

Microbial diversity – Bacteria

Miss: we look very different from an elephant, which looks different from a snail, which looks different from a sparrow, which looks different from a shrimp. From pictures of microbes I have seen, they do not look so different from one another. How different are they really?

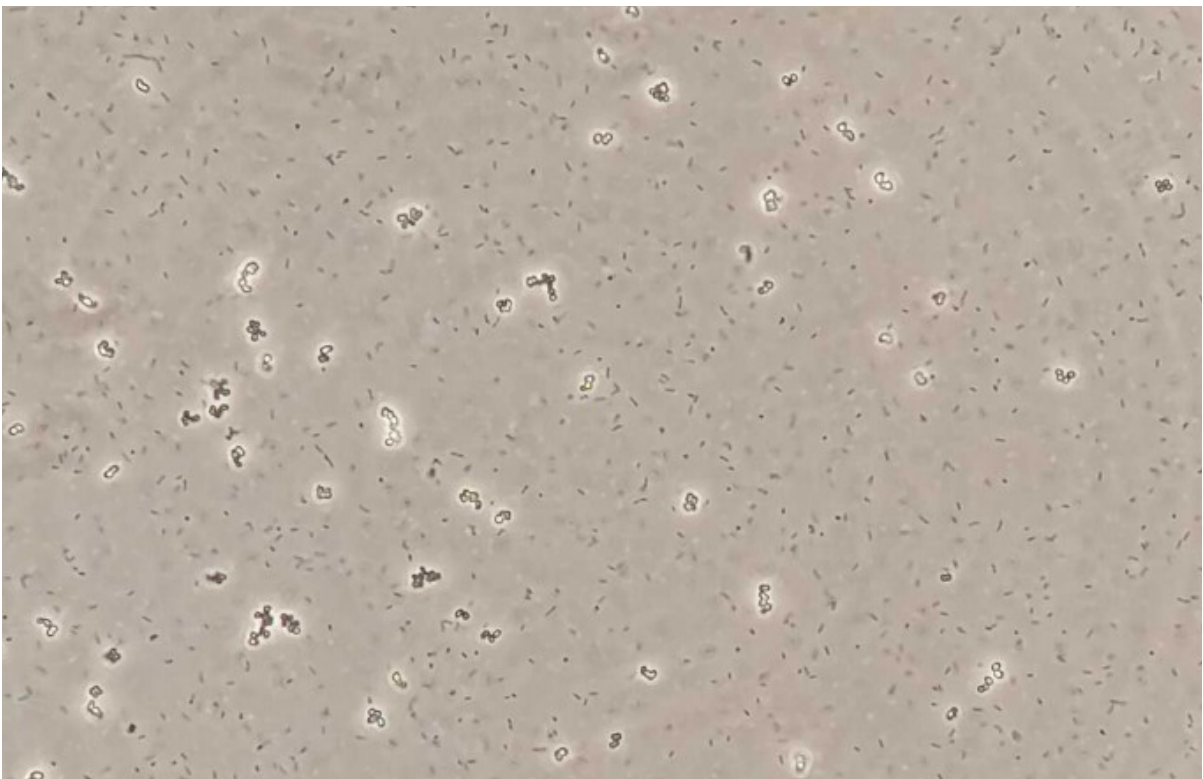


Photo credit: Alena di Primio, NIOZ, Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, Den Burg, The Netherlands

Pierre Offre

NIOZ, Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, P.O. Box 59, 1790 AB Den Burg, The Netherlands

Bacteria: look-alikes yet so different from one another

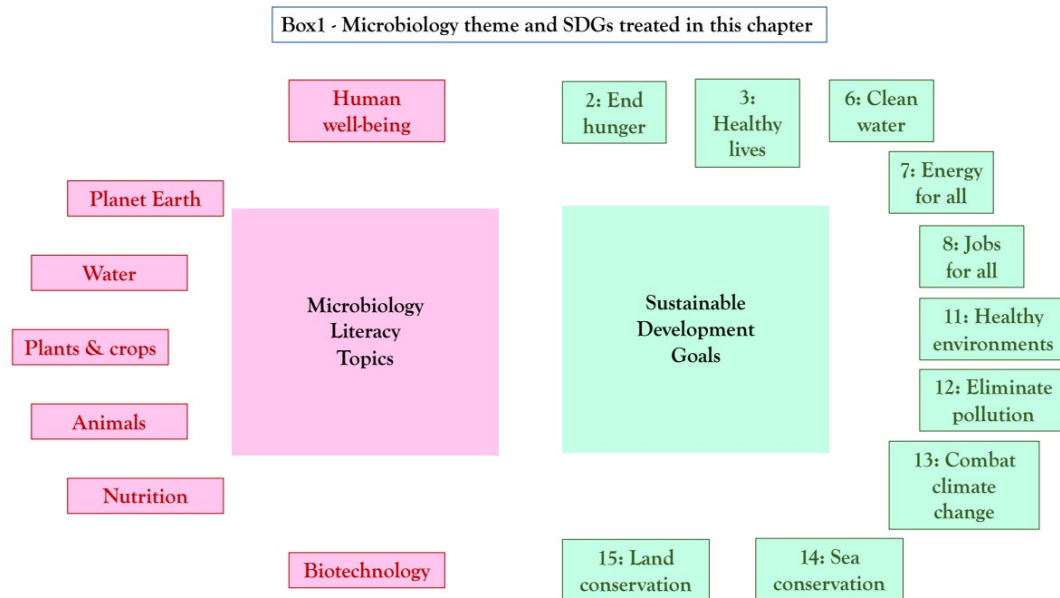
Storyline

Bacteria are tiny organisms generally invisible to the naked eye and usually consisting of a single cell no larger than 1 or 2 micrometers in length, width and height. Regardless of where they come from, be it an infected wound, a yoghurt or water from a garden pond, their shape appears to vary little when observed under a light microscope. Bacterial cells very often take the shape of a sphere (in which case they are referred to as cocci) or that of a short stick (referred to as rods), although various alternative yet rarer forms are known. The limited diversity of sizes and shapes commonly revealed by conventional microscopic observations of bacterial cells comes in direct contrast with the incredible variety of morphologies and complex behaviors that can be easily noticed without microscope among multicellular eukaryotes and especially members of the Animal Kingdom. Yet, bacteria are far more diverse than it seems, but their diversity is not of a type that can be easily observed. Decades of research and technological developments have now established that bacteria occur throughout the biosphere, having varied lifestyles supported by many unique traits including various strategies to extract energy and nutrients from their environments. Further, the total number of bacterial species in the biosphere could equal and even exceed the number of animal species as recently suggested by recent species inventories. Yet, most of these bacterial species remain uncharacterized and represent a huge reservoir of untapped biodiversity with multiple connections to Sustainable Development Goals.

The Microbiology and Societal Context

The microbiology: Number of described bacterial species; bacterial genome variations and classification of described bacteria; the different forms of nutrition in bacteria; variations in the growth of bacterial cells and their growth conditions; the different types of lifestyles and behavior in bacteria; estimates of the number of bacterial species inhabiting the biosphere; the classification of undescribed bacteria. *Societal and sustainability issues:* health; conservation; food; economy and employment; energy; environmental pollution; global warming.

A child-centric microbiology education framework






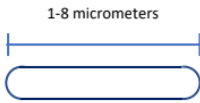
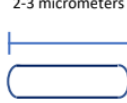
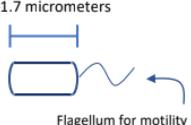
Bacteria: The Microbiology

There are currently 20,000 species of bacteria with validated names and descriptions. Based on research conducted over the past five years, this number increases by about 1,000 species every year. One of the two questions we would like to address here is: how much do these bacterial species differ from one another? Are they all very similar or, in contrast, can they be very different? By focusing on selected examples of bacteria and discussing features of their cells and lifestyles, it will become clear that bacteria vary broadly and in some ways that are unseen in eukaryotes. The second question then asks: how many bacterial species still lack a valid name and description? Although there is no doubt that most bacterial species have not been described, there is no clear consensus on their approximate number at the moment. Lowest estimates derived from recent inventories suggest there is well over 1,000,000 species of bacteria living in the biosphere.

1. How do bacterial cells differ from one another? Although much simpler than eukaryotic cells, even the simplest bacterial cell remains a marvel of complexity involving well over a thousand types of molecules (each present in a large number of copies) that interact with one another and determine the various traits of the cell: e.g., its size and shape, the type of nutrients it needs, how it consumes nutrients, the conditions under which the cell can grow and reproduce, how fast the cell grow and reproduce, the cell's ability to store nutrients, the cell's ability to move and explore its environment...etc. In order to illustrate the extent of the differences between all named bacterial species, I will first discuss variations in the genomes of bacteria before diving into the staggering degree of variation in their nutritional preferences, which is unparalleled in the biosphere. To conclude this discussion, I will then report on the differences in the growth and reproduction of bacteria and the variety of conditions under which bacteria reproduce. See **Figure 1** for a summary of the differences among selected examples of bacterial species.

A child-centric microbiology education framework

Figure 1 Selected examples of bacterial species having similar cell shape. Indicated features illustrate how broadly the biology and ecology of similar looking bacteria may differ. Note that detailed features of bacterial cells such as their exact size, shape and number of flagella are not easily revealed using conventional light microscopy.

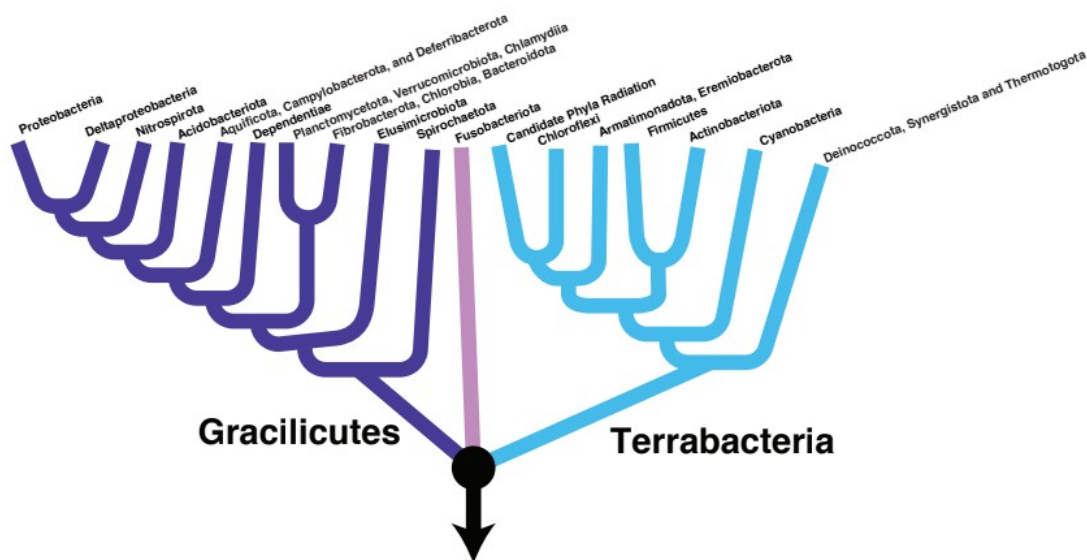
Cocci	<p><i>Magnetococcus marinus</i> MC-1</p>  <p>Flagella for motility</p> <p>1 micrometer</p> <p>Phylum: <i>Proteobacteria</i> Genome: 3,815 genes Diet: Mineral: CO₂ and swamp gas, requires O₂ Lifestyle: Free-living in marine waters <i>These bacteria contain small magnets that orientate the cells along the earth magnetic field</i></p>	<p><i>Opitutus terrae</i> PB90-1</p>  <p>Flagellum for motility</p> <p>0.5 micrometer</p> <p>Phylum: <i>Verrucomicrobia</i> Genome: 4,632 genes Diet: Organic: plant-derived carbohydrates, fermentative, O₂ intolerant Lifestyle: Free-living in flooded soils <i>Members of the phylum Verrucomicrobia are slow-growing, difficult to isolate bacteria</i></p>	<p><i>Staphylococcus aureus</i> Rosenbach</p>  <p>1 micrometer</p> <p>Phylum: <i>Firmicutes</i> Genome: 2,767 genes Diet: Organic: carbohydrates, amino acids, can switch between O₂ respiration and fermentation Lifestyle: Host-associated (mammals and birds), often commensal, sometimes pathogen <i>Infamous for their resistance to antibiotics</i></p>	
	Rods	<p><i>Mycobacterium leprae</i> TN</p>  <p>1-8 micrometers</p> <p>Phylum: <i>Actinobacteria</i> Genome: 1,614 genes Diet: Organic: lipids, requires O₂ Lifestyle: Host-associated (mammals), obligate intracellular pathogen <i>These slow-growing bacteria cause leprosy in humans</i></p>	<p><i>Geobacter sulfurreducens</i> PCA</p>  <p>2-3 micrometers</p> <p>Phylum: <i>Desulfobacterota</i> Genome: 3,466 genes Diet: Organic: acetate, O₂ intolerant, use various exotic forms of respirations including that of iron Lifestyle: Free-living, common in sub-surface environments (e.g., groundwater). Can form syntrophies <i>These bacteria produce appendages referred to as nanowires, which conduct electricity</i></p>	<p><i>Nitrosomonas europaea</i> ATCC 19718</p>  <p>1.1-1.7 micrometers</p> <p>Flagellum for motility</p> <p>Phylum: <i>Proteobacteria</i> Genome: 2,460 genes Diet: Mineral: CO₂ and ammonia (NH₃), requires O₂ Lifestyle: Free-living in soils, sewage and freshwater <i>These bacteria can produce large quantities of the greenhouse gas nitrous oxide (N₂O)</i></p>

Genomes: The genome of an organism is the entire set of (genetic) “instructions” that determine the organism’s traits, a bit like the source code of a computer program determines what the program does. The genome of every cell is made of DNA, a type of molecule. DNA molecules inside living cells consist of many smaller molecules (referred to as nucleotides) linked together in a linear nucleotide “string”. There are four types of nucleotides, which are symbolized by the letters A, T, G and C. DNA molecules are therefore characterized by the number of A, T, G and C nucleotides they contain and by the order of the nucleotides along the DNA molecule, which is referred to as the genome sequence. The genome sequence encodes information (i.e. “instructions”), like letters of the alphabet are used to make words, sentences and entire books. Every bacterial genome sequence includes meaningful strings of nucleotides referred to as genes separated along the genome sequence by short strings of nucleotides, referred to as intergenic regions. A typical gene consists on average of 1000 nucleotides and is equivalent to a word in a sentence. An intergenic region is equivalent to the space that separates words. While the simplest traits of an organism are determined by a single gene, some complex traits may involve several and sometimes many genes. In bacteria, but less so in other organisms, multiple genes specifying a function are usually clustered together in a sequence. A gene cluster is equivalent to a sentence. Complex organisms featuring many traits and elaborate behaviors tend to have large, gene-rich genomes while the simplest life forms have small, gene-poor genomes. So, how much does the genome sequence of bacteria vary? To illustrate the high degree of variation in the complexity of bacterial cells, the smallest bacterial genome known to date includes a total of 112,091 nucleotides and only 137 genes (it belongs to a member of the bacterial species *Nasuia deltocephalinicola*), while the largest one is almost 150-fold larger, including a total of 16,040,666 nucleotides and 14,018 genes (it belongs to a member of the species *Minicystis rosea*). Although such extreme genome sizes are rather unusual, the genome size of most bacteria commonly varies between 1 and 5 million nucleotides, including between 1,000 and 5,000 genes, approximately. For comparison, eukaryotic genomes such as those of the Baker’s yeast (*Saccharomyces cerevisiae*) and the vinegar

fly (*Drosophila melanogaster* often referred to as “the fruit fly”) include over 6,000 and 15,000 genes respectively.

Box2 - Genome sequences and the classification of bacteria: Bacterial genome sequences record the evolutionary history of bacteria and therefore provide some information on how different bacteria are related to one another: i.e. their phylogeny. Bacterial genome sequences are therefore used to reconstruct phylogenetic trees, which indicate how different species and groups of species are related to one another, in the same way family trees indicate how different family member are related to one another. Phylogenetic trees are used for classifying bacterial species into groups of species. Groups of bacterial species are given ranks on the basis of the degree of genetic similarity they share with other groups of bacteria. The most common ranks given to groups of bacterial species include in order of decreasing genetic similarity: genus, family, order, class and phylum (i.e., organisms within a genus share a higher degree of genetic similarity than those grouped within a family...etc). The 20,000 bacterial species that have been named and described until now are currently grouped in 30 to 40 phyla, depending on the classification scheme used (see Figure 2). For comparison, members of the animal Kingdom are currently classified in 34 phyla, which emphasizes how different bacteria are from one another. Please note that the wide variety of bacteria known to date share only few genes with one another. For example, the >200 species of bacteria grouped within the genus *Pseudomonas* share only slightly over 650 genes with one another. For comparison, the genome of a *Pseudomonas* species includes between 4,200 and 6,369 genes depending on the species but most genes in these genomes are only present in one or a small number of species. The genomes of the >200 *Pseudomonas* species

bacterial evolution” published in *Science*, 2021; 372 (6542): eabe0511 DOI: 10.1126/science.abe0511. For a comprehensive overview of bacterial phyla, please consult: https://gtdb.ecogenomic.org/tree?r=d_Bacteria



Nutrition: Many bacteria live off the organic constituents of other life forms (Figure 3A-3D). These organic constituents include a huge variety of chemical compounds, which can be grouped into distinct classes of molecules such as the proteins, carbohydrates and lipids, for example. Similar to fungi, bacteria feed on nutrients (i.e., molecules) dissolved in water (i.e., mixed and incorporated into water) and often have a role of decomposer, consuming the dead remains of once living organisms (yet see below for an overview of the different lifestyles of bacteria). However, these bacteria are extremely diverse and differ from one another in

A child-centric microbiology education framework

multiple ways. For example, some bacteria specialize on a limited number of compounds while others are capable of using a broad variety of molecules as food source. Further, some bacteria require oxygen (O_2) to assimilate their food, releasing carbon dioxide (CO_2) and water (H_2O) as waste products of the **cellular respiration** (Fig. 3A). Yet, other bacteria can replace O_2 by another compound suitable for cellular respiration and can therefore grow in the complete absence of O_2 (Fig. 3B). Various compounds are now known to be used by bacteria to fuel exotic forms of respiration. The vast majority of these alternative forms of respiration remain completely unobserved in eukaryotes. While some bacteria specialize on a single type of respiration to assimilate their food, others are more versatile and can switch between different types of respiration. In alternative respiration forms, H_2O is replaced by other waste products: e.g., in **arsenate** respiration, arsenate (AsO_4^{3-}) replaces O_2 and **arsenite** (AsO_3^{3-}) is produced instead of H_2O , while in the form of respiration referred to as **denitrification** (Fig. 3B), **nitrate** (NO_3^-) replaces O_2 and a mixture of **nitrogen gas** (N_2) and **nitrous oxide** (N_2O , a **greenhouse gas**) is excreted instead of H_2O . Finally, other modes of nutrition, referred to as **fermentations** result in the production of other waste products, which sometimes entirely replace the production of CO_2 . Like respirations, fermentations are varied. For example, the bacterial species *Acetobacter aceti*, which is used for the mass production of vinegar from wine and cider, produces **acetic acid** instead of CO_2 during the assimilation of ethanol (wine alcohol) (Fig. 3C). In contrast, the **lactic acid** bacterium *Lactococcus lactis*, used in the production of fermented milk products (e.g., cheeses), produces lactic acid instead of CO_2 during the assimilation of lactose (milk sugar) (Fig. 3D).

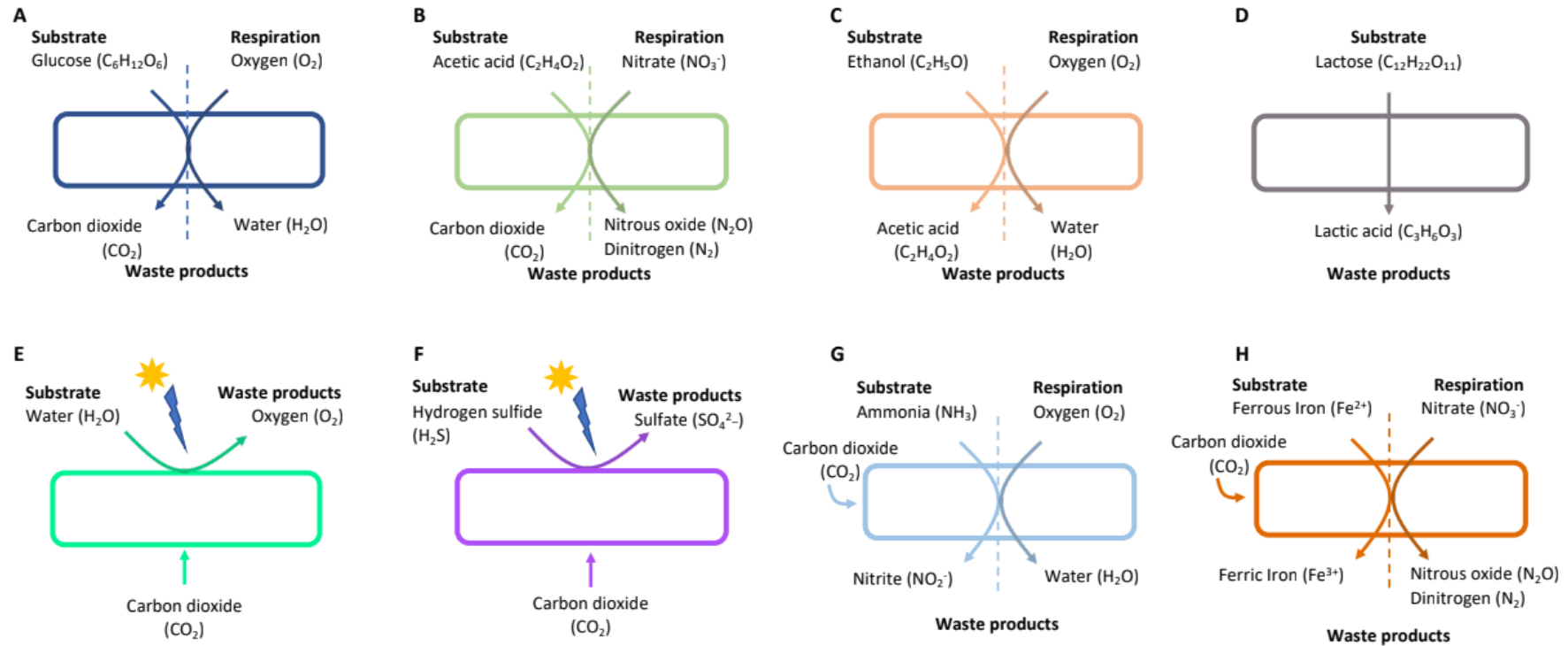
The nutritional diversity of bacteria does not stop at these differences. Various bacteria can live off organic molecules that are not or only rarely produced by other life forms. This includes for example, various constituents of crude oil (i.e., petroleum), plastics and all kinds of toxic compounds such as **pesticides** or **dioxins**. Other bacteria do not rely on the consumption of organic molecules, having instead a diet entirely based on inorganic (i.e., mineral) compounds like the wide majority of plants and algae. Such bacteria, like plants and algae, consume atmospheric CO_2 to produce their own organic constituents. Some of these bacteria require sunlight to assimilate their mineral diet and are referred to as **photosynthetic** bacteria. While plant and algae produce O_2 in the presence of light, only a single group among the various types of photosynthetic bacteria does (Fig. 3E). These bacteria are referred to as cyanobacteria although they are sometimes misleadingly called blue green algae. Photosynthetic bacteria that do not produce O_2 use different forms of photosynthesis (Fig. 3F), which is evidenced by their **pigmentation**: while cyanobacteria are blue green, other photosynthetic bacteria are either green (and referred to as 'green bacteria') or purple (and referred to as 'purple bacteria'). Green and purple bacteria require less light than cyanobacteria, plant and algae, and especially some green bacteria can grow with very dim light. Further, some members of the green and purple bacteria can grow in the complete absence of O_2 while cyanobacteria, plant and algae generally can't. The last category of bacteria includes those bacteria that have a mineral diet but do not require light. These bacteria are referred to as **chemosynthetic** bacteria and are also very diverse (Fig. 3G-3H). For example, some of these bacteria can derive energy from the degradation of gases (e.g., **hydrogen gas**, H_2) or from solid substrates (e.g., **metallic iron**). Bacteria that derive energy from metallic iron attach to the surface of sunken shipwrecks made of **steel** and **corrode** their metallic components. Chemosynthetic bacteria that eat **swamp gas** (H_2S) are key members of the fascinating and weird **chemosynthetic ecosystems** that develop around deep-sea **hydrothermal vents**.

A child-centric microbiology education framework

Growth and growth conditions: While bacterial cells are known to grow in size given appropriate conditions, bacterial growth refers, in most cases, to bacterial reproduction and more specifically to how long it takes for a bacterium to reproduce. Bacteria generally reproduce by binary fission (i.e., a “mother” cell divides into two “daughter” cells), although other modes of reproduction have been reported for a number of bacterial species (**Figure 4**). The length of time between the birth and division of a bacterial cell is referred to as the generation time, which varies greatly between distinct groups of bacteria. Given optimal conditions, some bacteria reproduce very quickly having a generation time of approximately 10 minutes, while others have a much slower growth, doubling once every second or third week. Recent research suggests that some bacteria living in conditions that contain very little food, such as deep-sea sediments, rock formations and salt crystals, may double only once every thousand or million years! The generation time of a bacterium is largely determined by its form of nutrition: i.e., bacteria living off organic molecules and using O₂ for respiration often grow faster than bacteria having a strictly mineral diet and an alternative form of respiration. However, environmental conditions also have a strong influence on the generation time of bacteria. In order to exploit all possible options for growth, some bacteria have specialized on less “attractive” settings and therefore need not only a food source to grow but also rather specific conditions. For example, some bacteria prefer environments in which nutrients are plentiful while others prefer environments in which nutrients are scarce, some prefer higher temperatures while others prefer lower temperatures, some prefer acidic conditions while others prefer neutral conditions...etc. Bacteria that do not encounter their optimal growth conditions have extended generation times and eventually stop growing once conditions differ too much from their optimal growth conditions. Therefore, even if bacteria thrive in almost any environment in the biosphere, bacteria inhabiting different environments are usually distinct from one another.

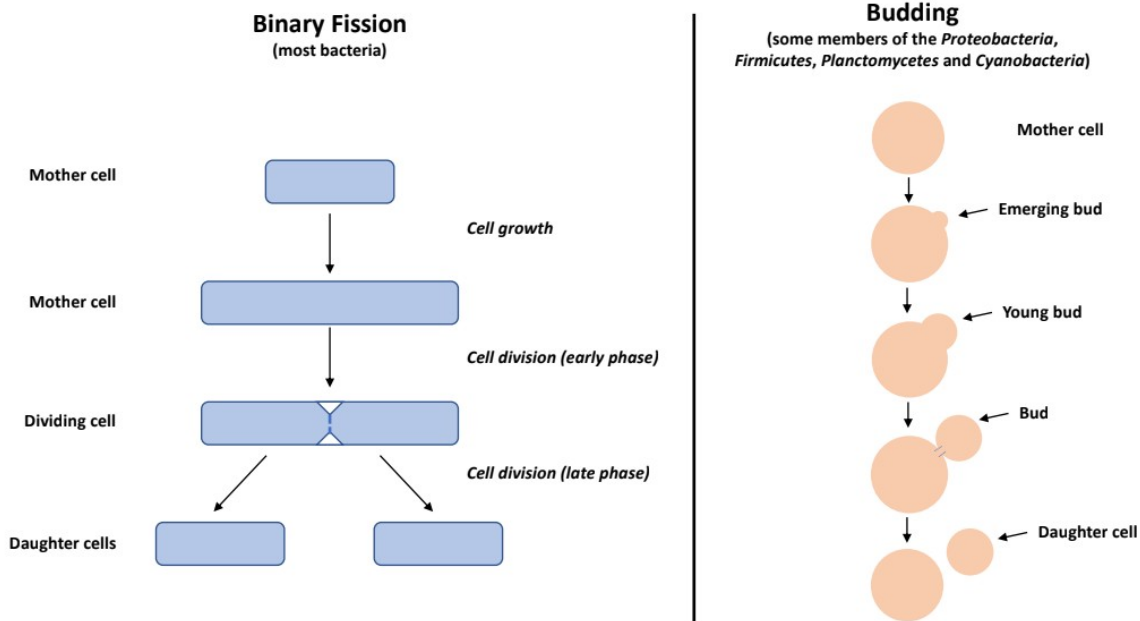
A child-centric microbiology education framework

Figure 3 Schematic representation of the principal forms of nutrition in bacteria. Each scheme illustrates a different form of nutrition using a specific example with the exception of (E) which always proceeds as illustrated. Schemes A-D illustrate forms of nutrition based on the consumption of organic nutrients, among which A-C are respiratory modes of nutrition and C-D are fermentations. Schemes E-H illustrate forms of nutrition involving a fully mineral diet, among which E-F are photosynthetic modes of nutrition while G-H are chemosynthetic modes of nutrition.



A child-centric microbiology education framework





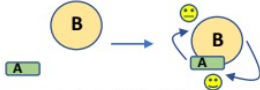
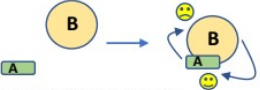
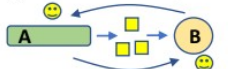


Figure 4 Schematic representation of two common modes of reproduction in bacteria.



2. *To what extent do the lifestyles of bacteria differ from one another?* The wide range of variations among bacterial cells supports many different lifestyles (Figure 5). Bacteria referred to as pathogens are usually well known to the broad public for their ability to cause diseases in eukaryotic hosts, especially humans. However, various bacteria are known to infect non-human hosts including both domesticated and wild animals, plants and some mushroom-forming fungi. Nevertheless, bacteria that live in association with a host do not always cause disease. Some, referred to as mutualists, benefit their host: e.g., they can contribute to the host's nutrition by producing essential nutrients that the host cannot produce or access while others improve host's defenses against pathogens or predators. Other host-associated bacteria do not appear to benefit nor harm the host; a relationship referred to as commensalism. Just like bacterial pathogens, host-associated mutualists and commensals are diverse and known to occur in a wide range of animals, plants, algae, fungi and protists. Among the broad variety of host-associated bacteria, some live on the surface of the host, others inside the host and sometimes even inside host cells. Further, host-associated bacteria are sometimes confined to their host, while in other cases they alternate between host-associated and free-living lifestyles. However, it should be noted that most bacteria have no known host and appear to be exclusively free-living and many of these bacteria do not have obligatory interactions with other organisms. Nevertheless, a wide variety of free-living bacteria engage in different types of interactions with other organisms. Some free-living bacteria trade nutrients with other free-living microorganisms in interactions referred to as syntrophies, some of which are obligate; meaning that partners in the syntrophy depend on each other. Others, in contrast, compete for resources, which sometimes involves the killing of one type of bacteria by another via the secretion of antibiotics or the injection of toxins. Finally, some free-living bacteria are known to actively prey on other organisms including other bacteria, microalgae, microfungi and perhaps even nematodes.

A child-centric microbiology education framework

Figure 5 Principal categories of interactions between bacteria and other organisms

				Impact of a bacterium on its interaction partner		
				Positive	Neutral	Negative
Obligately host dependent organisms	Obligate mutualism Bacterium A can only grow in association with organism B. A is beneficial to B		Obligate commensalism Bacterium A can only grow in association with organism B. A is neither beneficial nor harmful to B		Obligate Parasitism Bacterium A can only grow in association with organism B. A is harmful to B	
	A: e.g. <i>Buchnera aphidicola</i> B: Pea Aphid (<i>Acyrtosiphon pisum</i>) is the host of <i>B. aphidicola</i>		There is currently no bacteria known to have this lifestyle		A: e.g. <i>Rickettsia prowazekii</i> B: Humans, fleas and lice are the hosts of <i>R. prowazekii</i>	
Organisms having a host-associated life phase	Mutualism Bacterium A forms mutually beneficial associations with organism B.		Commensalism Bacterium A forms associations with organism B, which are neither beneficial nor harmful to B		Parasitism Bacterium A forms associations with organism B, which are harmful to B	
	A: e.g. <i>Bradyrhizobium japonicum</i> B: Soybean (<i>Glycine max</i>) is a host for <i>B. japonicum</i>		A: e.g. most strain of <i>Escherichia coli</i> B: Warm-blooded animals, including humans are the host of <i>E. coli</i>		A: e.g. <i>Pseudomonas aeruginosa</i> B: Various plants and animals, including humans can be hosts of <i>P. aeruginosa</i>	
Free-living organisms	Syntrophies Bacterium A can only grow when organism B eats its waste products		Cross-feeding Bacterium B feeds on wastes produced by organism A		Predation Bacterium A kills and feed on organism B	
	A: e.g. H ₂ -producing <i>Syntrophobacter</i> sp. B: e.g. H ₂ -eating methanogenic archaeon ■ H ₂		A: e.g. sugar-producing cyanobacterium B: e.g. sugar-eating flavobacterium ▲ sugar		A: e.g. <i>Myxococcus</i> sp., <i>Lysobacter</i> sp. B: Other bacteria, algae or nematodes ☠ Enzymes, antibiotics, toxins	

3. Why have researchers named and described only 20,000 species of bacteria?

Describing a new species of bacteria requires collecting a sufficient number of cells to test for several nutritional and cellular features and obtain the genetic information necessary to confirm that the cells under investigation are members of an undescribed species. Because bacteria are so small, and samples we can collect contain millions of bacteria in a complex mixture, it is essential to separate the one of interest from all the others. Various methods are available to do this but one cell is not enough, so once we isolate a bacterial cell, we need to amplify it to obtain more: a number of identical cells derived from one is called a pure culture or clone. We amplify the single cell by cultivating it (i.e., make it reproduce) using suitable nutrients. However, isolating and cultivating members of yet-undescribed species can prove slow and tedious, taking sometimes several years. Indeed, there is no fail-safe approach to quickly identify the right conditions to isolate and grow members of unnamed species, which generally involves a slow, trial-and-error research process. Nevertheless, depositing pure cultures (i.e., a culture free of any other organism including other members of the same species) in culture collections is a pre-requisite for the naming and description of a bacterial species, as defined by the [International Code of Nomenclature of Prokaryotes](#), in order to enable other researchers to independently confirm its characteristics and that it is indeed a new species.

4. Are there any bacterial species left to describe? The need to cultivate bacteria in order to describe and name them has been a major handicap because we do not yet know how to cultivate most bacteria. However, recently-developed technological developments now allow accessing the genetic information of bacterial cells, either in part or in its entirety, without having to isolate and grow bacteria outside their natural habitat. This novel, cultivation-independent approach allows investigation of bacteria in a wide variety of samples, including for example samples of water, sediment, soil and various type of animal and plant tissues. By comparing the genetic information of bacteria in a sample with the genetic information of bacteria with valid name and description, it is possible to determine which of the bacteria in

the sample are distinct from those that have been previously described. The combined results of bacterial species inventories that have been performed with the cultivation-independent approach provide essential information on the number of bacterial species that remain without description. These studies clearly indicate that the vast majority of bacterial species are still left without description but how many exactly, remains a matter of debate.

5. *How many bacterial species inhabit the Earth?* In 2019, a study that combined the results of 492 bacterial species inventories, which altogether investigated over 34,000 samples, concluded that there are about 1 million species of bacteria living in the entire biosphere, confirming that the vast majority of bacterial species have not been described so far. However, a few years earlier in 2016, a similar study predicted that the total number of bacterial species on Earth is a million times higher, i.e., in the order of 1 trillion (1,000,000,000,000). Why such a big difference between the two estimates? In fact, it all depends on how rare bacterial species are counted and on how the detection of rare bacterial species is interpreted (are rare bacterial species the result of an error of detection or not?). Although none of these estimates is final, they both point to a large number of undescribed species of bacteria. For comparison, while the number of described animal species is currently in excess of 1.5 million, estimates of the total number of animal species living in the biosphere ranges between 8 and 160 million.

6. *Do undescribed bacteria differ much from those that have been described?* One way to address this question consists of determining whether undescribed bacteria are genetically distant from described bacteria or, said in another way, whether there are entire phyla of bacteria that so far only consist of undescribed bacteria (see Box 2). Although there is no consensus on the number of recognized bacterial phyla, their current number usually ranges between 80 and 100 depending on the source of information. Considering that there are between 30 and 40 bacterial phyla including at least one described species, there are between 40 and 70 bacterial phyla that so far are only represented by undescribed bacteria. This means that there is a wide range of bacteria that profoundly differ from any of those that have been described until now. One striking example of this observation was the discovery in 2015 of the Candidate Phyla Radiation, a very broad group of small uncultivated bacteria, which possess genomes having unusual features and many of which might live as parasites of other bacteria.

Potential Implications for Decisions

Bacteria are hugely diverse; they have colonized almost every habitat in the Biosphere, and they play a wide range of roles in nature. In fact, bacteria are both friends and foes for nearly all other life forms on Earth. For example, where certain bacteria can be detrimental to human health, acting as producers of toxins or causing diseases, others are directly beneficial such as those inhabiting the human gut. The same can be said for bacteria that interact with farmed animals, crops and wildlife in general. Therefore, bacteria have an essential role to play in agriculture and conservation. Similarly, bacteria are important players in the food production chain: e.g., while some are essential in the production of fermented foods, others are food spoilage agents. Bacteria have also important beneficial roles in various industries. They have proven to be a valuable source of chemicals, which can be exploited for the development of therapeutic drugs and cosmetics for example. Their biosynthetic capacities can serve for the production of fine chemicals. In contrast, their biodegradative capabilities have applications in the development of bio-energies (e.g., the production of biofuels via the fermentation of plant **biomass**) or the **bioremediation** of a wide range of pollutants (e.g.,

A child-centric microbiology education framework

petroleum hydrocarbons, heavy metals). Yet, certain bacteria have an active role in the release of dangerous pollutants in the environment: e.g., bacteria play a role in the release of arsenic from rock minerals into groundwaters, which is a dramatic threat to public health in several countries. They also contribute to acid mine drainage, the creation of polluting toxic heavy metal-containing acidic water run-off from mine slag heaps/tailings and waste ponds, which severely impact aquatic wildlife. Last but not least, bacteria are important players in the climate crisis, since they have major roles both in the production and consumption of greenhouse gases. To sum up: studying bacteria is of prime importance to address many of the grand challenges that humanity is facing in the 21st century. The observation that humanity still knows very little about the tremendous diversity of bacteria inhabiting the biosphere emphasizes the urgency of intensifying the investigation of their biodiversity and developing microbiology education for present and future generations.

The Evidence Base, Further Reading and Teaching Aids

Introductory textbook

- Madigan, M.T., Bender, K.S., Buckley, D.H., Sattley, M. and Stahl, D.A. (2018) Brock Biology of Microorganisms, 15th Global Edition. Pearson Education Limited.

Comprehensive multivolume reference handbooks

- The Prokaryotes (2013 - 2014). Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E. and Thompson, F. (eds). Springer-Verlag Berlin Heidelberg
- Bergey's Manual of Systematic of Archaea and Bacteria (2015) Whitman, W.B. (ed). John Wiley & Sons, Inc.

Technics to cultivate bacteria and bacterial groups without cultivated representative

- Hug, L.A., Baker, B.J., Anantharaman, K., Brown, C.T., Probst, A.J., Castelle, C.J., *et al.* (2016) A new view of the tree of life. *Nature Microbiology* **1**:16048 doi: 10.1038/nmicrobiol.2016.48.
- Lewis, W.H., Tahon, G., Geesink, P., Sousa, D.Z. and Ettema, T.J.G. (2020) Innovations to culturing the uncultured microbial majority. *Nature Reviews Microbiology* <https://doi-org.proxy-ub.rug.nl/10.1038/s41579-020-00458-8>
- Overmann, J., Abt, B. and Sikorski, J. (2017) Present and future of culturing bacteria. *Annual Review of Microbiology* **71**: 711-730. doi: 10.1146/annurev-micro-090816-093449.

The International Code of Nomenclature of Prokaryotes

- Parker, C.T., Tindall, B.J. and Garrity, G.M. (2019) International Code of Nomenclature of Prokaryotes. *International Journal of Systematic and Evolutionary Microbiology* **69**: S1-S111.
- <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijsem.0.000778>
- <https://help.ezbiocloud.net/bacterial-nomenclature-101-and-how-to-describe-new-species/>

Bacterial species inventories

- Louca, S., Mazel, F., Doebeli, M. and Wegener Parfrey, L. (2019) A census-based estimate of Earth's bacterial and archaeal diversity. *PLOS Biology* **17**: e3000106 <https://doi.org/10.1371/journal.pbio.3000106>

A child-centric microbiology education framework

- Locey, K.J. and Lennon, J.T. (2016) Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America* 113: 5970-5975. <https://doi.org/10.1073/pnas.1521291113>

Animal species inventories

- Mora, C., Tittensor, D.R., Adl, S., Simpson, A.G.B. and Worm, B. (2011) How many species are there on earth and in the ocean? *PLOS Biology* 9: e1001127 <https://doi.org/10.1371/journal.pbio.1001127>
- Larsen, B.B., Miller, E.C., Rhodes, M.K. and Wiens, J.J. (2017) Inordinate fondness multiplied and redistributed: the number of species on Earth and the new pie of life. *Q Rev Biol.* 92: 229-265. <https://doi.org/10.1086/693564>.

Videos

Bacteria (Updated): a video from “the Amoeba Sisters” Youtube channel:

(<https://www.youtube.com/watch?v=ORB866QSGv8>)

“The Amoeba Sisters” Youtube channel gathers videos on a wide variety of topics relevant to this topic framework including for example, Cellular Respiration, Autotrophs and Heterotrophs, DNA and Genetics, Microscopes, Fermentation, Ecological Relationships and more. See the following link for an exhaustive list of videos:

(<https://www.youtube.com/c/AmoebaSisters/videos>)

DNA, Chromosomes, Genes, and Traits: An Intro to Heredity:

(<https://www.youtube.com/watch?v=8m6hHRIKwxY>)

Ecological Relationships: Although this video uses examples taken from the animal Kingdom, the concepts can be transposed to the Bacteria and their interaction partners as presented in this topic framework:

(<https://www.youtube.com/watch?v=rNjPI84sApQ>)

Fermentation: This video also discusses anaerobic respirations. Note that the video focuses on fermentations that occur in the absence of oxygen. Although this is a common definition of fermentation it is not fully correct as some lesser-known forms of fermentations referred to as oxidative fermentations are performed in presence of oxygen and use the respiratory machinery:

(https://www.youtube.com/watch?v=YbdkbCU20_M)

Autotrophs and Heterotrophs:

(<https://www.youtube.com/watch?v=f8G7IuLYxiA>)

Cellular Respiration (Updated): Although this video focuses on aerobic cellular respiration in Eukaryotes the process is similar in Bacteria excepting that it occurs in the Bacterial cell membrane instead of occurring in the dedicated organelle that is the mitochondrion:

([https://www.youtube.com/watch?v=e\]9Zjc-jdys](https://www.youtube.com/watch?v=e]9Zjc-jdys))

Please note that “the Amoeba Sisters” Youtube channel has also some video about other microbial groups include the Archaea, Fungi and Protists which are discussed in other topic frameworks.

Although the Youtube channel “Journey to the Microcosmos” generally discusses the diversity and lifestyles of protists and microscopic animals using beautiful live microscopic imaging videos of microorganisms, several videos discuss Bacteria:

(<https://www.youtube.com/c/microcosmos/videos>)

See for example: **Life Without Oxygen? Challenge Accepted**

(<https://www.youtube.com/watch?v=H8b09C1WPQk>)

The Youtube channel “SciShow” has also videos illustrating the diversity of bacteria and their amazing abilities:

6 Bacteria with Awesome Superpowers:

(<https://www.youtube.com/watch?v=cyOrx2o8IC0>)

6 Microbes Saving the Environment:

(<https://www.youtube.com/watch?v=hoiwlRrRW34>)

Thank Goodness for Bacterial Cannibalism:

(<https://www.youtube.com/watch?v=PADEFX47TK5I>)

5 of the Coolest Partnerships Between Animals and Bacteria:

(<https://www.youtube.com/watch?v=XvgdNQsmVE>)

Glossary

Acetic acid: An organic molecule of formula $C_2H_4O_2$ and a weak acid commonly produced by a wide variety of bacteria (and other organisms) through fermentation. Acetic acid is a major component of vinegar (4 to 8% by volume).

Arsenate: An inorganic ion (electrically charged molecule) which contains arsenic and oxygen atoms as indicated by its formula AsO_4^{3-} . Arsenate ions are toxic and occur naturally either as such in water or in solid form as part of several types of minerals such as erythrite. Some bacteria can use arsenate in place of O_2 for cellular respiration.

Arsenite: An inorganic ion (electrically charged molecule) which contains arsenic and oxygen atoms as indicated by its formula AsO_3^{3-} . Arsenite ions are toxic and more so than arsenate ions. Arsenite ions occur naturally either as such in water (e.g., groundwater) or in solid form as part of several types of minerals such as reinerite. Arsenite ions are produced via the respiration of arsenate ions.

Biodiversity: This term generally refers to the variety of life forms on Earth. It can refer for example to the genetic variations within groups of organisms but also to the variety of species present in a specific geographic location or the variety of ecosystems within a geographic area.

Biomass: As used in this chapter, the term biomass refers to plant material used for the production of (bio)-fuels via the microbial fermentation of the plant material. More generally, the term biomass refers to material derived from any life form.

Bioremediation: The process of decontamination of a polluted area via the stimulation of life forms (generally microorganisms) capable of degrading or removing the pollutant from the contaminated area.

Biosphere: Called the “zone of life”, it is the part of the Earth, which is inhabited by living organisms.

Cell: It is the smallest unit of life or said differently, it is the smallest “object” or “entity”, which has the property of being alive. A cell is a compartment containing various types of molecules and delimited by a membrane, which is made of lipids. For more information see here: <https://www.nature.com/scitable/topicpage/what-is-a-cell-14023083/>

Cellular respiration: The process by which nutrients are converted into a form of energy that can be used by a cell to stay alive and maintain its function.

A child-centric microbiology education framework

Chemosynthesis (chemosynthetic): A form of nutrition by which an organism produces its constituents (i.e., biomass) from carbon dioxide (CO₂). Unlike photosynthetic organisms, chemosynthetic organisms do not use sunlight as energy source to power the conversion of CO₂ into cellular constituents. Instead, they use energy they recover from the chemical oxidation of (generally inorganic) nutrients during the cellular respiration process.

Chemosynthetic ecosystem: The organic constituents of the life forms inhabiting a chemosynthetic ecosystem are produced by chemosynthetic organisms. The organic constituents of life forms inhabiting non-chemosynthetic ecosystems are produced by photosynthetic organisms. Chemosynthetic ecosystems are generally secluded in remote locations, underground or at the bottom of the ocean. For example, chemosynthetic ecosystems develop around deep-sea hydrothermal vents and include a wide variety of unusual life forms like the giant tube worm *Riftia pachyptila*, the nutrition of which is dependent on chemosynthetic organisms.

Corrosion (corrode): A process in which a metal is altered, taking a different chemical form. For example, the corrosion of metallic iron produces rust, which consists of iron oxides (compounds of iron and oxygen atoms whereas metallic iron solely consists of iron atoms).

Dioxins: A group of organic compounds, some of which are extremely toxic and act as pollutants. These compounds are generally produced by industrial processes although some natural sources are also known. Some dioxins have been used as herbicides in the context of warfare.

Eukaryotes: A broad group of organisms that include the animals, plants, fungi and protists. Eukaryotic cells have an internal compartment referred to as nucleus, which is delimited by two, lipid-containing membranes known as an “envelope”. The nucleus contains the genetic material (DNA) of eukaryotic organisms. Eukaryotes are one of the three domains of life, the other two include the Bacteria on one hand and the Archaea on the other hand. The genetic material of bacterial and archaeal cells is not enclosed in a nucleus.

Evolutionary history: This refers to the history of genetic changes that led to the evolution of current life forms from the first life forms that emerge in the early stages of Earth history.

Fermentation: A mode of nutrition, by which an organism living off organic compounds releases at least one organic compound among the main waste products of its nutrition (the organic compound released as waste product differs from those that are consumed). Some forms of fermentations produce hydrogen gas (H₂) as an additional, inorganic waste product. Although fermentations are very often thought to occur in the absence of O₂, some forms of fermentation occur in its presence (e.g., the production of acetic acid by *Acetobacter aceti*).

Genome: The entirety of the genetic information of an organism. The genetic information determines the features of an organism and how it functions. The genetic material in which the genetic information is stored is made of DNA, which stands for DeoxyriboNucleic Acid.

Greenhouse gas: An atmospheric gas which causes the greenhouse effect, i.e., gases that absorb heat, which otherwise would escape from the Earth into space. The heat absorbed by greenhouse gases therefore keep the heat near the Earth surface.

Growth: Although growth of an organism often refers to an increase in the mass and size of this organism, the growth of a bacterial culture usually refers to the proliferation of bacterial cells, i.e., the generation of new bacterial cells by the binary fission of a mother cell into two daughter cells.

Hydrogen gas: A gas made of molecules of dihydrogen, i.e., H₂. It is only present in trace amounts in the Earth’s atmosphere but can be present in high concentration in the fluids erupting from hydrothermal vents. Hydrogen gas is also produced biologically by some fermentative bacteria and it can fuel the growth of various types of chemosynthetic organisms.

A child-centric microbiology education framework

Hydrothermal vent: A crack on the seafloor from which geothermally heated water erupts. The geothermal heating of the water is generally due to volcanic activity occurring in the area where the vent is located. The heated water that erupts from the vent is usually enriched in minerals and gases that can serve as nutrients for chemosynthetic organisms.

International Code of Nomenclature of Prokaryotes: The set of rules for naming groups (referred to as “taxa”) of bacterial and archaeal organisms.

Kingdom: A rank given to a group of species. This rank is intermediate between the phylum rank (Kingdom > Phylum i.e., the Animal Kingdom includes 34 phyla) and the domain rank (Domain > Kingdom i.e., the domain Eukaryota includes the Plant, Animal and Fungal Kingdoms and several additional groups). It is only used in the classification of eukaryotic organisms.

Lactic acid: An organic molecule of formula $C_3H_6O_3$ and a weak acid commonly produced by a wide variety of bacteria (and other organisms) through fermentation. Lactic acid is produced during the fermentation of milk and is therefore present in fermented milk products. Lactic acid is responsible for the sour taste of plain, non-sweetened yogurt.

Light microscope: Also referred to as an optical microscope, it uses visible light focused on a small (i.e., microscopic) object and lenses to obtain a magnified image of the small object. Other types of microscopes referred to as electron microscopes do not focus a beam of visible light on the microscopic object to be observed but instead focus a beam of electrons and special lenses to produce a magnified image of the microscopic object.

Metallic iron: The solid state of pure iron is a metal (i.e., a material that conducts electricity and heat) and is therefore referred to as metallic iron. At temperatures and pressures commonly found on the Earth’s surface, pure iron appears as metallic iron. However, deposits of metallic iron are rather rare. Iron occurs more often in forms that are not purely consisting of iron atoms. Iron appears very often in the form of iron oxides, i.e., in compounds that include oxygen atoms in addition to iron atoms.

Micrometer: A unit of length, exactly a million times smaller than a meter (1 micrometer = 0.000001 meter) or a thousand times smaller than a millimeter (1 micrometer = 0.001 millimeter)

Morphology: The external form and internal structure (anatomy) of an organism.

Nematodes: A broad group of worms colloquially referred to as “roundworms”. Nematodes are one of the most diverse (i.e., species-rich) groups among the animals. The group currently includes 20,000 described species but there may be up to 1,000,000 species of nematodes in existence. Many of these worms are small (millimeter size range) but larger forms are known. The group includes both free-living and parasitic forms: the group includes several important parasites of humans, domesticated animals and crops.

Nitrate: An inorganic ion (electrically charged molecule) which contains nitrogen and oxygen atoms as indicated by its formula NO_3^- . Nitrate ions occur naturally either as such in marine and freshwaters or in solid form as part of minerals referred to generally as nitrate minerals (e.g., Nitratine). Some bacteria can use nitrate in place of O_2 for cellular respiration.

Nitrogen gas: A gas made of molecules of dinitrogen, i.e., N_2 . It is the dominant gas in the Earth’s atmosphere, which is a gas mixture that includes 78% of nitrogen gas. The form of respiration referred to as denitrification is a source of nitrogen gas.

Nitrous oxide: A chemical compound made of nitrogen and oxygen atoms as indicated by its formula N_2O . At the temperatures and pressures commonly found on the Earth’s surface, nitrous oxide is a gas. Although it is only present in trace amounts in the Earth’s atmosphere, it is an extremely potent greenhouse gas that contributes to climate change. The form of respiration referred to as denitrification is a major source of nitrous oxide.

A child-centric microbiology education framework

Nutrition: The process by which an organism uses food to sustain its own life. The process involves (1) taking up nutrients, (2) converting some of the energy contained in the nutrients in a form that is usable by the organism and (3) using that form of energy and some of the nutrients to fuel cellular processes involved in the survival and growth of the organism and finally (4) excreting the waste products that have been generated by the conversion of some of the nutrients into a form of usable energy.

Organic: Refers to a chemical substance, which originates from living organisms although in organic chemistry it often refers to a chemical compound in which carbon (C) atoms are bound to hydrogen (H) atoms. Nevertheless, the latter definition is not without caveat since urea ((NH₂)₂CO) is generally considered an organic compound but its carbon atom is not bound to a hydrogen atom (it is carbon atom is bound to two nitrogen atoms and to an oxygen atom).

Pesticide: A chemical aimed at controlling a pest, which can be broadly defined as any organism, which is directly or indirectly harmful to humans. The term “pest” is especially used to refer to organisms that damage crops, livestock and forests.

Photosynthesis: A form of nutrition by which an organism produces its constituents (i.e., biomass) from carbon dioxide (CO₂). Photosynthetic organisms use sunlight as energy source to power the conversion of CO₂ into cell components.

Pigmentation: The natural coloring of a living organism.

Protist: Any eukaryotic organism that is not an animal, plant or fungus.

Species: The basic unit for the classification of organisms and the basic unit of biodiversity.

Steel: An alloy made of iron and small amounts of carbon. An alloy is a mixture of several metals or a mixture of one metal and another non-metallic element. Steel falls in the second category.

Swamp gas: A mixture of gases including methane (CH₄), hydrogen sulfide (H₂S) and carbon dioxide (CO₂), which is commonly produced in swamps, bogs and marshes. Swamp gas is often used to solely refer to hydrogen sulfide, which has a foul odor of rotten eggs whereas methane and carbon dioxide have no odor.

Trait: An observable characteristic or feature of an organism.

Validated name: The name of a group of organisms is validated once it appears on the list of approved/validated names published by the *International Journal of Systematic and Evolutionary Microbiology* referred to as the IJSEM.