grow between some of the molars and teeth, a little white matter, about as thick as batter. Observing it I judged that, although I could not see anything moving in it, there were yet living animalcules in it. I then mixed it several times with pure rain-water, in which there were no animalcules, and also with saliva that I took from my mouth after eliminating the air-bubbles lest these should stir the spittle. I then again and again saw to my great astonishment, that there were many very small living animalcules in the said matter, which moved very prettily. The big sort had the shape of fig. A; these had a very strong and swift motion, and shot through the water or spittle like a pike through the water. These were mostly few in number. The second sort had the shape of fig. B. These often spun round like a top and every now and then took a course like that shown between C and D. These were far more in number. I could not make out the shape of the third sort, for at one time they seemed to be long and round while at another time they appeared to be round. These were so small that I could see them no bigger than fig. E and therewithal they went forward so rapidly and whirled about among one another so densely that one might imagine to see a big swarm of gnats or flies flying about together. These last at times appeared to me so numerous that I judged that I saw several thousands of them in a quantity of water or spittle (mixed with the aforesaid matter) no bigger than a sand-grain, although there were quite nine parts of water or spittle to one part of the matter taken from between my front-teeth and grinders. Furthermore the matter consisted for the greater part of a great number of fibres, some greatly differing from others in their length, yet of one and the same thickness, some bent crooked, some straight as in fig. F and which lay about in disorderly confusion. And because I had formerly seen in water live animalcules that had the same figure, I made every endeavour to see if there was any life in them, but I could not make out the least motion in any of them that at all looked like life.”

Dental samples from other people, of different gender, age, oral hygiene, and drinking/smoking habits:

“I also took spittle from the mouths of two different women, who, I am convinced, daily cleaned their mouths, and I examined it as closely as I could. But in this, I could not discern any living animalcules. I then mixed the same saliva with a little of the matter that I picked with a needle from between their teeth and then discovered as many living animalcules and also the long particles, as before related.

I have also examined the spittle of a child about 8 years old, but there also could not discover any living animalcules; and after that I mixed the spittle with some of the matter taken from between the child’s teeth and discovered as great a number of animalcules and other particles as mentioned before.”

“While an old man who leads a sober life and never drinks aqua vitae (ethanol solution) or tobacco and very seldom any wine was talking to me, my eye fell on his teeth, which were all coated over; this made me ask him when he had

Box 1.1 Dental bacteria observed by Leeuwenhoek in 1683—cont'd

last cleaned his mouth and the reply was, that he had never washed his mouth all his life. So I took spittle from his mouth and examined it, but could not find in it anything but what I had seen in my own spittle or that of the others.

I took also the matter that stuck between and against his teeth; on mixing this with clean water in which there were no animalcules, and also with his spittle, I observed an incredible number of living animalcules, swimming more nimbly than I had ever seen up to this time. The big sort which were very plentiful, bent their body into curves while going forward, as in fig. G. Furthermore the other animalcules were so excessively numerous that all the water seemed to live, although only very little matter - taken from the teeth - had been mixed with it. The long particles, mentioned before, were also numerous.

I also took the spittle and the white matter, lodged upon and between his teeth from an old man who is in the habit of taking aqua vitae in the morning and of drinking wine and tobacco in the afternoon, wondering whether the little animals could live in spite of this continual drinking. I judged that this man, because his teeth were so uncommonly dirty, would not clean his mouth; when I asked him, he answered: never in all my life with water, but every day by flushing it with aqua vitae and wine. Yet I could not find anything in his spittle in addition to what I found in other saliva. I also mixed his spittle with the matter sticking to the front side of his teeth, but did not find anything in it save a few only of the smallest sort of living animalcules repeatedly mentioned heretofore. However, in the matter which I had taken from between his front-teeth (for he had not a back-tooth in his mouth) I saw many more animalcules, consisting of two of the smallest sort."

Intervention on his own dental sample:

"I did not clean my mouth on purpose for three days and then took the matter that, in a small quantity, had stuck to the gum above my front-teeth; this I mixed both with spittle and with clean water and discovered a few living animalcules in it."

"Furthermore I took some strong wine-vinegar into my mouth, set my teeth, and let the vinegar run between them several times; after this I rinsed three times with clean water. I then once more took some of the foresaid matter both from between my front-teeth and my grinders, mixing it as before several times with spittle as well as with clean rain-water; nearly always I discovered an incredible number of living animalcules, but mostly in the matter which I took from between my back-teeth. Few, however, had the shape of fig. A. I also mixed a little wine-vinegar with the mingled spittle and with the water; the little animals therein died at once. From this I drew the conclusion that the vinegar which I had in my mouth did not penetrate through all the matter which was firmly lodged between and against my front-teeth and my grinders, and only killed those animalcules that were in the outermost parts of the white matter."

On the number of oral bacteria:

"I have had several gentlewomen in my house, who were eager to see the little eels in vinegar. Some of them were so disgusted at what they saw that they resolved never to take vinegar again. But what if in future one should tell such people that there are living more animals in the unclean matter on the teeth in one's mouth than there are men in a whole Kingdom? Especially in those who never clean their mouths, owing to which such a stench comes from the mouth of many that one can hardly bear talking to them. Many call this a stenching breath, but actually it is in most cases a stinking mouth. For my part, I judge from my own case, although I clean my mouth in the manner heretofore described, that there are not living in our United Netherlands so many people as I carry living animals in my mouth this very day. For when I saw that one of my back-teeth was coated against the gum with the said matter about the thickness of a horse-hair, where to all appearance the salt had not scoured this matter for a few days, there were so enormous a number of living animalcules, that I imagined that I could discern as many as 1000 living little animals in a quantity of this matter no bigger than 1/100 part of a sandgrain." [3] (Remember the cube in volume calculations.)

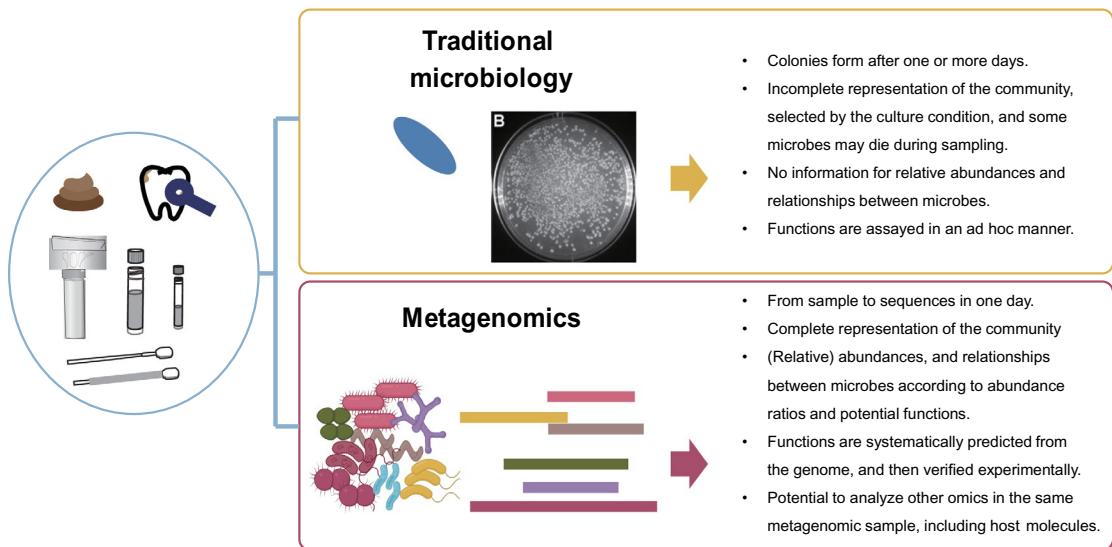


Fig. 1.2 Metagenomics vs traditional microbiology. Absolute abundances can be obtained if quantitatively performed at each step (more in [Chapter 3](#)). Relative abundances are important for ecological studies ([Chapter 2](#)). Credit: Huijue Jia.

sample, piece together genomic information for individual microbes, and quantify how abundant each microbe is in the sample. Before the advent of high-throughput sequencing technologies, impressive work has been done using traditional microbiology, and later using molecular biology techniques. Without having to guess on the culture conditions and grow out each microbe in a plate, metagenomics make it possible for researchers and clinicians to know all the microbes that are there and what they could do. Sequencing platforms both large and small, along with developments in bioinformatics, are making metagenomic studies more accessible for researchers and clinicians who have different needs for the cost and for the time to results.

Our quest to understand the human microbiome ([Box 1.2](#)) will bring about more technology. Taking better care of the microbes that live inside or on the surface of the human body will help us better cope with diseases in populations around the world.

1.2 How many microbial cells can a human body have?

Bacteria are typically in the low micrometer range ([Fig. 1.1](#)), if not in longer filaments. Fungi and viruses span a wider range on the larger and the smaller sides. At the smallest volume, free-living bacteria are limited by the volume of DNA and proteins inside; at the largest

Box 1.2 “Metagenome”, “microbiota” and “microbiome”

Metagenome:

Dr. Jo Handelsman introduced the word “metagenome” in a 1998 publication on the soil microbiome [5], for which only a fraction of the microbes had been cultured in isolation. The article advocates for direct cloning of metagenomic DNA from soil samples into *E. coli* BAC (Bacterial Artificial Chromosome) libraries for further analyses on the gene functions, without culturing each microbe [5]. Now with high-throughput sequencing and bioinformatic analyses, we use the term “metagenomics” to refer to unbiased direct sequencing of the microbial community in any sample.

Microbiota or microbiome:

A popular assumption is that the Nobel Laureate Joshua Lederberg coined the term “microbiome” in 2001. At face value, the terms “microbiota” or “microflora” are more about the microbial ecosystem. The term “Microbiome,” however, is not a product of the genomics era and already includes everything in the ecosystem, a “biome” after all [6]. The words for microbial communities in fact date back earlier [6,7].

Back to work in 1923, Sergei Nikolaievich Winogradsky advocated for the study of interacting microbes in their natural contexts, following his discovery by the end of the 19th century, of how aerobic nitrification (the oxidation of ammonium salts to nitrites and nitrites to nitrates) bacteria locally depleted oxygen, so that the anaerobic nitrogen fixer *Clostridium pasteurianum* could live in the niche created by its neighbors and finish the two-step nitrification process.

Some interesting comments on pure culture from a 1949 paper:

“... condition of pure culture in an artificial environment is never comparable to that in a natural environment ... one cannot challenge the notion that a microbe cultivated sheltered from any living competitors and luxuriously fed becomes a hot-house culture, and is induced to become in a short period of time a new race that could not be identified with its prototype without special study” [6,8].

Specific pathogen-free and germ-free animal models become a common laboratory practice in 1960s. To determine the selected microbiota that is compatible for the sustained health of the animal models has been a goal:

“to endow them, in short, with a selected microbiota compatible with sustained health and conferring some ability to withstand the assaults of other micro-organisms that would almost inevitably be encountered outside the germ-free environment” wrote Lane-Petter in 1962 [9]. In 1986, Linda R. Hegstrand and Roberta Jean Hine discovered a difference in hypothalamic histamine levels between germ-free and conventionally raised animals, an early example of the gut-brain axis [7].

When studying plant diseases, John M. Whipps wrote in 1988:

“A convenient ecological framework in which to examine biocontrol systems is that of the microbiome. This may be defined as a characteristic microbial community occupying a reasonably well defined habitat which has distinct physio-chemical properties. This term thus not only refers to the microorganisms involved but also encompasses their theatre of activity” [6,10].

volume, bacteria are limited by the number of ribosomes required for maintaining such a size, which would be too much to fit into the cell volume (Fig. 1.3) [11]. The cytoplasmic volume of *E. coli* shrank by 17% upon nutrient starvation [12]. The same principles should also apply to archaea. The smallest observed archaea are comparable to the smallest bacteria, with a volume of about $3.41 \times 10^{-20} \text{ m}^3$, 0.5 megabases (Mb) genome, and about 92 ribosomes per cell [11].

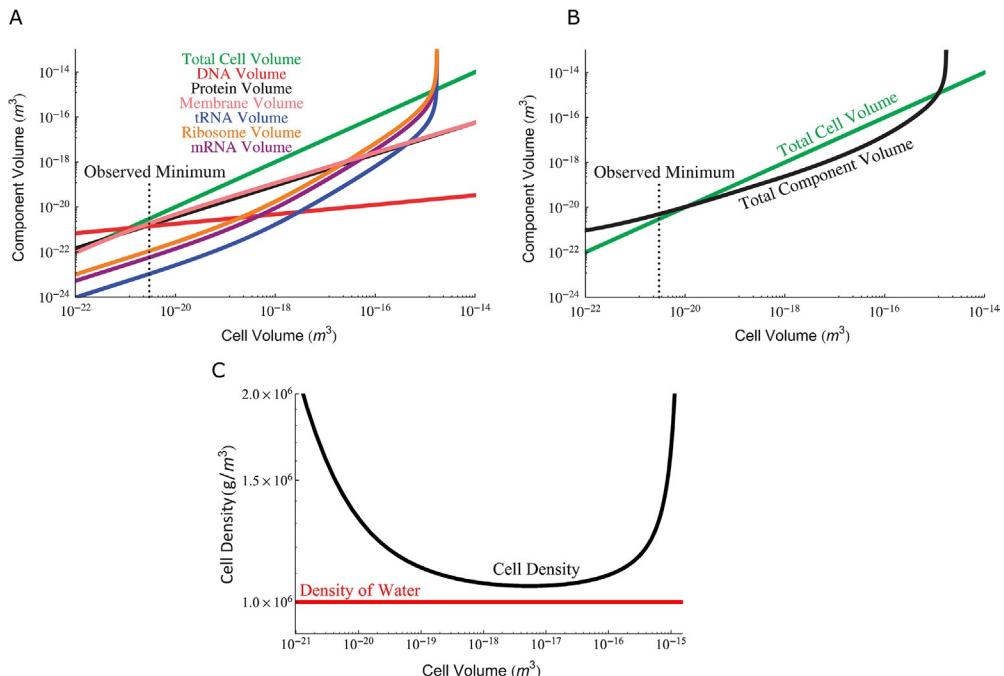
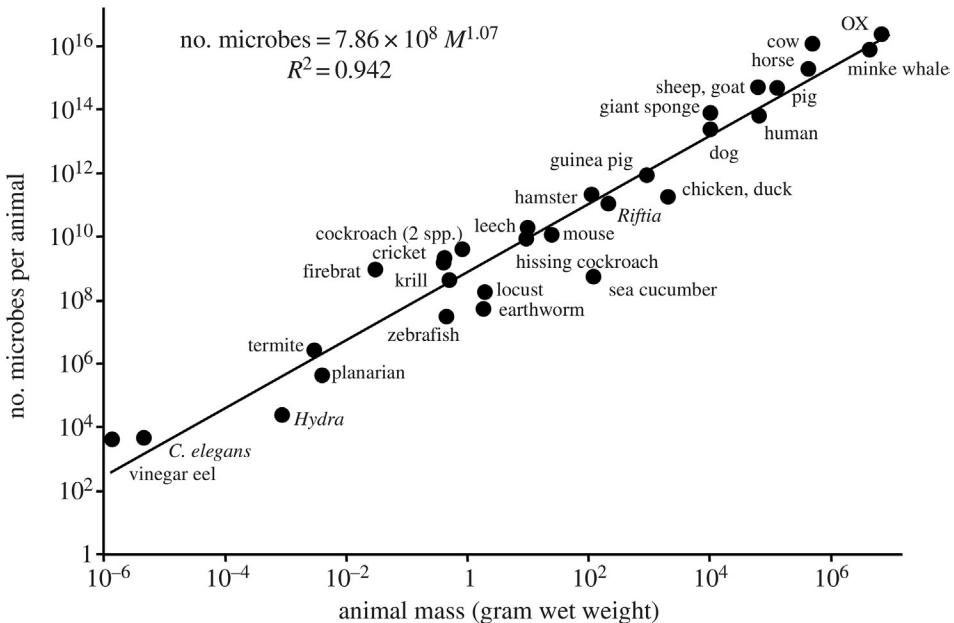


Fig. 1.3 Theoretical limitations for free-living bacteria and archaea to not go much smaller or larger. For example, a typical *E. coli* cell is $2\ \mu\text{m}$ long, $0.5\ \mu\text{m}$ in diameter. (A) The volume-dependent scaling of each of the major cellular components for bacteria. (B) The total cell volume was compared with the volume of all cellular components as a function of cell size. (C) The volume-dependent scaling for the calculated total cellular density. The black curve is the calculated density and the red curve is the reference value for the density of water. Credit: (A and B) Fig. 3a,b, (C) Fig. S5 of Kempes CP, Wang L, Amend JP, Doyle J, Hoehler T. Evolutionary tradeoffs in cellular composition across diverse bacteria. ISME J 2016;10:2145–57. <https://doi.org/10.1038/ismej.2016.21>.

We like to talk about tigers, elephants, and whales, but humans are actually among the large animals on earth. There is an interesting linear relationship between the logarithm of the bodyweight of an animal and the logarithm of the number of microbes (Fig. 1.4A). Each gram of an animal averages approximately 3.4×10^9 of associated prokaryotes (bacteria or archaea), and animals are approximately 0.34% prokaryotes by weight [13]. Animal-associated microbes total about $2.1\text{--}2.3 \times 10^{25}$ of the $9.2\text{--}31.7 \times 10^{29}$ prokaryotic cells on earth. Total gut volume has been estimated to scale with animal body mass with exponents of 1.0–1.08, and the surface area of the gut scales with body mass with an exponent of 0.75. However, counts of microbes per unit volume or mass of gut contents vary over several orders of magnitude, and the proportion of the gut (gut in zoology sense, the entire alimentary tract) devoted to intensive microbial activities also varies extensively among animals.

Most of the 3.8×10^{13} microbial cells for a healthy adult human resides in the colon (Figs. 1.4B and 1.5) [14,15]. The number of microbial

A



B

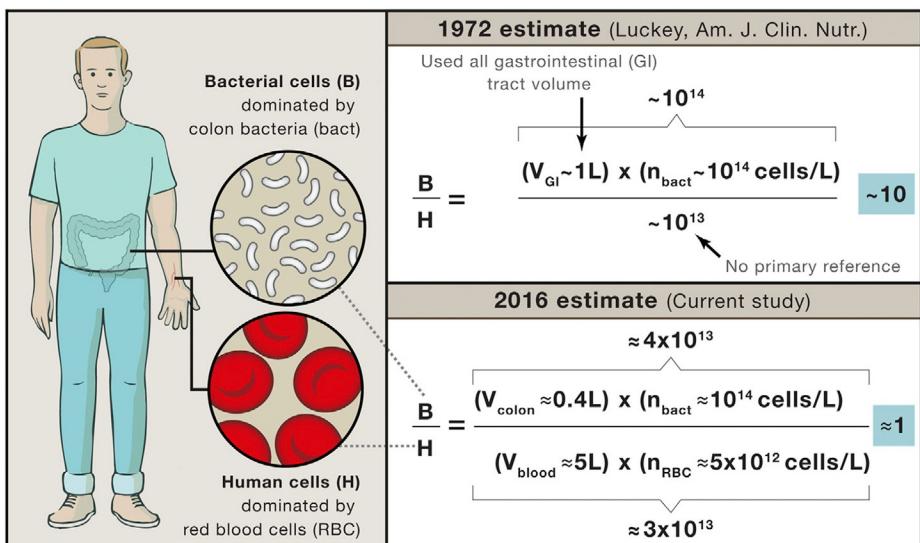


Fig. 1.4 Current estimate for the number of microbes in an adult human, and the general trend in animals of different sizes. (A) Counts of microorganisms per individual animal versus individual animal body mass (M , wet weight in gram), log-log plot. (B) The total number of bacterial cells was estimated according to the typical volume of the adult human colon, instead of volume of the entire gastrointestinal tract, as the bacterial community is much denser in the colon. Credit: (A) Fig. 1 of Kieft TL, Simmons KA. Allometry of animal-microbe interactions and global census of animal-associated microbes. Proc R Soc B Biol Sci 2015;282:20150702. <https://doi.org/10.1098/rspb.2015.0702>. (B) Fig. 1b of Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? revisiting the ratio of bacterial to host cells in humans. Cell 2016;164:337–40. <https://doi.org/10.1016/j.cell.2016.01.013>.

species in the human colon is over 600, and their number of cells could range from 1 to more than 10^{13} , corresponding to relative abundances of less than 10^{-13} to over 0.3 (The relative abundances of all species sum up to one). The skin has a large surface area, but has relatively simple microbial communities in humans (Fig. 1.6A and B) [16,17]. The lung is topologically an outer surface, with an even larger surface area and

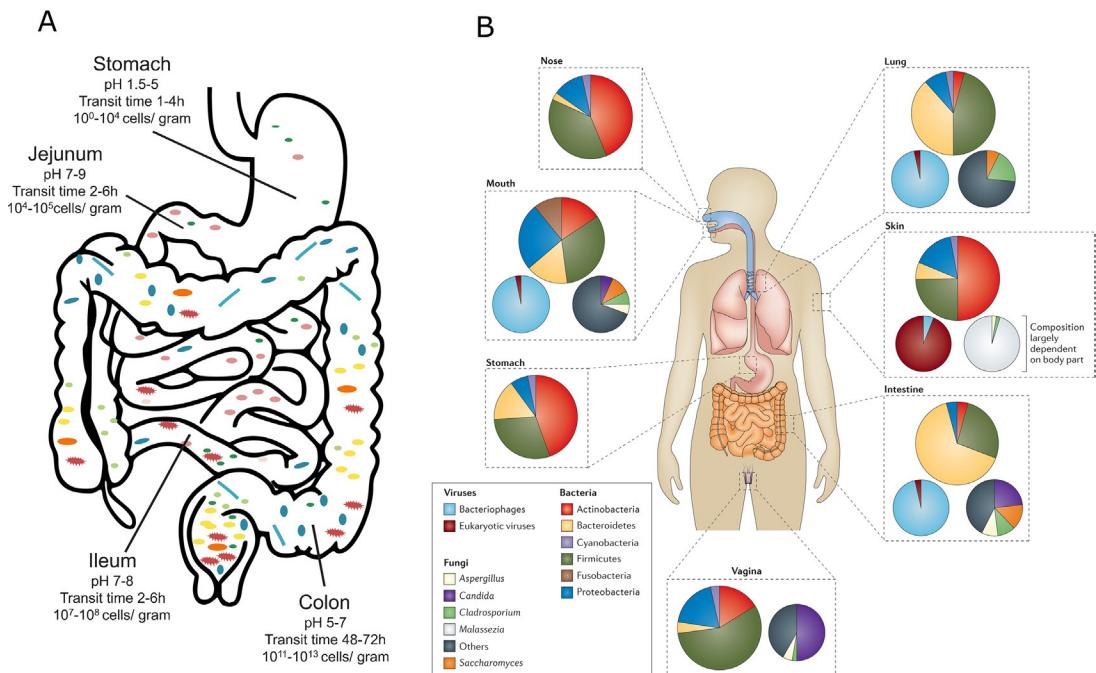


Fig. 1.5 Mucosal sites are major habitats for the human microbiome. (A) Estimates for the number of microbes in different segments of the gastrointestinal tract, together with major factors that influence the community such as pH and transit time. (B) Bacteria phyla, fungi genera, and viruses at different body sites. The figure shows the relative abundance of bacterial, fungal, and viral communities at different body sites exposed to the external environment—the nose, mouth, skin, stomach, intestinal tract, vagina, and lungs. Bacterial composition is represented by the six most commonly detected phyla—Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Fusobacteria, and Proteobacteria. Fungal composition includes Aspergillus, Candida, Cladosporium, Malassezia, and Saccharomyces as the most prominent genera. Additional types of fungi are summarized as “Others.” Viral composition is classified simply as bacteriophages or eukaryotic viruses. Data based on Erb-Downward JR, Thompson DL, Han MK, Freeman CM, McCloskey L, Schmidt LA, et al. Analysis of the lung microbiome in the “healthy” smoker and in COPD. PLoS One 2011;6:e16384. <https://doi.org/10.1371/journal.pone.0016384>; Underhill DM, Iliev ID. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol 2014;14:405–16. <https://doi.org/10.1038/nri3684>; Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol 2011;9:279–90. <https://doi.org/10.1038/nrmicro2540>; Abeles SR, Pride DT. Molecular bases and role of viruses in the human microbiome. J Mol Biol 2014;426:3892–906. <https://doi.org/10.1016/j.jmb.2014.07.002>. Credit: (A) Fig. 1a of Belzer C, de Vos WM. Microbes inside—from diversity to function: the case of Akkermansia. ISME J 2012;6:1449–58. <https://doi.org/10.1038/ismej.2012.6>. (B) Fig. 1 of Marsland BJ, Gollwitzer ES. Host-microorganism interactions in lung diseases. Nat Rev Immunol 2014;14:827–35. <https://doi.org/10.1038/nri3769>.

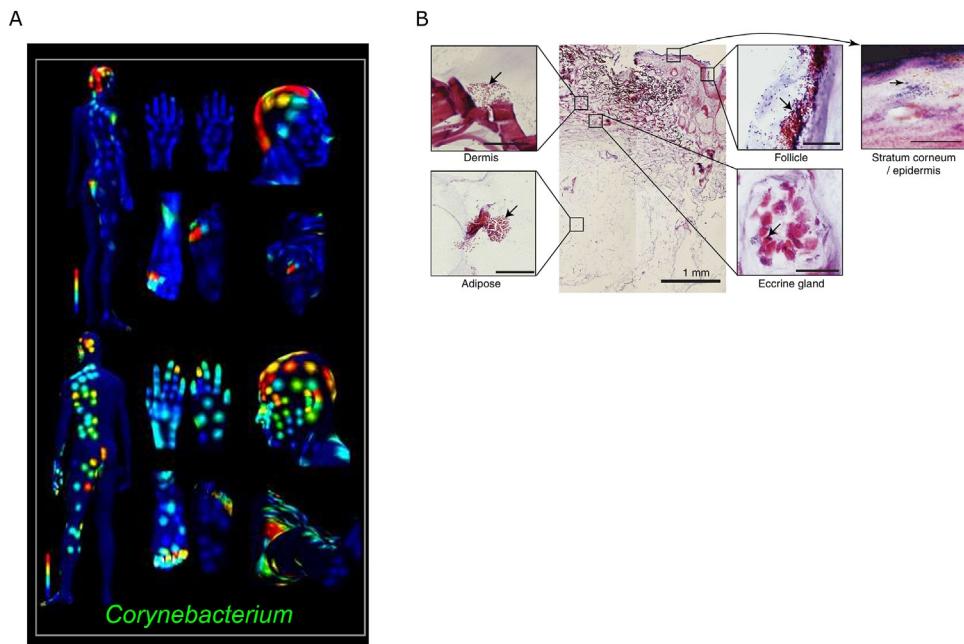


Fig. 1.6 Bacteria on and under the skin. (A) Distribution of *Corynebacterium* spp. on the human skin. (B) Gram stain for bacteria inside the skin. Cut from normal areas of melanoma samples. Credit: (A) Cropped from Fig. 3 of Bouslimani A, Porto C, Rath CM, Wang M, Guo Y, Gonzalez A, et al. Molecular cartography of the human skin surface in 3D. Proc Natl Acad Sci U S A 2015;112:E2120–9. <https://doi.org/10.1073/pnas.1424409112>. (B) Fig. 1c of Nakatsuji T, Chiang H-I, Jiang SB, Nagarajan H, Zengler K, Gallo RL. The microbiome extends to subepidermal compartments of normal skin. Nat Commun 2013;4:1431. <https://doi.org/10.1038/ncomms2441>.

a sparse population of microbes [18,19]. The oral cavity and the vagina are densely populated with bacteria, along with some viruses and sometimes fungi, but the total number of microbial cells would be 1 or 2 orders of magnitude lower than the estimate for the colon, even if the bladder and the uterus are also considered (Fig. 1.7). Semen samples can contain more than 10^6 – 10^7 bacteria per mL (milliliter) [20]. Microbes are found in more traditionally “sterile” places, including in tumors (Fig. 1.8), but these are not large numbers. If one day we see each of the 3×10^{13} cells in the human body to contain more than 2 intracellular bacteria, we may start to worry about the total estimate (Fig. 1.4).

Worked sample 1.1

What physiological conditions do you think might change the number of microbes in a given body site?

How many microbial cells are we losing from a given body site every day? What does that predict regarding their growth rate? Or they are repleted by microbes from other places (More in Chapters 2 and 4).

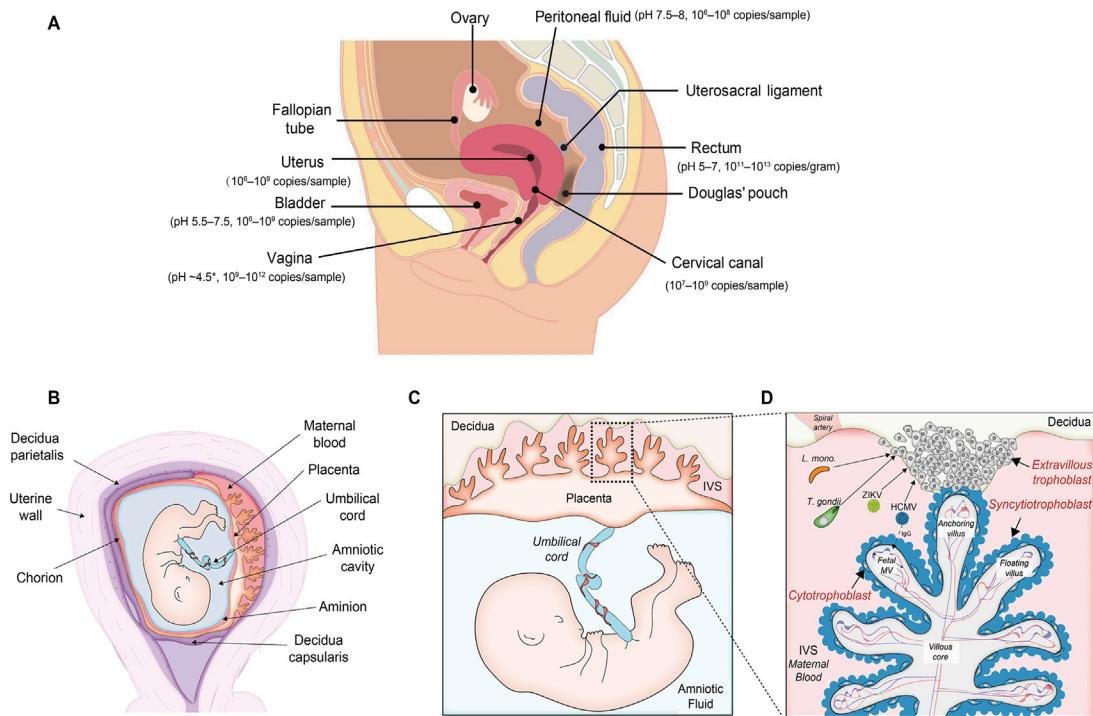


Fig. 1.7 Microbiome of the female reproductive tract. (A) Tentative estimates for the number of microbes in different regions of the female reproductive tract in nonpregnant volunteers operated for reasons not known to involve infection (e.g., uterine fibroids) [21]. Copies of total bacterial genomes were calculated according to qPCR against *Lactobacillus* species in the samples, which constitute a much smaller portion of the community in the upper reproductive tract where the pH is no longer acidic. (B–D) More research would be necessary for different regions of the placenta (more in Chapter 3). Amniotic fluid is typically only available for preterm birth. (B) Schematic of the uterine cavity during pregnancy. The developing fetus is encased within the amniotic cavity, surrounded by the chorion and amnion, and anchored to the maternal decidua by the placenta (at the site of attachment, the decidua basalis). (C) Maternal blood fills the intervillous space (IVS) via spiral arteries that bathe the surfaces of the placenta in maternal blood (once the maternal microvasculature has been established). (D) The human hemochorionic placenta is formed by villous trees composed of both floating villi and anchoring villi, which attach directly to the decidua basalis by the invasion of extravillous trophoblasts (EVTs). The human placenta villous trees are covered by syncytiotrophoblasts, with a layer of cytotrophoblasts (which become discontinuous throughout pregnancy) below this layer. Several pathogens, including *Listeria monocytogenes* (*L. mono*), *Toxoplasma gondii* (*T. gondii*), human CMV (HCMV), and Zika virus (ZIKV), are thought to access the villous core following replication in EVTs. Credit: (A) Chen Chen from BGI-Shenzhen. (B–D) Fig. 1A–C of Arora N, Sadovsky Y, Dermody TS, Coyne CB. Microbial vertical transmission during human pregnancy. *Cell Host Microbe* 2017;21:561–7. <https://doi.org/10.1016/j.chom.2017.04.007>.

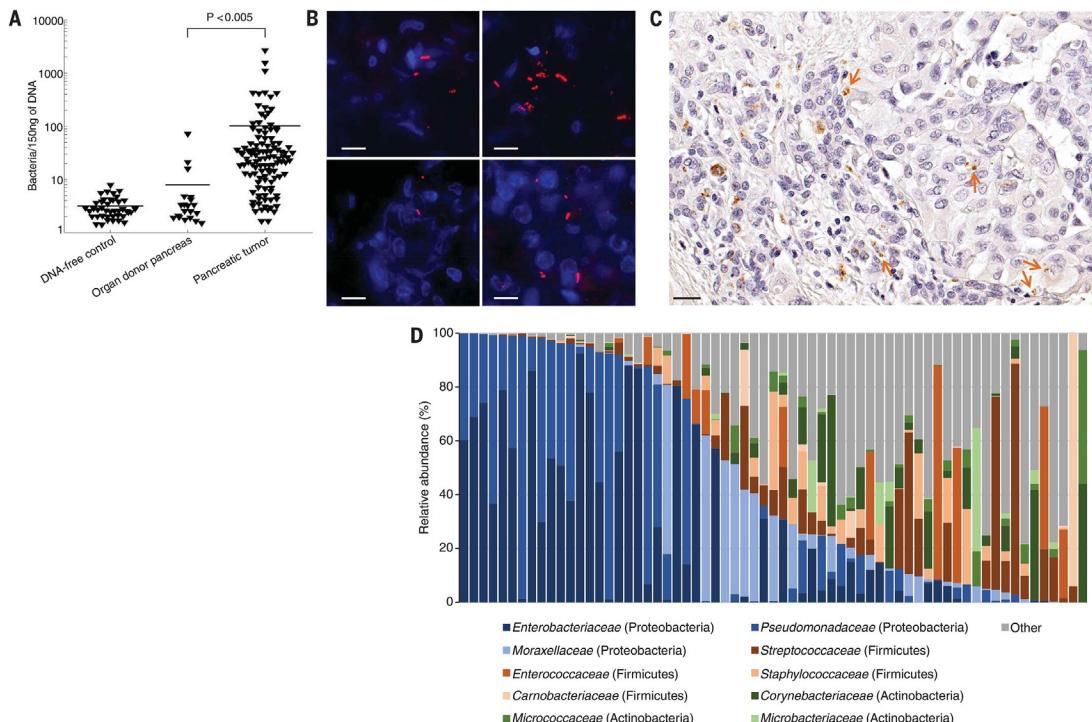


Fig. 1.8 Bacteria in pancreatic ductal adenocarcinoma (PDAC) studied by multiple methods. (A) The presence of bacteria in human pancreatic tumors or in healthy pancreatic tissue from organ donors was assessed by bacterial 16S rDNA qPCR. A calibration curve, generated by spiking bacterial DNA into human DNA, was used to estimate bacterial numbers. Bars represent the mean. (B) Fluorescence in situ hybridization was used to detect bacterial 16S rRNA sequences in a human PDAC tumor (red). Cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Four sections from one tumor are presented. Scale bars, 10 mm. (C) Immunohistochemistry of a human PDAC tumor using an antibacterial LPS antibody. Arrows point to LPS staining in the tumor tissue. Scale bar, 20 mm. (D) Distribution of family-level phylotypes in 65 human PDAC tumors. Relative abundance (%) is plotted for each tumor. Credit: Fig. 4 of Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* 2017;357:1156–60. <https://doi.org/10.1126/science.aah5043>.

1.3 Viral particles in the human body

Viral particles have also been counted from many kinds of samples. There are 10^9 virus-like particles (VLPs) per gram of feces, 10^7 VLPs per milliliter of urine, and 10^8 VLPs per milliliter of saliva [22].

Besides bacteriophages, there are viruses for eukaryotes. Anelloviridae is a family of nonenveloped, single-stranded DNA viruses with a circular genome of 2–4 kilobases (kb). Anelloviridae is commonly detected in all the major mucosal sites, as well as in blood and semen [22]. Members of the Anelloviridae family include torque teno virus, torque teno mini virus and torque teno midi virus.

Worked sample 1.2

Do you think the total number of microbial species in the human body will be more than a few thousands?

Where do you think the current number may be underestimated?

1.4 Microbiome in other species

This book focuses on the human microbiome and diseases. With the basic principles covered, it is up to the readers to take inspirations from microbiome studies on animals or even plants (Fig. 1.9) [23], in the process of investigating a particular question.

For example, a switch of microbiome components may appear drastic in a specific lineage of the host. The insect *Cicadas* depend on the essential bacterial symbionts *Sulcia* and *Hodgkinia*, but in many Japanese *Cicadas*, *Hodgkinia* has been replaced by a fungus that is likely recruited from cicada-parasitizing *Ophiocordyceps* fungi. The fungal symbiont encodes all the pathways for B vitamins and nitrogen recycling, and could synthesize all essential and nonessential amino acids, which is more versatile than histidine and methionine synthesis provided by *Hodgkinia* [25]. Flies (*Drosophila melanogaster*) actively seek food to replenish their gut bacteria, and the taxonomic preference can be modified by early exposure to the bacteria [26].

Factors such as pH, oxygen, temperature, minerals, carbon, and nitrogen sources are well established in traditional microbiology to impact growth (Fig. 1.10) [27], and some of the bacteria can fix carbon dioxide or fix nitrogen themselves. In a community setting, and being on the surface of or inside a host presents both opportunities and challenges (more in Chapter 2). Functions such as competitive exclusion against pathogens, digestion of complex organic substrates, providing nutrients and growth factors, promoting development, and affecting behavior, are evolutionarily conserved service of the microbiome to the host, and there may be manipulation of the host for better survival of the microbes [28–31].

Compared to nutrient-poor environments such as deep ocean sediments, where a bacterium takes more than 1000 years to replicate once [33], life is fast for the human-associated microbes, despite possible differences between taxa.

1.5 Microbiome from ancient times

Paleogenomics is telling us a lot about the evolution and migration of modern humans as well as other species. Fragments of microbial DNA can also be extracted from dental calculus and coprolites (fossil feces) from ancient times (Figs. 1.11 and 1.12), which provide exciting data points to offer a glimpse into the evolution of the microbiome.

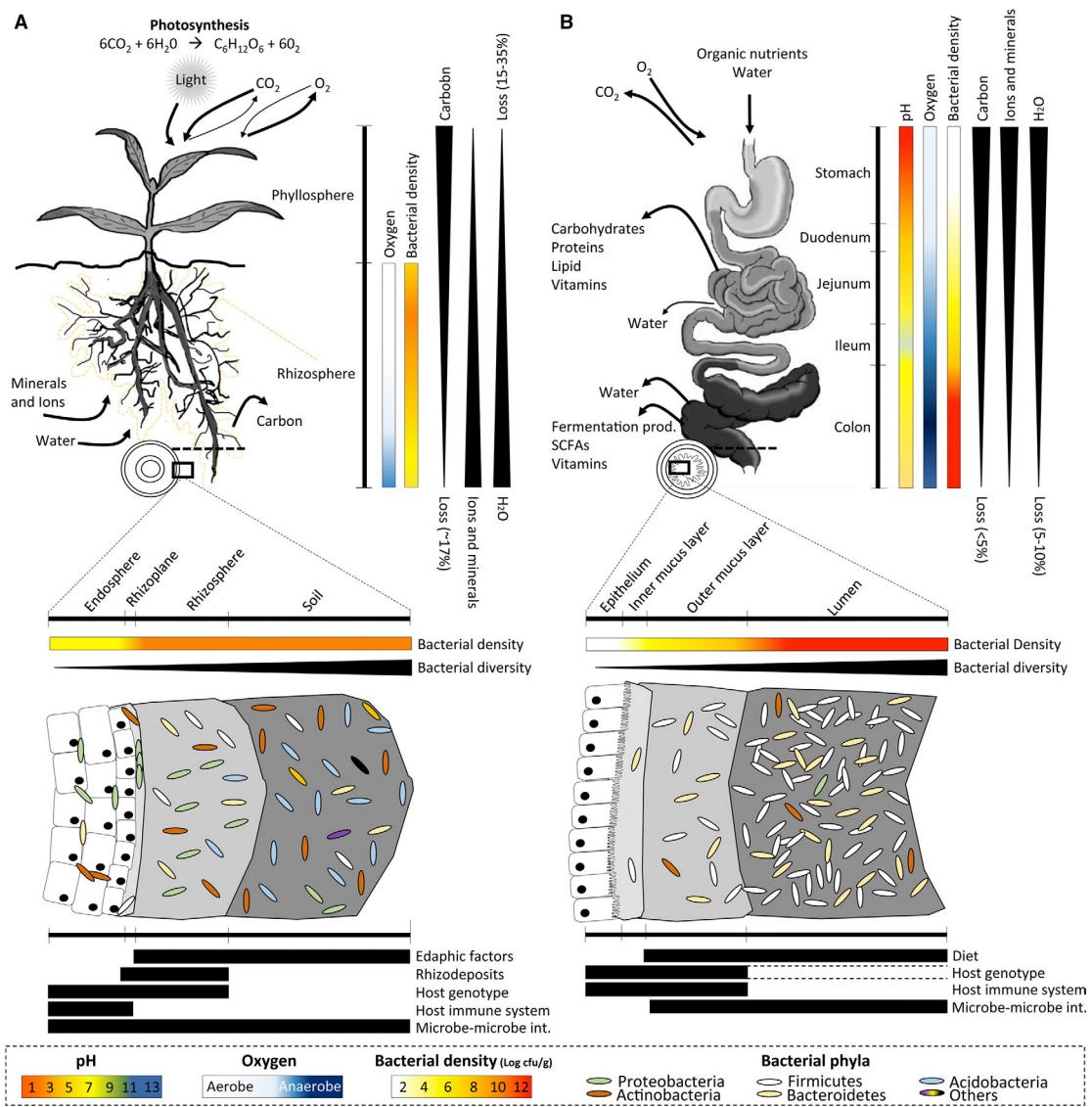


Fig. 1.9 Physiological functions of the plant roots and human gut in nutrient uptake, spatial aspects of microbiota composition, and factors driving community establishment. Spatial compartmentalization of the plant root microbiota (A) and the human gut microbiota (B). Upper panels: the major nutrient fluxes are indicated, as well as pH and oxygen gradients in relation to the bacterial density. Lower panels: compartmentalization of the microbiota along the lumen-epithelium continuum in the gut or along the soil-endosphere continuum in the root. For each compartment, the bacterial density, the bacterial diversity, and the major represented phyla are represented for both the gut and the root organs. The main factors driving community establishment in these distinct compartments are depicted with black bars. The gut drawing was adapted by [23] from Tsabouri et al. [24] with permission from the publisher. Credit: Fig. 1 of Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, et al. Microbiota and host nutrition across plant and animal kingdoms. *Cell Host Microbe* 2015;17:603–16. <https://doi.org/10.1016/j.chom.2015.04.009>.

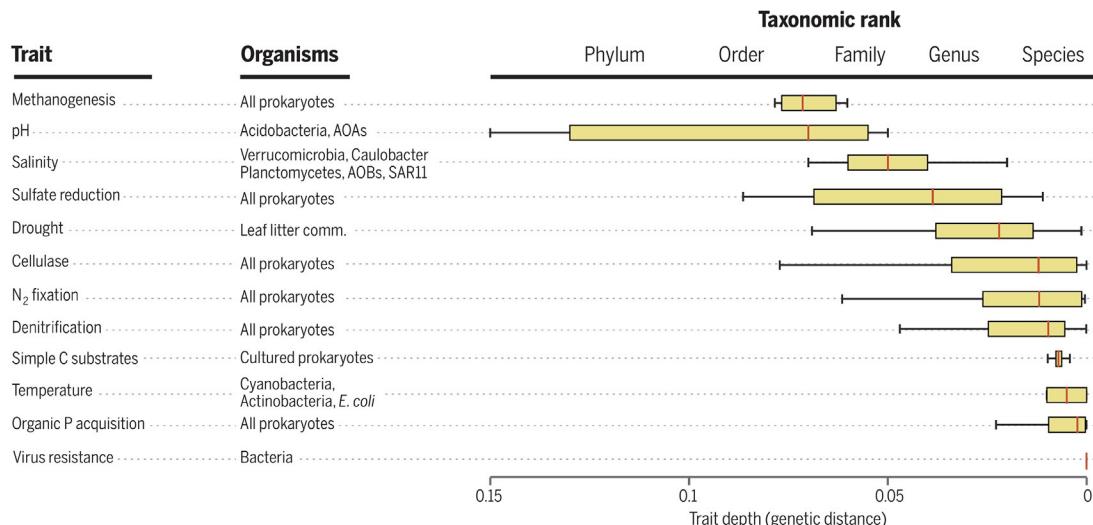


Fig. 1.10 Prokaryotic traits are conserved at different phylogenetic depths. A box plot of the depth of clades within which taxa consistently share a trait measured as the genetic distance to the root node of a clade (bottom axis; usually of the 16S rRNA gene). For some traits, the distribution was based on several studies, each with one estimate. For other traits, the authors of the original figure [32] reported the distribution calculated by a single study. For comparison, the authors of the original figure showed rough taxonomic levels on the top axis. Credit: Fig. 3 of Martiny JBH, Jones SE, Lennon JT, Martiny AC. Microbiomes in light of traits: a phylogenetic perspective. Science 2015;350:aac9323. <https://doi.org/10.1126/science.aac9323>.



Fig. 1.11 Supra-gingival dental calculus is identifiable in a concave ring on a lower molar from a Medieval specimen, York, United Kingdom. Credit: Fig. 1 of Weyrich LS, Dobney K, Cooper A. Ancient DNA analysis of dental calculus. J Hum Evol 2015;79:119–24. <https://doi.org/10.1016/j.jhevol.2014.06.018>.

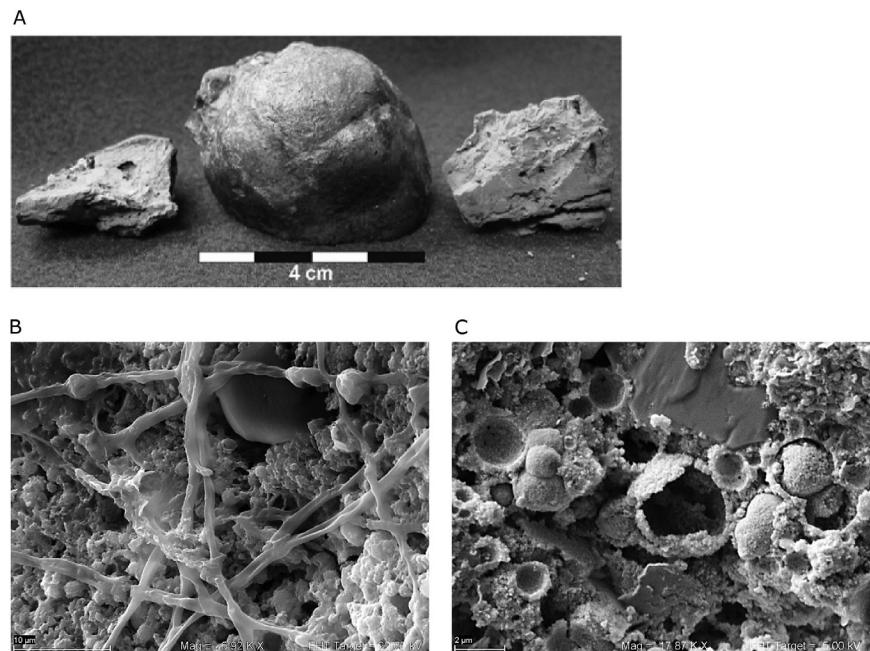


Fig. 1.12 Microbe-like structures in a coprolite (fossil feces) from the Cretaceous period, even though DNA would not have survived that long. (A) The Coprolite from a *Triceratops* dinosaur (*Sternoholophus* Marsh) site preserved in fluvial deposits in the Upper Cretaceous Hell Creek Formation of eastern Montana, United States. The host animal is unknown. The coprolite contains small quantities of minute bone or tooth fragments, kerogenized plant residues (pollen, spores, sporangia, cuticle, etc.), hyphae of probable fungal origin, and small detrital mineral grains in a fine-grained, highly porous matrix. (B) Branching, fungal or bacterial hyphae, and numerous small spherical objects resembling bacteria in size and shape; scale bar 10 µm; M = silicate mineral grain. (C) Structure of the coprolite matrix showing hollow, thin-walled mineral spheres, some of which have double shells with a thin void space between that may be the former location of bacterial cell walls; scale bar 2 µm; M = silicate mineral grain. Credit: Fig. 1 and Fig. 3D,F of Hollocher KT, Hollocher TC, Rigby JK. A phosphatic coprolite lacking diagenetic permineralization from the upper cretaceous hell creek formation, northeastern montana: importance of dietary calcium phosphate in preservation. *Palaios* 2010;25:132–40. <https://doi.org/10.2110/palo.2008.p08-132r>.

Leeuwenhoek's account gave us some idea about how hygiene practices have changed in the developed countries (Box 1.1). Ancient dental, fecal, and environmental samples can show diet and microbes. Neanderthal coprolite samples from a cave in Spain showed many of the same genera as we do in the gut microbiome [34]. Pathogens including viruses, bacteria, fungi, and parasitic worms might have played important roles in shaping both the genome and the microbiome [35,36]. Such historical questions are also up to the readers themselves to further explore.

1.6 Summary

Metagenomics is at its prime for human microbiome studies. Beginning with Antoni van Leeuwenhoek's observation on microorganisms in dental plaques (Box 1.1), this chapter introduces metagenomic studies of the human microbiome in the context of technological development for the advancement of microbiology. Logarithm of the number of microbial cells scales with the biomass of an animal. The current estimate of 3.8×10^{13} microbial cells for an adult human is based on the volume of the colon, where the overwhelming majority of microorganisms reside. A lot of other epithelia, tissues or body fluids have smaller populations of microbes. As detailed in Box 1.2, we will use the term microbiome to include meanings of microbiota or microflora throughout this book.

Besides technology and cost, there is no limit to where we can study for the microbiome. This book is not organized according to the traditional human microbiome body sites, and we expect to accommodate more new discoveries in the years to come. The number of microbial cells, or the microbial biomass in each sample would be an important consideration for metagenomic studies (Chapters 3–5). After covering ecological principles in Chapter 2, this book goes on with more practical knowledge for designing metagenomic studies (Chapters 3 and 4), the taxonomic resolution (Chapter 5), and how we can draw causal conclusions for the role of the microbiome in diseases (Chapter 6). We can then put the knowledge into clinical practice (Chapter 7) and more long-term health management (Chapter 8).

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