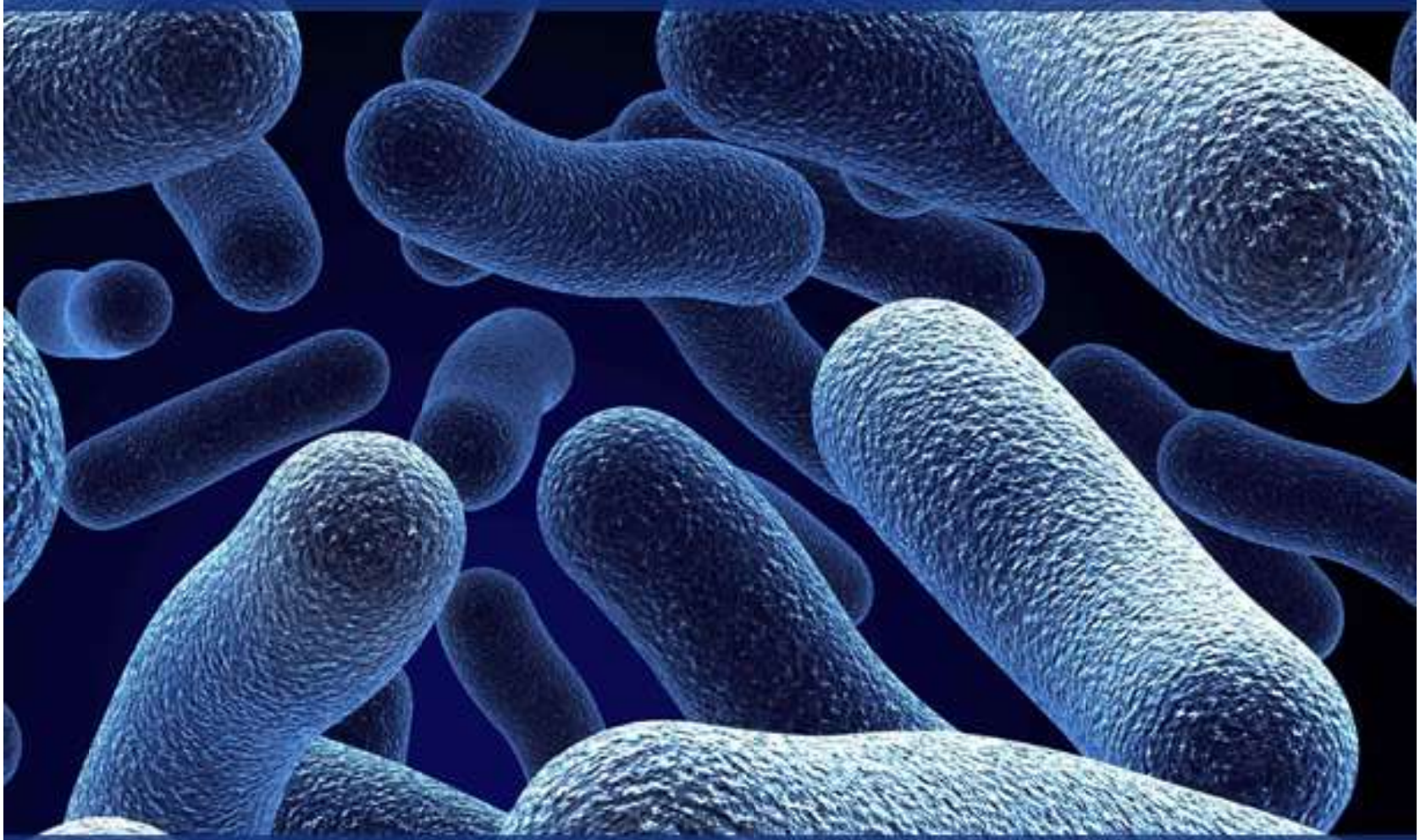
Bacteria and Fungus Microbiology



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Chapter 1

Bacteria

Bacteria are a large domain of single-celled, prokaryote microorganisms. Typically a few micrometres in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals. Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water; in all, there are approximately five nonillion (5×10^{30}) bacteria on Earth, forming a biomass on Earth, which exceeds that of all plants and animals. Bacteria are vital in recycling nutrients, with many steps in nutrient cycles depending on these organisms, such as the fixation of nitrogen from the atmosphere and putrefaction. However, most bacteria have not been characterised, and only about half of the phyla of bacteria have species that can be grown in the laboratory. The study of bacteria is known as bacteriology, a branch of microbiology.

There are approximately ten times as many bacterial cells in the human flora as there are human cells in the body, with large numbers of bacteria on the skin and as gut flora. The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and a few are beneficial. However, a few species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy and bubonic plague. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people a year, mostly in sub-Saharan Africa. In developed countries, antibiotics are used to treat bacterial infections and in agriculture, so antibiotic resistance is becoming common. In industry, bacteria are important in sewage treatment, the production of cheese and yogurt through fermentation, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.

Once regarded as plants constituting the Class Schizomycetes, bacteria are now classified as prokaryotes. Unlike cells of animals and other eukaryotes, bacterial cells do not contain a nucleus and rarely harbour membrane-bound organelles. Although the term *bacteria* traditionally included all prokaryotes, the scientific classification changed after the discovery in the 1990s that prokaryotes consist of two very different groups of

organisms that evolved independently from an ancient common ancestor. These evolutionary domains are called Bacteria and Archaea.

Etymology

The word *bacteria* is the plural of the New Latin *bacterium*, which is the latinisation of the Greek βακτήριον (*baktērion*), the diminutive of βακτηρία (*baktēria*), meaning "staff, cane", because the first ones to be discovered were rod-shaped.

History of bacteriology

Antonie van Leeuwenhoek, the first microbiologist and the first person to observe bacteria using a microscope.

Bacteria were first observed by Antonie van Leeuwenhoek in 1676, using a single-lens microscope of his own design. He called them "animalcules" and published his observations in a series of letters to the Royal Society. The name *bacterium* was introduced much later, by Christian Gottfried Ehrenberg in 1838.

Louis Pasteur demonstrated in 1859 that the fermentation process is caused by the growth of microorganisms, and that this growth is not due to spontaneous generation. (Yeasts and molds, commonly associated with fermentation, are not bacteria, but rather fungi.)

Along with his contemporary, Robert Koch, Pasteur was an early advocate of the germ theory of disease. Robert Koch was a pioneer in medical microbiology and worked on cholera, anthrax and tuberculosis. In his research into tuberculosis, Koch finally proved the germ theory, for which he was awarded a Nobel Prize in 1905. In *Koch's postulates*, he set out criteria to test if an organism is the cause of a disease, and these postulates are still used today.

Though it was known in the nineteenth century that bacteria are the cause of many diseases, no effective antibacterial treatments were available. In 1910, Paul Ehrlich developed the first antibiotic, by changing dyes that selectively stained *Treponema pallidum*—the spirochaete that causes syphilis—into compounds that selectively killed the pathogen. Ehrlich had been awarded a 1908 Nobel Prize for his work on immunology, and pioneered the use of stains to detect and identify bacteria, with his work being the basis of the Gram stain and the Ziehl-Neelsen stain.

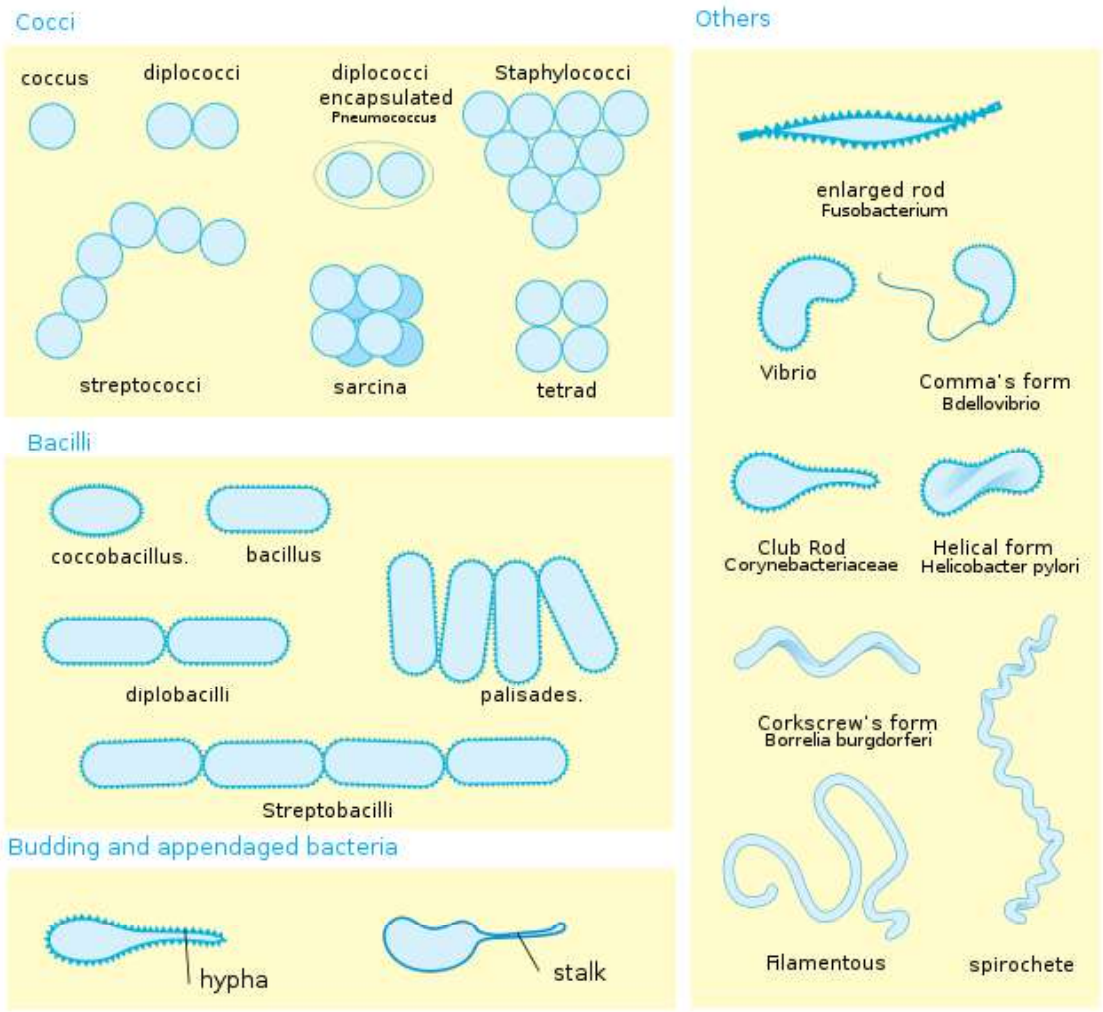
A major step forward in the study of bacteria was the recognition in 1977 by Carl Woese that archaea have a separate line of evolutionary descent from bacteria. This new phylogenetic taxonomy was based on the sequencing of 16S ribosomal RNA, and divided prokaryotes into two evolutionary domains, as part of the three-domain system.

Origin and early evolution

The ancestors of modern bacteria were single-celled microorganisms that were the first forms of life to appear on Earth, about 4 billion years ago. For about 3 billion years, all organisms were microscopic, and bacteria and archaea were the dominant forms of life. Although bacterial fossils exist, such as stromatolites, their lack of distinctive morphology prevents them from being used to examine the history of bacterial evolution, or to date the time of origin of a particular bacterial species. However, gene sequences can be used to reconstruct the bacterial phylogeny, and these studies indicate that bacteria diverged first from the archaeal/eukaryotic lineage.

Bacteria were also involved in the second great evolutionary divergence, that of the archaea and eukaryotes. Here, eukaryotes resulted from ancient bacteria entering into endosymbiotic associations with the ancestors of eukaryotic cells, which were themselves possibly related to the Archaea. This involved the engulfment by proto-eukaryotic cells of alpha-proteobacterial symbionts to form either mitochondria or hydrogenosomes, which are still found in all known Eukarya (sometimes in highly reduced form, e.g. in ancient "amitochondrial" protozoa). Later on, some eukaryotes that already contained mitochondria also engulfed cyanobacterial-like organisms. This led to the formation of chloroplasts in algae and plants. There are also some algae that originated from even later endosymbiotic events. Here, eukaryotes engulfed a eukaryotic algae that developed into a "second-generation" plastid. This is known as secondary endosymbiosis.

Morphology



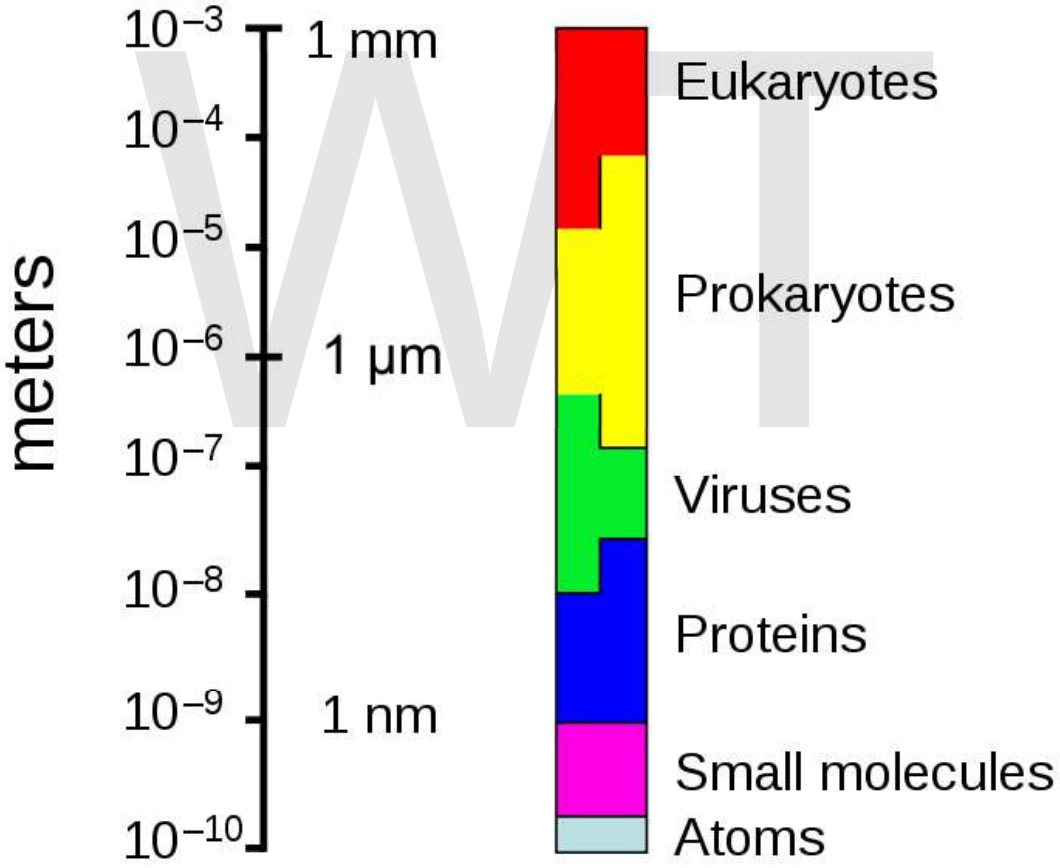
Bacteria display many cell morphologies and arrangements

Bacteria display a wide diversity of shapes and sizes, called *morphologies*. Bacterial cells are about one tenth the size of eukaryotic cells and are typically 0.5–5.0 micrometres in length. However, a few species–for example *Thiomargarita namibiensis* and *Epulopiscium fishelsoni*–are up to half a millimetre long and are visible to the unaided eye. Among the smallest bacteria are members of the genus *Mycoplasma*, which measure only 0.3 micrometres, as small as the largest viruses. Some bacteria may be even smaller, but these ultramicrobacteria are not well-studied.

Most bacterial species are either spherical, called cocci (*sing*. coccus, from Greek *κόκκος-kókkos*, grain, seed) or rod-shaped, called bacilli (*sing*. bacillus, from Latin *baculus*, stick). Elongation is associated with swimming. Some rod-shaped bacteria, called vibrio, are slightly curved or comma-shaped; others, can be spiral-shaped, called spirilla, or tightly coiled, called spirochaetes. A small number of species even have

tetrahedral or cuboidal shapes. More recently, bacteria were discovered deep under the Earth's crust that grow as long rods with a star-shaped cross-section. The large surface area to volume ratio of this morphology may give these bacteria an advantage in nutrient-poor environments. This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton, and is important because it can influence the ability of bacteria to acquire nutrients, attach to surfaces, swim through liquids and escape predators.

Many bacterial species exist simply as single cells, others associate in characteristic patterns: *Neisseria* form diploids (pairs), *Streptococcus* form chains, and *Staphylococcus* group together in "bunch of grapes" clusters. Bacteria can also be elongated to form filaments, for example the Actinobacteria. Filamentous bacteria are often surrounded by a sheath that contains many individual cells. Certain types, such as species of the genus *Nocardia*, even form complex, branched filaments, similar in appearance to fungal mycelia.



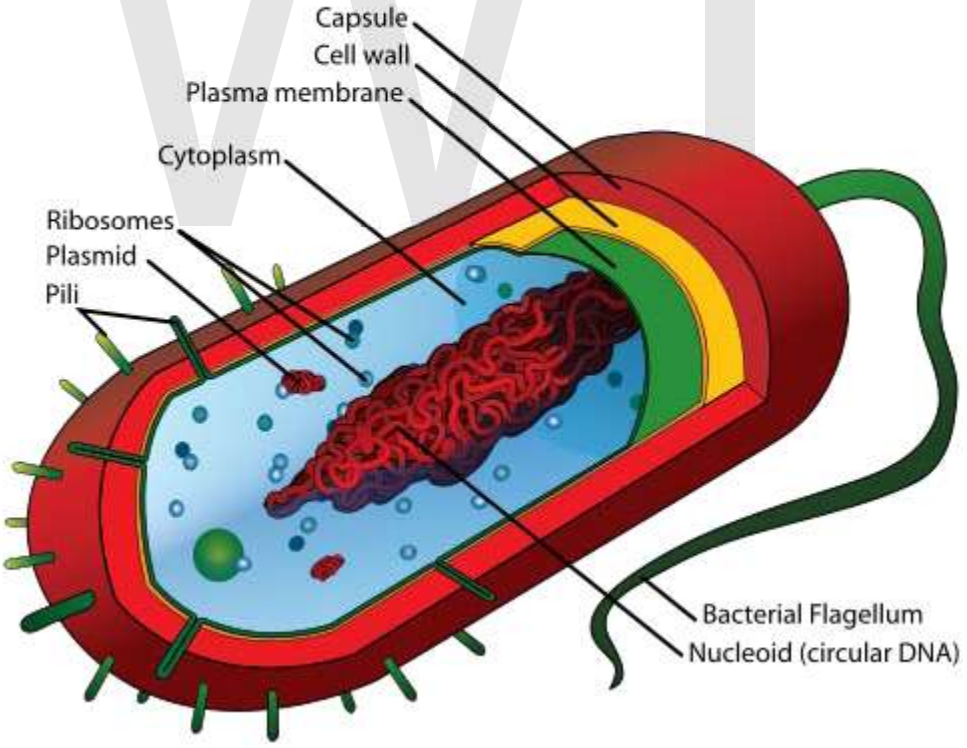
The range of sizes shown by prokaryotes, relative to those of other organisms and biomolecules

Bacteria often attach to surfaces and form dense aggregations called biofilms or bacterial mats. These films can range from a few micrometers in thickness to up to half a meter in

depth, and may contain multiple species of bacteria, protists and archaea. Bacteria living in biofilms display a complex arrangement of cells and extracellular components, forming secondary structures such as microcolonies, through which there are networks of channels to enable better diffusion of nutrients. In natural environments, such as soil or the surfaces of plants, the majority of bacteria are bound to surfaces in biofilms. Biofilms are also important in medicine, as these structures are often present during chronic bacterial infections or in infections of implanted medical devices, and bacteria protected within biofilms are much harder to kill than individual isolated bacteria.

Even more complex morphological changes are sometimes possible. For example, when starved of amino acids, Myxobacteria detect surrounding cells in a process known as quorum sensing, migrate towards each other, and aggregate to form fruiting bodies up to 500 micrometres long and containing approximately 100,000 bacterial cells. In these fruiting bodies, the bacteria perform separate tasks; this type of cooperation is a simple type of multicellular organisation. For example, about one in 10 cells migrate to the top of these fruiting bodies and differentiate into a specialised dormant state called myxospores, which are more resistant to drying and other adverse environmental conditions than are ordinary cells.

Cellular structure



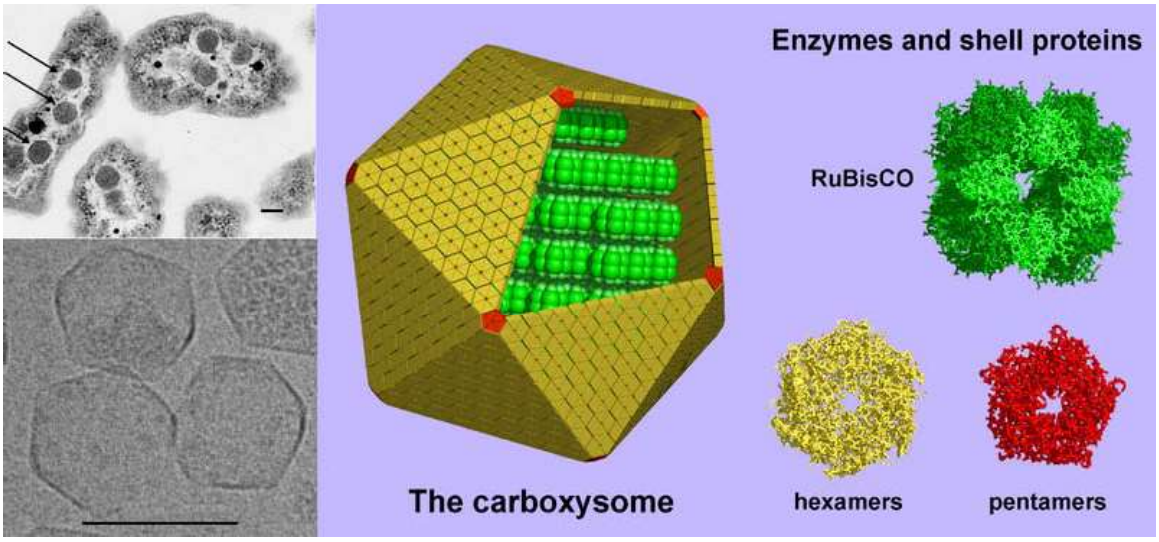
Structure and contents of a typical Gram positive bacterial cell

Intracellular structures

The bacterial cell is surrounded by a lipid membrane, or cell membrane, which encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell. As they are prokaryotes, bacteria do not tend to have membrane-bound organelles in their cytoplasm and thus contain few large intracellular structures. They consequently lack a nucleus, mitochondria, chloroplasts and the other organelles present in eukaryotic cells, such as the Golgi apparatus and endoplasmic reticulum. Bacteria were once seen as simple bags of cytoplasm, but elements such as prokaryotic cytoskeleton, and the localization of proteins to specific locations within the cytoplasm have been found to show levels of complexity. These subcellular compartments have been called "bacterial hyperstructures".

Micro-compartments such as carboxysome provides a further level of organization, which are compartments within bacteria that are surrounded by polyhedral protein shells, rather than by lipid membranes. These "polyhedral organelles" localize and compartmentalize bacterial metabolism, a function performed by the membrane-bound organelles in eukaryotes.

Many important biochemical reactions, such as energy generation, occur by concentration gradients across membranes, a potential difference also found in a battery. The general lack of internal membranes in bacteria means reactions such as electron transport occur across the cell membrane between the cytoplasm and the periplasmic space. However, in many photosynthetic bacteria the plasma membrane is highly folded and fills most of the cell with layers of light-gathering membrane. These light-gathering complexes may even form lipid-enclosed structures called chlorosomes in green sulfur bacteria. Other proteins import nutrients across the cell membrane, or to expel undesired molecules from the cytoplasm.



Carboxysomes are protein-enclosed bacterial organelles. Top left is an electron microscope image of carboxysomes in *Halothiobacillus neapolitanus*, below is an image of purified carboxysomes. On the right is a model of their structure. Scale bars are 100 nm.

Bacteria do not have a membrane-bound nucleus, and their genetic material is typically a single circular chromosome located in the cytoplasm in an irregularly shaped body called the nucleoid. The nucleoid contains the chromosome with associated proteins and RNA. The order Planctomycetes are an exception to the general absence of internal membranes in bacteria, because they have a membrane around their nucleoid and contain other membrane-bound cellular structures. Like all living organisms, bacteria contain ribosomes for the production of proteins, but the structure of the bacterial ribosome is different from those of eukaryotes and Archaea.

Some bacteria produce intracellular nutrient storage granules, such as glycogen, polyphosphate, sulfur or polyhydroxyalkanoates. These granules enable bacteria to store compounds for later use. Certain bacterial species, such as the photosynthetic Cyanobacteria, produce internal gas vesicles, which they use to regulate their buoyancy – allowing them to move up or down into water layers with different light intensities and nutrient levels.

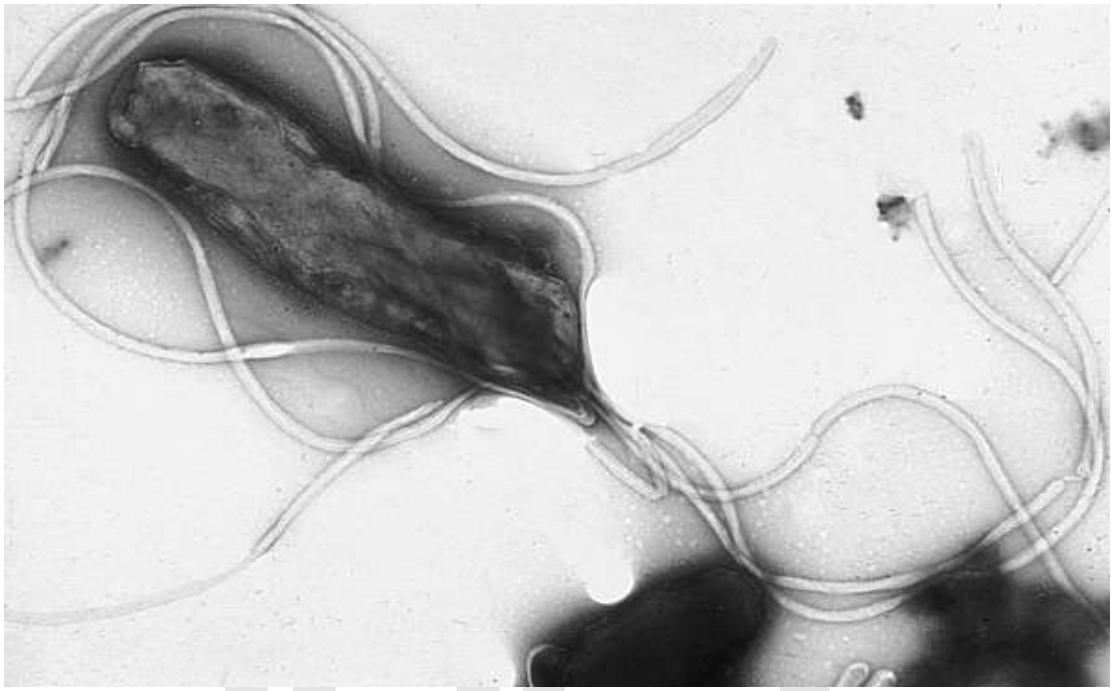
Extracellular structures

Around the outside of the cell membrane is the bacterial cell wall. Bacterial cell walls are made of peptidoglycan (called murein in older sources), which is made from polysaccharide chains cross-linked by unusual peptides containing D-amino acids. Bacterial cell walls are different from the cell walls of plants and fungi, which are made of cellulose and chitin, respectively. The cell wall of bacteria is also distinct from that of Archaea, which do not contain peptidoglycan. The cell wall is essential to the survival of many bacteria, and the antibiotic penicillin is able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan.

There are broadly speaking two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species.

Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. Most bacteria have the Gram-negative cell wall, and only the Firmicutes and Actinobacteria (previously known as the low G+C and high G+C Gram-positive bacteria, respectively) have the alternative Gram-positive arrangement. These differences in structure can produce differences in antibiotic susceptibility; for instance, vancomycin can kill only Gram-positive bacteria and is ineffective against Gram-negative pathogens, such as *Haemophilus influenzae* or *Pseudomonas aeruginosa*.

In many bacteria an S-layer of rigidly arrayed protein molecules covers the outside of the cell. This layer provides chemical and physical protection for the cell surface and can act as a macromolecular diffusion barrier. S-layers have diverse but mostly poorly understood functions, but are known to act as virulence factors in *Campylobacter* and contain surface enzymes in *Bacillus stearothermophilus*.



Helicobacter pylori electron micrograph, showing multiple flagella on the cell surface

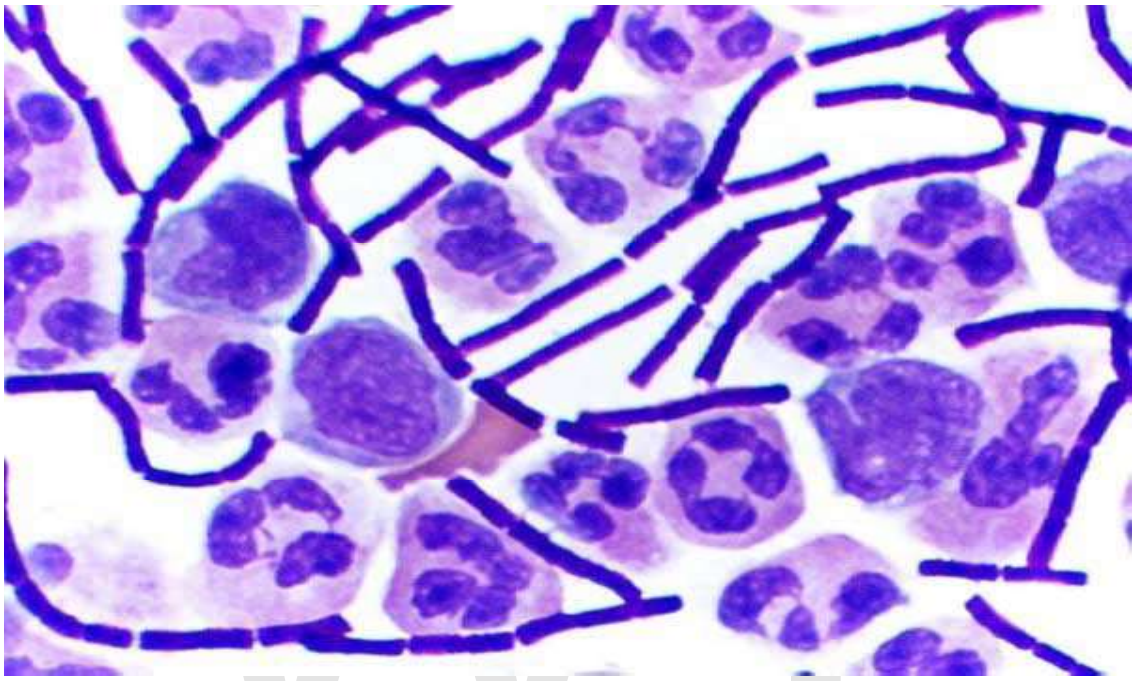
Flagella are rigid protein structures, about 20 nanometres in diameter and up to 20 micrometres in length, that are used for motility. Flagella are driven by the energy released by the transfer of ions down an electrochemical gradient across the cell membrane.

Fimbriae are fine filaments of protein, just 2–10 nanometres in diameter and up to several micrometers in length. They are distributed over the surface of the cell, and resemble fine hairs when seen under the electron microscope. Fimbriae are believed to be involved in attachment to solid surfaces or to other cells and are essential for the virulence of some bacterial pathogens. Pili (*sing*. pilus) are cellular appendages, slightly larger than fimbriae, that can transfer genetic material between bacterial cells in a process called conjugation.

Capsules or slime layers are produced by many bacteria to surround their cells, and vary in structural complexity: ranging from a disorganised slime layer of extra-cellular polymer, to a highly structured capsule or glycocalyx. These structures can protect cells from engulfment by eukaryotic cells, such as macrophages. They can also act as antigens and be involved in cell recognition, as well as aiding attachment to surfaces and the formation of biofilms.

The assembly of these extracellular structures is dependent on bacterial secretion systems. These transfer proteins from the cytoplasm into the periplasm or into the environment around the cell. Many types of secretion systems are known and these structures are often essential for the virulence of pathogens, so are intensively studied.

Endospores



Bacillus anthracis (stained purple) growing in cerebrospinal fluid

Certain genera of Gram-positive bacteria, such as *Bacillus*, *Clostridium*, *Sporohalobacter*, *Anaerobacter* and *Heliobacterium*, can form highly resistant, dormant structures called endospores. In almost all cases, one endospore is formed and this is not a reproductive process, although *Anaerobacter* can make up to seven endospores in a single cell. Endospores have a central core of cytoplasm containing DNA and ribosomes surrounded by a cortex layer and protected by an impermeable and rigid coat.

Endospores show no detectable metabolism and can survive extreme physical and chemical stresses, such as high levels of UV light, gamma radiation, detergents, disinfectants, heat, freezing, pressure and desiccation. In this dormant state, these organisms may remain viable for millions of years, and endospores even allow bacteria to survive exposure to the vacuum and radiation in space. Endospore-forming bacteria can also cause disease: for example, anthrax can be contracted by the inhalation of *Bacillus anthracis* endospores, and contamination of deep puncture wounds with *Clostridium tetani* endospores causes tetanus.

Metabolism

Bacteria exhibit an extremely wide variety of metabolic types. The distribution of metabolic traits within a group of bacteria has traditionally been used to define their taxonomy, but these traits often do not correspond with modern genetic classifications. Bacterial metabolism is classified into nutritional groups on the basis of three major criteria: the kind of energy used for growth, the source of carbon, and the electron donors used for growth. An additional criterion of respiratory microorganisms are the electron acceptors used for aerobic or anaerobic respiration.

Nutritional types in bacterial metabolism

Nutritional type	Source of energy	Source of carbon	Examples
Phototrophs	Sunlight	Organic compounds (photoheterotrophs) or carbon fixation (photoautotrophs)	Cyanobacteria, Green sulfur bacteria, Chloroflexi, or Purple bacteria
Lithotrophs	Inorganic compounds	Organic compounds (lithoheterotrophs) or carbon fixation (lithoautotrophs)	Thermodesulfobacteria, *Hydrogenophilaceae*, or Nitrospirae
Organotrophs	Organic compounds	Organic compounds (chemoheterotrophs) or carbon fixation (chemoautotrophs)	*Bacillus*, *Clostridium* or *Enterobacteriaceae*

Carbon metabolism in bacteria is either heterotrophic, where organic carbon compounds are used as carbon sources, or autotrophic, meaning that cellular carbon is obtained by fixing carbon dioxide. Heterotrophic bacteria include parasitic types. Typical autotrophic bacteria are phototrophic cyanobacteria, green sulfur-bacteria and some purple bacteria, but also many chemolithotrophic species, such as nitrifying or sulfur-oxidising bacteria. Energy metabolism of bacteria is either based on phototrophy, the use of light through photosynthesis, or on chemotrophy, the use of chemical substances for energy, which are mostly oxidised at the expense of oxygen or alternative electron acceptors (aerobic/anaerobic respiration).

Filaments of photosynthetic cyanobacteria

Finally, bacteria are further divided into lithotrophs that use inorganic electron donors and organotrophs that use organic compounds as electron donors. Chemotrophic organisms use the respective electron donors for energy conservation (by aerobic/anaerobic respiration or fermentation) and biosynthetic reactions (e.g. carbon dioxide fixation), whereas phototrophic organisms use them only for biosynthetic purposes. Respiratory organisms use chemical compounds as a source of energy by taking electrons from the reduced substrate and transferring them to a terminal electron acceptor in a redox reaction. This reaction releases energy that can be used to synthesise ATP and drive metabolism. In aerobic organisms, oxygen is used as the electron acceptor. In anaerobic organisms other inorganic compounds, such as nitrate, sulfate or carbon dioxide are used as electron acceptors. This leads to the ecologically important processes of denitrification, sulfate reduction and acetogenesis, respectively.

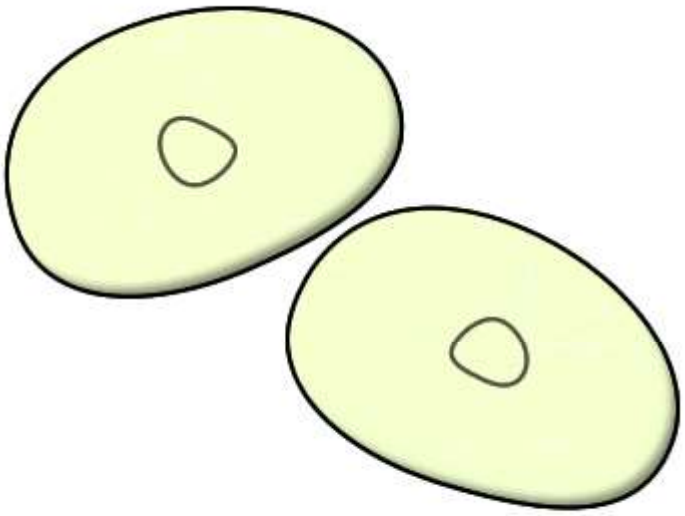
Another way of life of chemotrophs in the absence of possible electron acceptors is fermentation, where the electrons taken from the reduced substrates are transferred to oxidised intermediates to generate reduced fermentation products (e.g. lactate, ethanol, hydrogen, butyric acid). Fermentation is possible, because the energy content of the substrates is higher than that of the products, which allows the organisms to synthesise ATP and drive their metabolism.

These processes are also important in biological responses to pollution; for example, sulfate-reducing bacteria are largely responsible for the production of the highly toxic forms of mercury (methyl- and dimethylmercury) in the environment. Non-respiratory anaerobes use fermentation to generate energy and reducing power, secreting metabolic by-products (such as ethanol in brewing) as waste. Facultative anaerobes can switch between fermentation and different terminal electron acceptors depending on the environmental conditions in which they find themselves.

Lithotrophic bacteria can use inorganic compounds as a source of energy. Common inorganic electron donors are hydrogen, carbon monoxide, ammonia (leading to nitrification), ferrous iron and other reduced metal ions, and several reduced sulfur compounds. Unusually, the gas methane can be used by methanotrophic bacteria as both a source of electrons and a substrate for carbon anabolism. In both aerobic phototrophy and chemolithotrophy, oxygen is used as a terminal electron acceptor, while under anaerobic conditions inorganic compounds are used instead. Most lithotrophic organisms are autotrophic, whereas organotrophic organisms are heterotrophic.

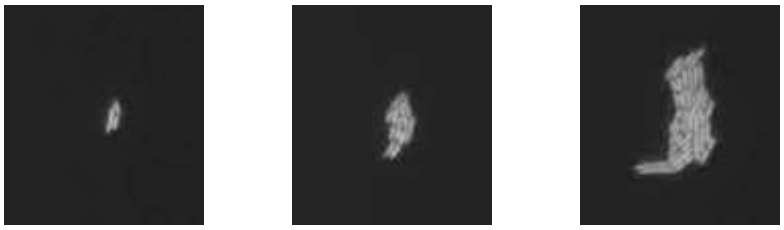
In addition to fixing carbon dioxide in photosynthesis, some bacteria also fix nitrogen gas (nitrogen fixation) using the enzyme nitrogenase. This environmentally important trait can be found in bacteria of nearly all the metabolic types listed above, but is not universal.

Growth and reproduction



Many bacteria reproduce through *binary fission*

Unlike multicellular organisms, increases in the size of bacteria (cell growth) and their reproduction by cell division are tightly linked in unicellular organisms. Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction. Under optimal conditions, bacteria can grow and divide extremely rapidly, and bacterial populations can double as quickly as every 9.8 minutes. In cell division, two identical clone daughter cells are produced. Some bacteria, while still reproducing asexually, form more complex reproductive structures that help disperse the newly formed daughter cells. Examples include fruiting body formation by *Myxobacteria* and aerial hyphae formation by *Streptomyces*, or budding. Budding involves a cell forming a protrusion that breaks away and produces a daughter cell.



A colony of *Escherichia coli*.

In the laboratory, bacteria are usually grown using solid or liquid media. Solid growth media such as agar plates are used to isolate pure cultures of a bacterial strain. However, liquid growth media are used when measurement of growth or large volumes of cells are

required. Growth in stirred liquid media occurs as an even cell suspension, making the cultures easy to divide and transfer, although isolating single bacteria from liquid media is difficult. The use of selective media (media with specific nutrients added or deficient, or with antibiotics added) can help identify specific organisms.

Most laboratory techniques for growing bacteria use high levels of nutrients to produce large amounts of cells cheaply and quickly. However, in natural environments nutrients are limited, meaning that bacteria cannot continue to reproduce indefinitely. This nutrient limitation has led the evolution of different growth strategies. Some organisms can grow extremely rapidly when nutrients become available, such as the formation of algal (and cyanobacterial) blooms that often occur in lakes during the summer. Other organisms have adaptations to harsh environments, such as the production of multiple antibiotics by *Streptomyces* that inhibit the growth of competing microorganisms. In nature, many organisms live in communities (e.g. biofilms) which may allow for increased supply of nutrients and protection from environmental stresses. These relationships can be essential for growth of a particular organism or group of organisms (syntrophy).

Bacterial growth follows three phases. When a population of bacteria first enter a high-nutrient environment that allows growth, the cells need to adapt to their new environment. The first phase of growth is the lag phase, a period of slow growth when the cells are adapting to the high-nutrient environment and preparing for fast growth. The lag phase has high biosynthesis rates, as proteins necessary for rapid growth are produced. The second phase of growth is the logarithmic phase (log phase), also known as the exponential phase. The log phase is marked by rapid exponential growth. The rate at which cells grow during this phase is known as the *growth rate* (*k*), and the time it takes the cells to double is known as the *generation time* (*g*). During log phase, nutrients are metabolised at maximum speed until one of the nutrients is depleted and starts limiting growth. The final phase of growth is the *stationary phase* and is caused by depleted nutrients. The cells reduce their metabolic activity and consume non-essential cellular proteins. The stationary phase is a transition from rapid growth to a stress response state and there is increased expression of genes involved in DNA repair, antioxidant metabolism and nutrient transport.

Genetics

Most bacteria have a single circular chromosome that can range in size from only 160,000 base pairs in the endosymbiotic bacteria *Candidatus Carsonella ruddii*, to 12,200,000 base pairs in the soil-dwelling bacteria *Sorangium cellulosum*. Spirochaetes of the genus *Borrelia* are a notable exception to this arrangement, with bacteria such as *Borrelia burgdorferi*, the cause of Lyme disease, containing a single linear chromosome. The genes in bacterial genomes are usually a single continuous stretch of DNA and although several different types of introns do exist in bacteria, these are much more rare than in eukaryotes.

Bacteria may also contain plasmids, which are small extra-chromosomal DNAs that may contain genes for antibiotic resistance or virulence factors.

Bacteria, as asexual organisms, inherit identical copies of their parent's genes (i.e., they are clonal). However, all bacteria can evolve by selection on changes to their genetic material DNA caused by genetic recombination or mutations. Mutations come from errors made during the replication of DNA or from exposure to mutagens. Mutation rates vary widely among different species of bacteria and even among different clones of a single species of bacteria. Genetic changes in bacterial genomes come from either random mutation during replication or "stress-directed mutation", where genes involved in a particular growth-limiting process have an increased mutation rate.

Some bacteria also transfer genetic material between cells. This can occur in three main ways. Firstly, bacteria can take up exogenous DNA from their environment, in a process called transformation. Genes can also be transferred by the process of transduction, when the integration of a bacteriophage introduces foreign DNA into the chromosome. The third method of gene transfer is bacterial conjugation, where DNA is transferred through direct cell contact. This gene acquisition from other bacteria or the environment is called horizontal gene transfer and may be common under natural conditions. Gene transfer is particularly important in antibiotic resistance as it allows the rapid transfer of resistance genes between different pathogens.

Bacteriophages

Bacteriophages are viruses that infect bacteria. Many types of bacteriophage exist, some simply infect and lyse their host bacteria, while others insert into the bacterial chromosome. A bacteriophage can contain genes that contribute to its host's phenotype: for example, in the evolution of *Escherichia coli* O157:H7 and *Clostridium botulinum*, the toxin genes in an integrated phage converted a harmless ancestral bacterium into a lethal pathogen. Bacteria resist phage infection through restriction modification systems that degrade foreign DNA, and a system that uses CRISPR sequences to retain fragments of the genomes of phage that the bacteria have come into contact with in the past, which allows them to block virus replication through a form of RNA interference. This CRISPR system provides bacteria with acquired immunity to infection.

Behavior

Secretion

Bacteria frequently secrete chemicals into their environment in order to modify it favorably. The secretions are often proteins and may act as enzymes that digest some form of food in the environment.

Bioluminescence

A few bacteria have chemical systems that generate light. This bioluminescence often occurs in bacteria that live in association with fish, and the light probably serves to attract fish or other large animals.

Multicellularity

Bacteria often function as multicellular aggregates known as biofilms, exchanging a variety of molecular signals for inter-cell communication, and engaging in coordinated multicellular behavior.

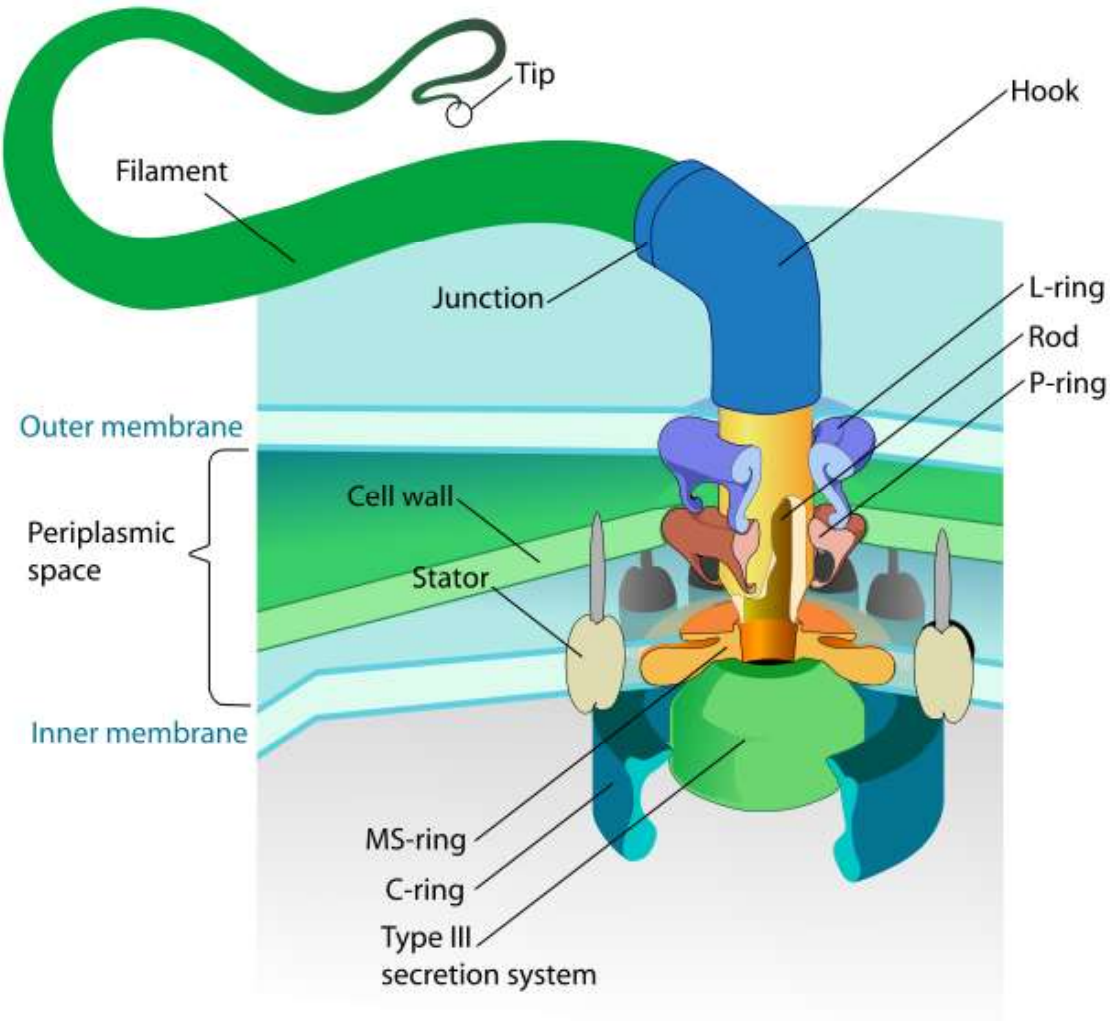
The communal benefits of multicellular cooperation include a cellular division of labor, accessing resources that cannot effectively be utilized by single cells, collectively defending against antagonists, and optimizing population survival by differentiating into distinct cell types. For example, bacteria in biofilms can have more than 500 times increased resistance to antibacterial agents than individual "planktonic" bacteria of the same species.

One type of inter-cellular communication by a molecular signal is called quorum sensing, which serves the purpose of determining whether there is a local population density that is sufficiently high that it is productive to invest in processes that are only successful if large numbers of similar organisms behave similarly, as in excreting digestive enzymes or emitting light.

Quorum sensing allows bacteria to coordinate gene expression, and enables them to produce, release and detect autoinducers or pheromones which accumulate with the growth in cell population.

Movement

Many bacteria can move using a variety of mechanisms: flagella are used for swimming through water; bacterial gliding and twitching motility move bacteria across surfaces; and changes of buoyancy allow vertical motion.



Flagellum of Gram-negative Bacteria. The base drives the rotation of the hook and filament.

Swimming bacteria frequently move near 10 body lengths per second and a few as fast as 100. This makes them at least as fast as fish, on a relative scale.

In twitching motility, bacterial use their type IV pili as a grappling hook, repeatedly extending it, anchoring it and then retracting it with remarkable force (>80 pN).

Flagella are semi-rigid cylindrical structures that are rotated and function much like the propeller on a ship. Objects as small as bacteria operate a low Reynolds number and cylindrical forms are more efficient than the flat, paddle-like, forms appropriate at human size scale.

Bacterial species differ in the number and arrangement of flagella on their surface; some have a single flagellum (monotrichous), a flagellum at each end (amphitrichous), clusters of flagella at the poles of the cell (lophotrichous), while others have flagella distributed over the entire surface of the cell (peritrichous). The bacterial flagella is the best-

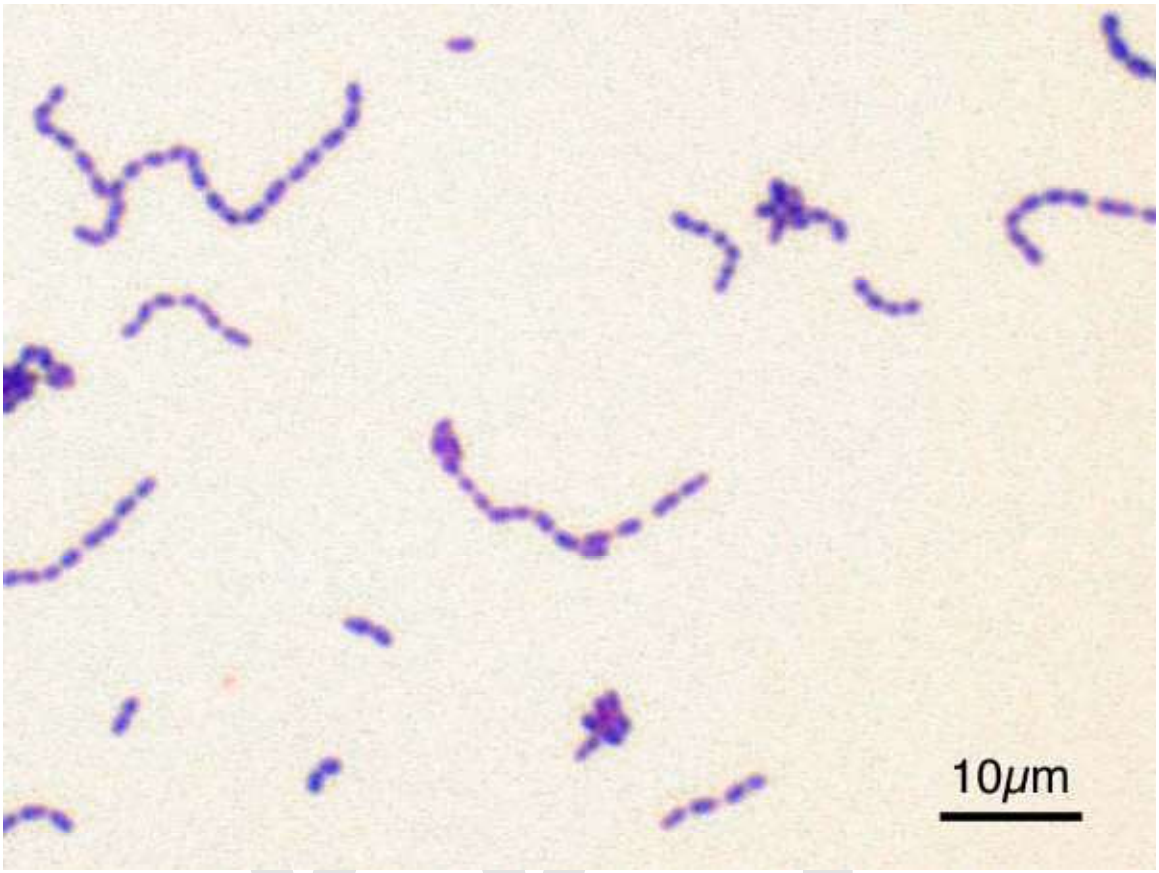
understood motility structure in any organism and is made of about 20 proteins, with approximately another 30 proteins required for its regulation and assembly. The flagellum is a rotating structure driven by a reversible motor at the base that uses the electrochemical gradient across the membrane for power. This motor drives the motion of the filament, which acts as a propeller.

Many bacteria (such as *E. coli*) have two distinct modes of movement: forward movement (swimming) and tumbling. The tumbling allows them to reorient and makes their movement a three-dimensional random walk. The flagella of a unique group of bacteria, the spirochaetes, are found between two membranes in the periplasmic space. They have a distinctive helical body that twists about as it moves.

Motile bacteria are attracted or repelled by certain stimuli in behaviors called *taxes*: these include chemotaxis, phototaxis, energy taxis and magnetotaxis. In one peculiar group, the myxobacteria, individual bacteria move together to form waves of cells that then differentiate to form fruiting bodies containing spores. The myxobacteria move only when on solid surfaces, unlike *E. coli* which is motile in liquid or solid media.

Several *Listeria* and *Shigella* species move inside host cells by usurping the cytoskeleton, which is normally used to move organelles inside the cell. By promoting actin polymerization at one pole of their cells, they can form a kind of tail that pushes them through the host cell's cytoplasm.

Classification and identification

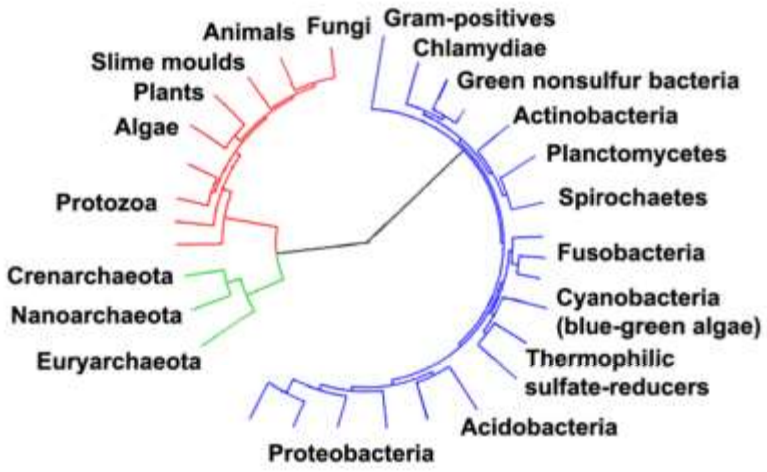


Streptococcus mutans visualized with a Gram stain

Classification seeks to describe the diversity of bacterial species by naming and grouping organisms based on similarities. Bacteria can be classified on the basis of cell structure, cellular metabolism or on differences in cell components such as DNA, fatty acids, pigments, antigens and quinones. While these schemes allowed the identification and classification of bacterial strains, it was unclear whether these differences represented variation between distinct species or between strains of the same species. This uncertainty was due to the lack of distinctive structures in most bacteria, as well as lateral gene transfer between unrelated species. Due to lateral gene transfer, some closely related bacteria can have very different morphologies and metabolisms. To overcome this uncertainty, modern bacterial classification emphasizes molecular systematics, using genetic techniques such as guanine cytosine ratio determination, genome-genome hybridization, as well as sequencing genes that have not undergone extensive lateral gene transfer, such as the rRNA gene. Classification of bacteria is determined by publication in the International Journal of Systematic Bacteriology, and Bergey's Manual of Systematic Bacteriology. The International Committee on Systematic Bacteriology (ICSB) maintains international rules for the naming of bacteria and taxonomic categories and for the ranking of them in the International Code of Nomenclature of Bacteria.

The term "bacteria" was traditionally applied to all microscopic, single-celled prokaryotes. However, molecular systematics showed prokaryotic life to consist of two separate domains, originally called *Eubacteria* and *Archaebacteria*, but now called *Bacteria* and *Archaea* that evolved independently from an ancient common ancestor. The archaea and eukaryotes are more closely related to each other than either is to the bacteria. These two domains, along with Eukarya, are the basis of the three-domain system, which is currently the most widely used classification system in microbiolology. However, due to the relatively recent introduction of molecular systematics and a rapid increase in the number of genome sequences that are available, bacterial classification remains a changing and expanding field. For example, a few biologists argue that the Archaea and Eukaryotes evolved from Gram-positive bacteria.

Identification of bacteria in the laboratory is particularly relevant in medicine, where the correct treatment is determined by the bacterial species causing an infection. Consequently, the need to identify human pathogens was a major impetus for the development of techniques to identify bacteria.



Phylogenetic tree showing the diversity of bacteria, compared to other organisms. Eukaryotes are colored red, archaea green and bacteria blue.

The Gram stain, developed in 1884 by Hans Christian Gram, characterises bacteria based on the structural characteristics of their cell walls. The thick layers of peptidoglycan in the "Gram-positive" cell wall stain purple, while the thin "Gram-negative" cell wall appears pink. By combining morphology and Gram-staining, most bacteria can be classified as belonging to one of four groups (Gram-positive cocci, Gram-positive bacilli, Gram-negative cocci and Gram-negative bacilli). Some organisms are best identified by stains other than the Gram stain, particularly mycobacteria or *Nocardia*, which show acid-fastness on Ziehl–Neelsen or similar stains. Other organisms may need to be identified by their growth in special media, or by other techniques, such as serology.

Culture techniques are designed to promote the growth and identify particular bacteria, while restricting the growth of the other bacteria in the sample. Often these techniques are designed for specific specimens; for example, a sputum sample will be treated to identify organisms that cause pneumonia, while stool specimens are cultured on selective media to identify organisms that cause diarrhoea, while preventing growth of non-pathogenic bacteria. Specimens that are normally sterile, such as blood, urine or spinal fluid, are cultured under conditions designed to grow all possible organisms. Once a pathogenic organism has been isolated, it can be further characterised by its morphology, growth patterns such as (aerobic or anaerobic growth, patterns of hemolysis) and staining.

As with bacterial classification, identification of bacteria is increasingly using molecular methods. Diagnostics using such DNA-based tools, such as polymerase chain reaction, are increasingly popular due to their specificity and speed, compared to culture-based methods. These methods also allow the detection and identification of "viable but nonculturable" cells that are metabolically active but non-dividing. However, even using these improved methods, the total number of bacterial species is not known and cannot even be estimated with any certainty. Following present classification, there are fewer than 9,000 known species of bacteria (including cyanobacteria), but attempts to estimate the true level of bacterial diversity have ranged from 10^7 to 10^9 total species – and even these diverse estimates may be off by many orders of magnitude.

Interactions with other organisms

Despite their apparent simplicity, bacteria can form complex associations with other organisms. These symbiotic associations can be divided into parasitism, mutualism and commensalism. Due to their small size, commensal bacteria are ubiquitous and grow on animals and plants exactly as they will grow on any other surface. However, their growth can be increased by warmth and sweat, and large populations of these organisms in humans are the cause of body odor.

Predators

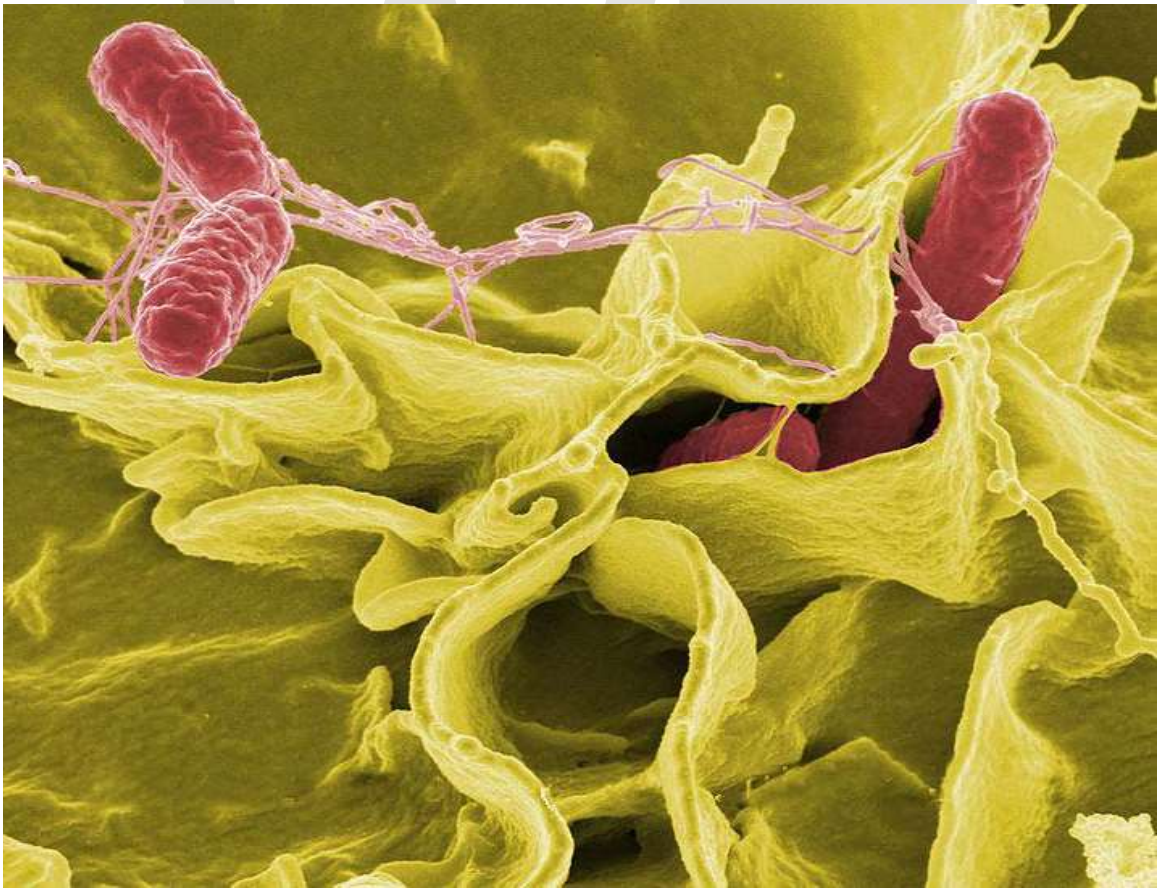
Some species of bacteria kill and then consume other microorganisms, these species called *predatory bacteria*. These include organisms such as *Myxococcus xanthus*, which forms swarms of cells that kill and digest any bacteria they encounter. Other bacterial predators either attach to their prey in order to digest them and absorb nutrients, such as *Vampirococcus*, or invade another cell and multiply inside the cytosol, such as *Daptobacter*. These predatory bacteria are thought to have evolved from saprophages that consumed dead microorganisms, through adaptations that allowed them to entrap and kill other organisms.

Mutualists

Certain bacteria form close spatial associations that are essential for their survival. One such mutualistic association, called interspecies hydrogen transfer, occurs between clusters of anaerobic bacteria that consume organic acids such as butyric acid or

propionic acid and produce hydrogen, and methanogenic Archaea that consume hydrogen. The bacteria in this association are unable to consume the organic acids as this reaction produces hydrogen that accumulates in their surroundings. Only the intimate association with the hydrogen-consuming Archaea keeps the hydrogen concentration low enough to allow the bacteria to grow.

In soil, microorganisms which reside in the rhizosphere (a zone that includes the root surface and the soil that adheres to the root after gentle shaking) carry out nitrogen fixation, converting nitrogen gas to nitrogenous compounds. This serves to provide an easily absorbable form of nitrogen for many plants, which cannot fix nitrogen themselves. Many other bacteria are found as symbionts in humans and other organisms. For example, the presence of over 1,000 bacterial species in the normal human gut flora of the intestines can contribute to gut immunity, synthesise vitamins such as folic acid, vitamin K and biotin, convert milk protein to lactic acid, as well as fermenting complex undigestible carbohydrates. The presence of this gut flora also inhibits the growth of potentially pathogenic bacteria (usually through competitive exclusion) and these beneficial bacteria are consequently sold as probiotic dietary supplements.

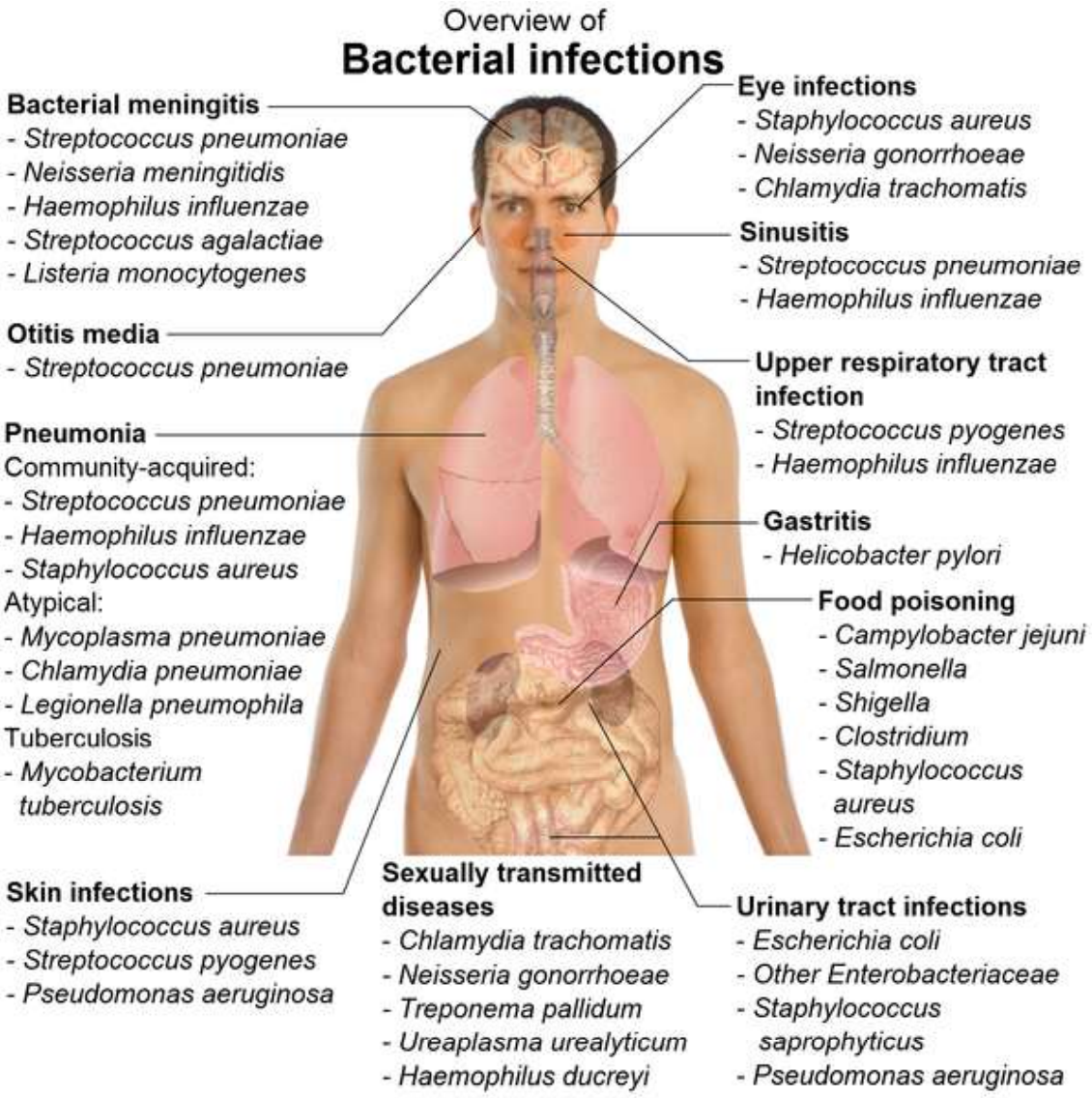


Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells

Pathogens

If bacteria form a parasitic association with other organisms, they are classed as pathogens. Pathogenic bacteria are a major cause of human death and disease and cause infections such as tetanus, typhoid fever, diphtheria, syphilis, cholera, foodborne illness, leprosy and tuberculosis. A pathogenic cause for a known medical disease may only be discovered many years after, as was the case with *Helicobacter pylori* and peptic ulcer disease. Bacterial diseases are also important in agriculture, with bacteria causing leaf spot, fire blight and wilts in plants, as well as Johne's disease, mastitis, salmonella and anthrax in farm animals.

Each species of pathogen has a characteristic spectrum of interactions with its human hosts. Some organisms, such as *Staphylococcus* or *Streptococcus*, can cause skin infections, pneumonia, meningitis and even overwhelming sepsis, a systemic inflammatory response producing shock, massive vasodilation and death. Yet these organisms are also part of the normal human flora and usually exist on the skin or in the nose without causing any disease at all. Other organisms invariably cause disease in humans, such as the Rickettsia, which are obligate intracellular parasites able to grow and reproduce only within the cells of other organisms. One species of Rickettsia causes typhus, while another causes Rocky Mountain spotted fever. *Chlamydia*, another phylum of obligate intracellular parasites, contains species that can cause pneumonia, or urinary tract infection and may be involved in coronary heart disease. Finally, some species such as *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, and *Mycobacterium avium* are opportunistic pathogens and cause disease mainly in people suffering from immunosuppression or cystic fibrosis.



Overview of bacterial infections and main species involved.

Bacterial infections may be treated with antibiotics, which are classified as bacteriocidal if they kill bacteria, or bacteriostatic if they just prevent bacterial growth. There are many types of antibiotics and each class inhibits a process that is different in the pathogen from that found in the host. An example of how antibiotics produce selective toxicity are chloramphenicol and puromycin, which inhibit the bacterial ribosome, but not the structurally different eukaryotic ribosome. Antibiotics are used both in treating human disease and in intensive farming to promote animal growth, where they may be contributing to the rapid development of antibiotic resistance in bacterial populations. Infections can be prevented by antiseptic measures such as sterilizating the skin prior to piercing it with the needle of a syringe, and by proper care of indwelling catheters. Surgical and dental instruments are also sterilized to prevent contamination by bacteria. Disinfectants such as bleach are used to kill bacteria or other pathogens on surfaces to prevent contamination and further reduce the risk of infection.

Significance in technology and industry

Bacteria, often lactic acid bacteria such as *Lactobacillus* and *Lactococcus*, in combination with yeasts and molds, have been used for thousands of years in the preparation of fermented foods such as cheese, pickles, soy sauce, sauerkraut, vinegar, wine and yoghurt.

The ability of bacteria to degrade a variety of organic compounds is remarkable and has been used in waste processing and bioremediation. Bacteria capable of digesting the hydrocarbons in petroleum are often used to clean up oil spills. Fertilizer was added to some of the beaches in Prince William Sound in an attempt to promote the growth of these naturally occurring bacteria after the infamous 1989 *Exxon Valdez* oil spill. These efforts were effective on beaches that were not too thickly covered in oil. Bacteria are also used for the bioremediation of industrial toxic wastes. In the chemical industry, bacteria are most important in the production of enantiomerically pure chemicals for use as pharmaceuticals or agrichemicals.

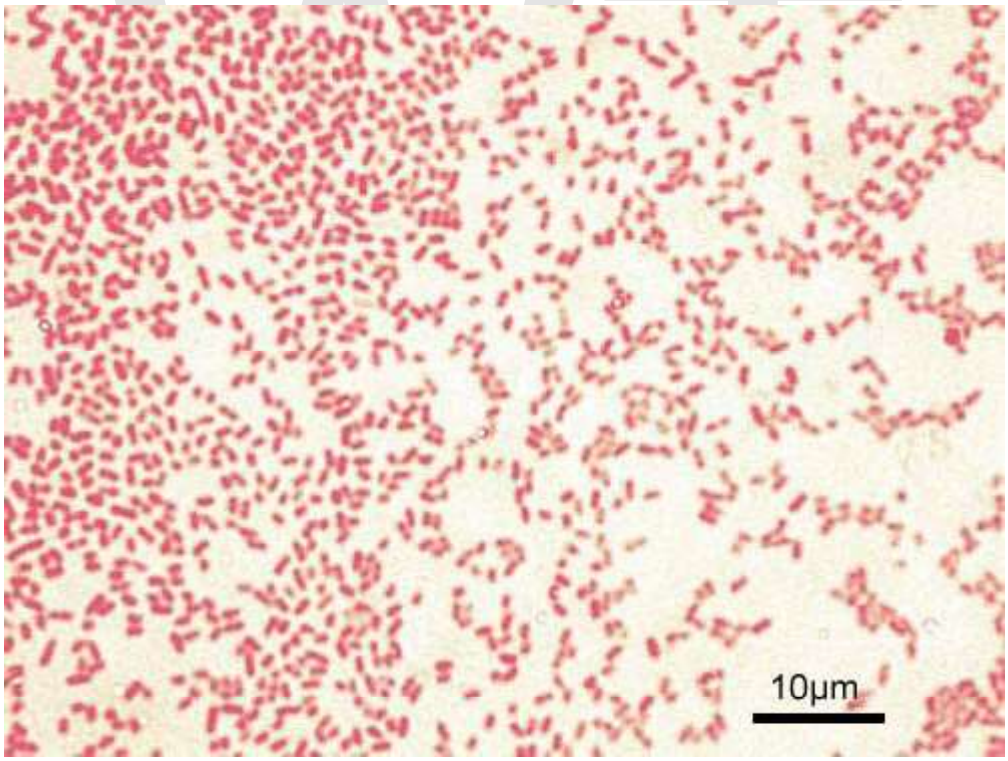
Bacteria can also be used in the place of pesticides in the biological pest control. This commonly involves *Bacillus thuringiensis* (also called BT), a Gram-positive, soil dwelling bacterium. Subspecies of this bacteria are used as a Lepidopteran-specific insecticides under trade names such as Dipel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators and most other beneficial insects.

Because of their ability to quickly grow and the relative ease with which they can be manipulated, bacteria are the workhorses for the fields of molecular biology, genetics and biochemistry. By making mutations in bacterial DNA and examining the resulting phenotypes, scientists can determine the function of genes, enzymes and metabolic pathways in bacteria, then apply this knowledge to more complex organisms. This aim of understanding the biochemistry of a cell reaches its most complex expression in the synthesis of huge amounts of enzyme kinetic and gene expression data into mathematical models of entire organisms. This is achievable in some well-studied bacteria, with models of *Escherichia coli* metabolism now being produced and tested. This understanding of bacterial metabolism and genetics allows the use of biotechnology to bioengineer bacteria for the production of therapeutic proteins, such as insulin, growth factors, or antibodies.

Chapter 2

Gram-Negative Bacteria and Gram-Positive Bacteria

Gram-negative bacteria



Microscopic image of Gram-negative *Pseudomonas aeruginosa* bacteria (pink-red rods).

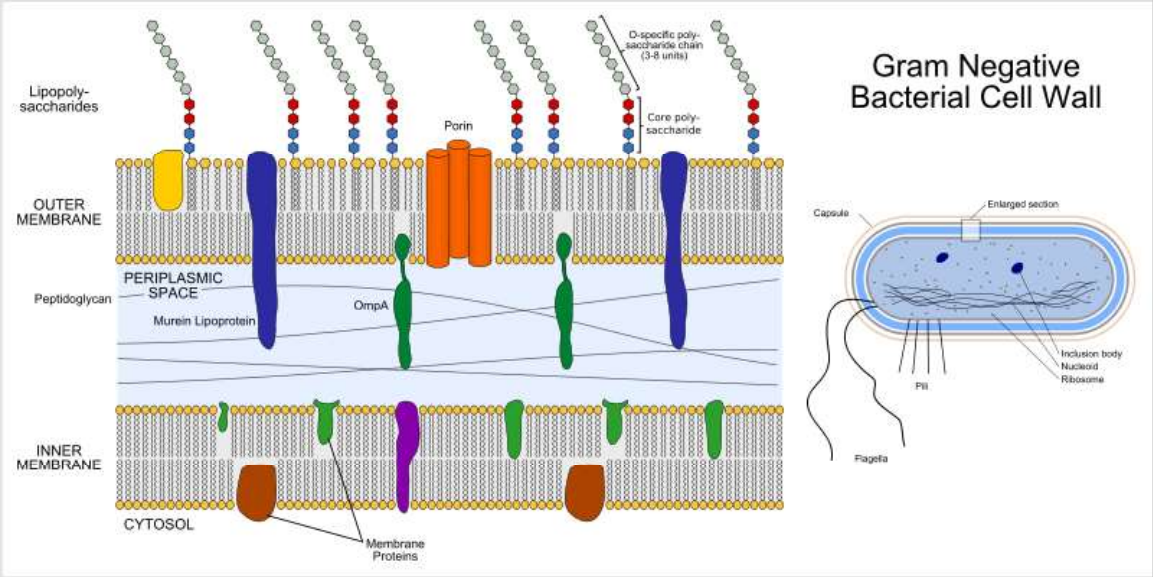
Gram-negative bacteria are bacteria that do not retain crystal violet dye in the Gram staining protocol. In a Gram stain test, a counterstain (commonly safranin) is added after the crystal violet, coloring all Gram-negative bacteria with a red or pink color. The test itself is useful in classifying two distinct types of bacteria based on the structural

differences of their bacterial cell walls. Gram-positive bacteria will retain the crystal violet dye when washed in a decolorizing solution.

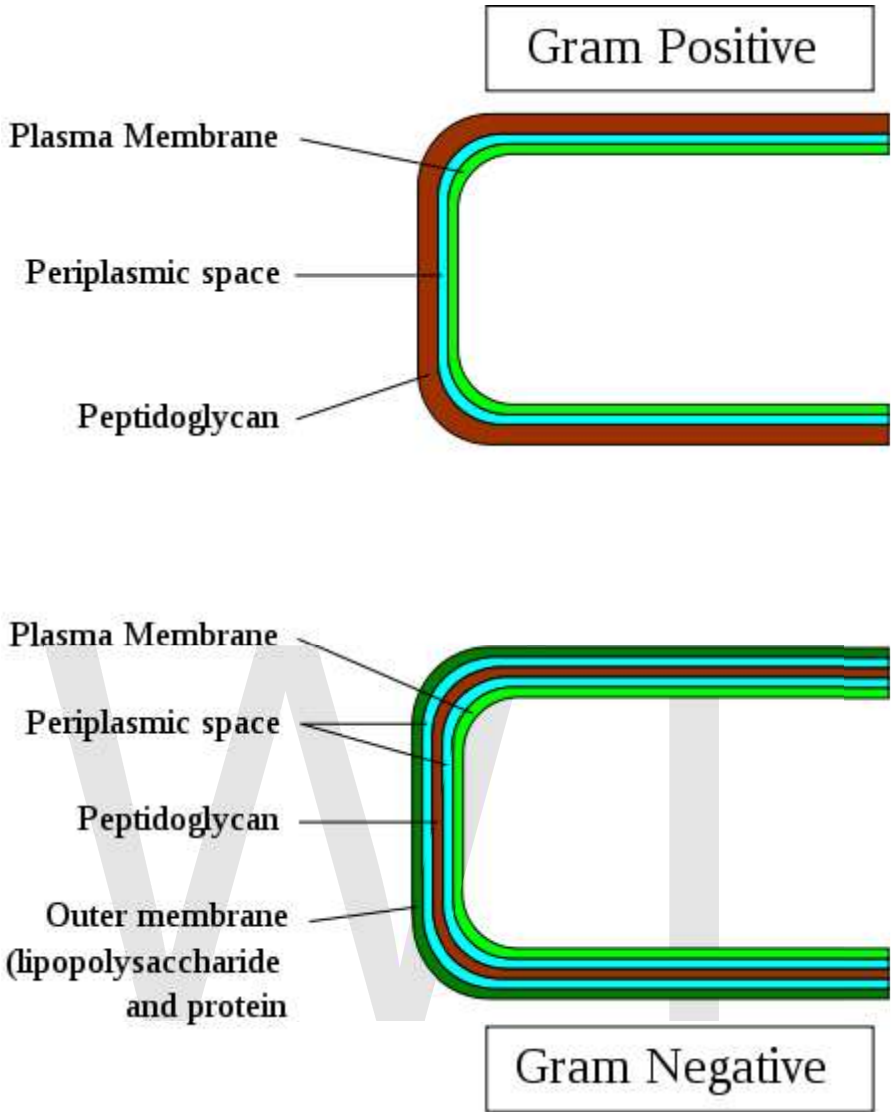
The pathogenic capability of Gram-negative bacteria is often associated with certain components of Gram-negative cell walls, in particular, the lipopolysaccharide (also known as LPS or endotoxin) layer. In humans, LPS triggers an innate immune response characterized by cytokine production and immune system activation. Inflammation is a common result of cytokine (from the Greek *cyto*, cell and *kinesis*, movement) production, which can also produce host toxicity.

When treated as a clade, the term "negibacteria" is sometimes used.

Characteristics



Structure of gram-negative cell wall

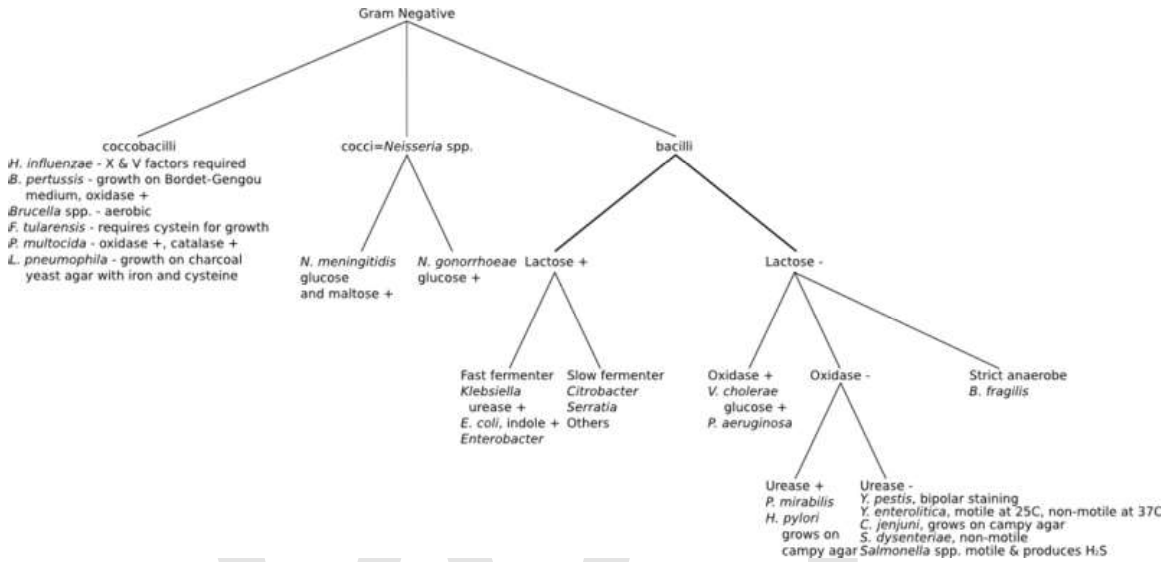


Gram-positive- and negative bacteria are chiefly differentiated by their cell wall structure.

The following characteristics are displayed by Gram-negative bacteria:

1. Cytoplasmic membrane
2. Thin peptidoglycan layer (which is much thinner than in Gram-positive bacteria)
3. Outer membrane containing lipopolysaccharide (LPS, which consists of lipid A, core polysaccharide, and O antigen) outside the peptidoglycan layer
4. Porins exist in the outer membrane, which act like pores for particular molecules
5. There is a space between the layers of peptidoglycan and the secondary cell membrane called the periplasmic space
6. The S-layer is directly attached to the outer membrane, rather than the peptidoglycan
7. If present, flagella have four supporting rings instead of two
8. No teichoic acids or lipoteichoic acids are present

9. Lipoproteins are attached to the polysaccharide backbone.
10. Most of them contain Braun's lipoprotein, which serves as a link between the outer membrane and the peptidoglycan chain by a covalent bound
11. Most do not sporulate (*Coxiella burnetii*, which produces spore-like structures, is a notable exception)



Example species

The proteobacteria are a major group of Gram-negative bacteria, including *Escherichia coli*, *Salmonella*, *Shigella*, and other Enterobacteriaceae, *Pseudomonas*, *Moraxella*, *Helicobacter*, *Stenotrophomonas*, *Bdellovibrio*, acetic acid bacteria, *Legionella* and alpha-proteobacteria as *Wolbachia* and numerous others. Other notable groups of Gram-negative bacteria include the cyanobacteria, spirochaetes, green sulfur and green non-sulfur bacteria.

Medically relevant Gram-negative cocci include three organisms, which cause a sexually transmitted disease (*Neisseria gonorrhoeae*), a meningitis (*Neisseria meningitidis*), and respiratory symptoms (*Moraxella catarrhalis*).

Medically relevant Gram-negative bacilli include a multitude of species. Some of them primarily cause respiratory problems (*Hemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*), primarily urinary problems (*Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*), and primarily gastrointestinal problems (*Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi*).

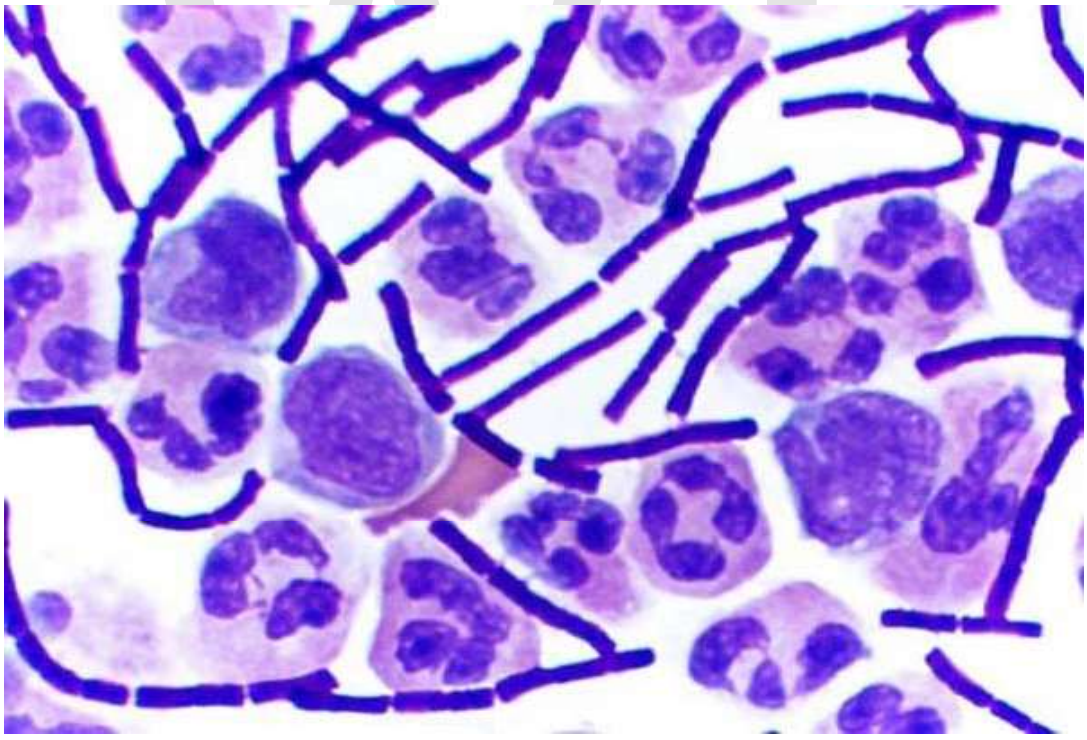
Gram-negative bacteria associated with nosocomial infections include *Acinetobacter baumannii*, which cause bacteremia, secondary meningitis, and ventilator-associated pneumonia in intensive-care units of hospital establishments.

Medical treatment

One of the several unique characteristics of Gram-negative bacteria is the structure of the outer membrane. The outer leaflet of the membrane comprises a complex lipopolysaccharide whose lipid portion acts as an endotoxin. If endotoxin enters the circulatory system, it causes a toxic reaction, with the sufferer developing a high temperature, high respiration rate, and low blood pressure. This may lead to endotoxic shock, which may be fatal.

This outer membrane protects the bacteria from several antibiotics, dyes, and detergents that would normally damage the inner membrane or cell wall (peptidoglycan). The outer membrane provides these bacteria with resistance to lysozyme and penicillin. However, alternative medicinal treatments such as lysozyme with EDTA and the antibiotic ampicillin have been developed to combat the protective outer membrane of some pathogenic Gram-negative organisms. Other drugs can be used, significant ones being chloramphenicol, streptomycin, and nalidixic acid.

Gram-positive bacteria



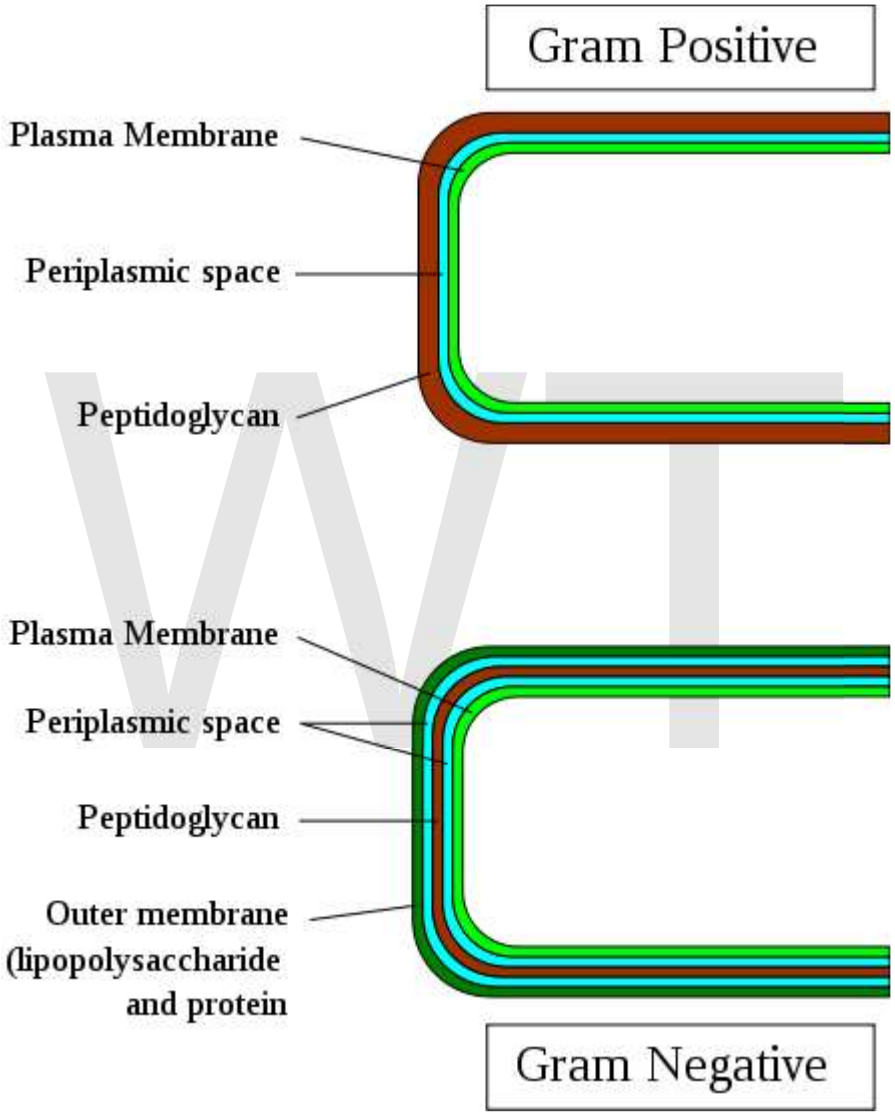
Gram-positive Bacillus anthracis bacteria (purple rods) in cerebrospinal fluid sample. The other cells are white blood cells.

Gram-positive bacteria are those that are stained dark blue or violet by Gram staining. This is in contrast to Gram-negative bacteria, which cannot retain the crystal violet stain, instead taking up the counterstain (safranin or fuchsine) and appearing red or pink. Gram-

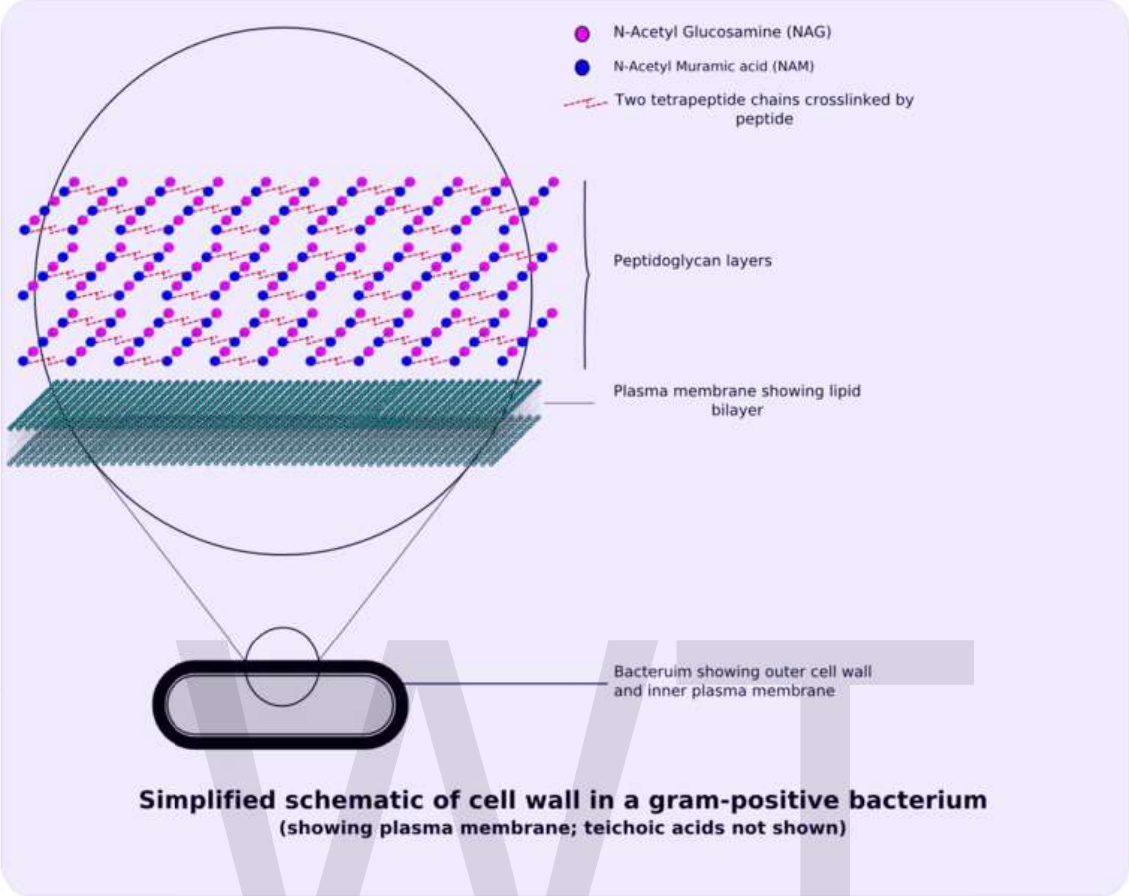
positive organisms are able to retain the crystal violet stain because of the high amount of peptidoglycan in the cell wall. Gram-positive cell walls typically lack the outer membrane found in Gram-negative bacteria.

When treated as a clade, the term "posibacteria" is sometimes used.

Characteristics



Gram-positive and -negative cell wall structure



Structure of Gram-positive cell wall

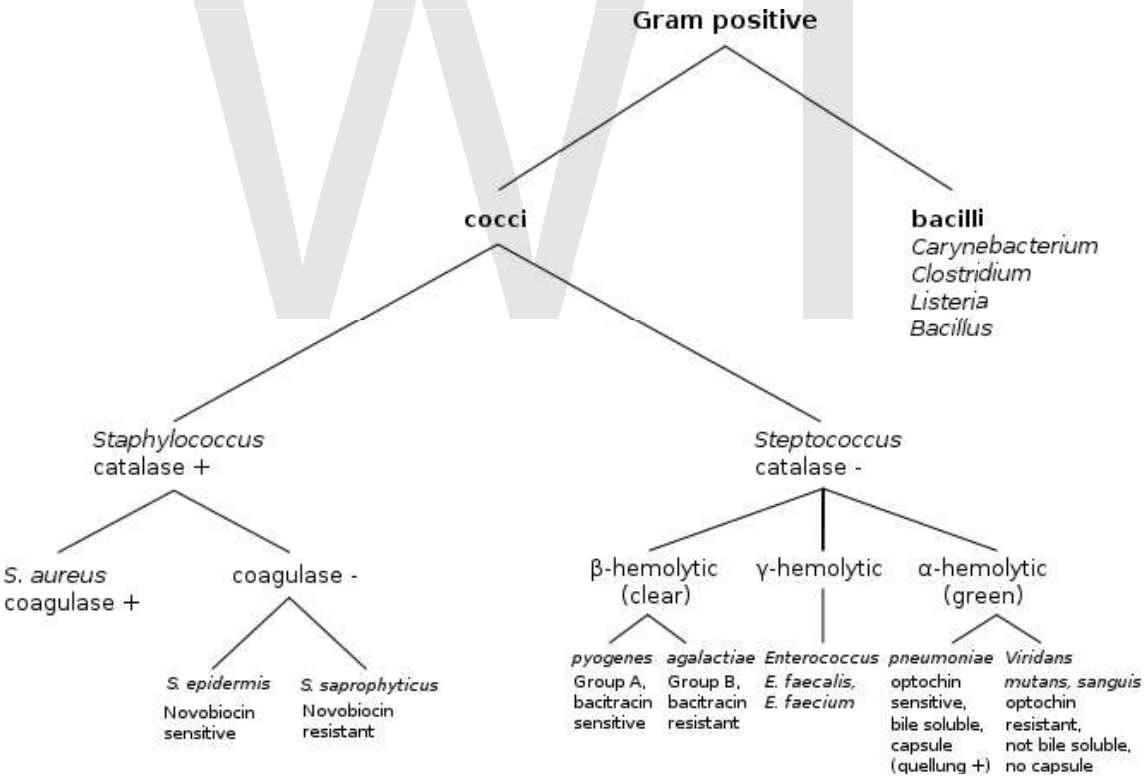
The following characteristics are generally present in a Gram-positive bacterium:

1. cytoplasmic lipid membrane
2. thick peptidoglycan layer
    - teichoic acids and lipoids are present, forming lipoteichoic acids, which serve to act as chelating agents, and also for certain types of adherence.
3. capsule polysaccharides (only in some species)
4. flagellum (only in some species)
    - if present, it contains two rings for support as opposed to four in Gram-negative bacteria because Gram-positive bacteria have only one membrane layer.
5. The individual peptidoglycan molecules are cross-linked by pentaglycine chains by a DD-transpeptidase enzyme. In gram-negative bacteria, the transpeptidase creates a covalent bond directly between peptidoglycan molecules, with no intervening bridge.

Classification

In the original bacterial phyla, the Gram-positive organisms made up the phylum Firmicutes, a name now used for the largest group. It includes many well-known genera such as *Staphylococcus, Streptococcus, Enterococcus,* (which are cocci) and *Bacillus, Corynebacterium, Nocardia, Clostridium, Actinobacteria,* and *Listeria* (which are rods and can be remembered by the mnemonic obconical). It has also been expanded to include the Mollicutes, bacteria-like *Mycoplasma* that lack cell walls and cannot be Gram stained, but are derived from such forms. Actinobacteria are the other major group of Gram-positive bacteria, which have a high guanine and cytosine content in their genomes (high G+C group). This contrasts with the Firmicutes, which have a low G+C content.

Both Gram-positive and Gram-negative bacteria may have a membrane called an S-layer. In Gram-negative bacteria, the S-layer is directly attached to the outer membrane. In Gram-positive bacteria, the S-layer is attached to the peptidoglycan layer. Unique to Gram-positive bacteria is the presence of teichoic acids in the cell wall. Some particular teichoic acids, lipoteichoic acids, have a lipid component and can assist in anchoring peptidoglycan, as the lipid component is embedded in the membrane.



Exceptions

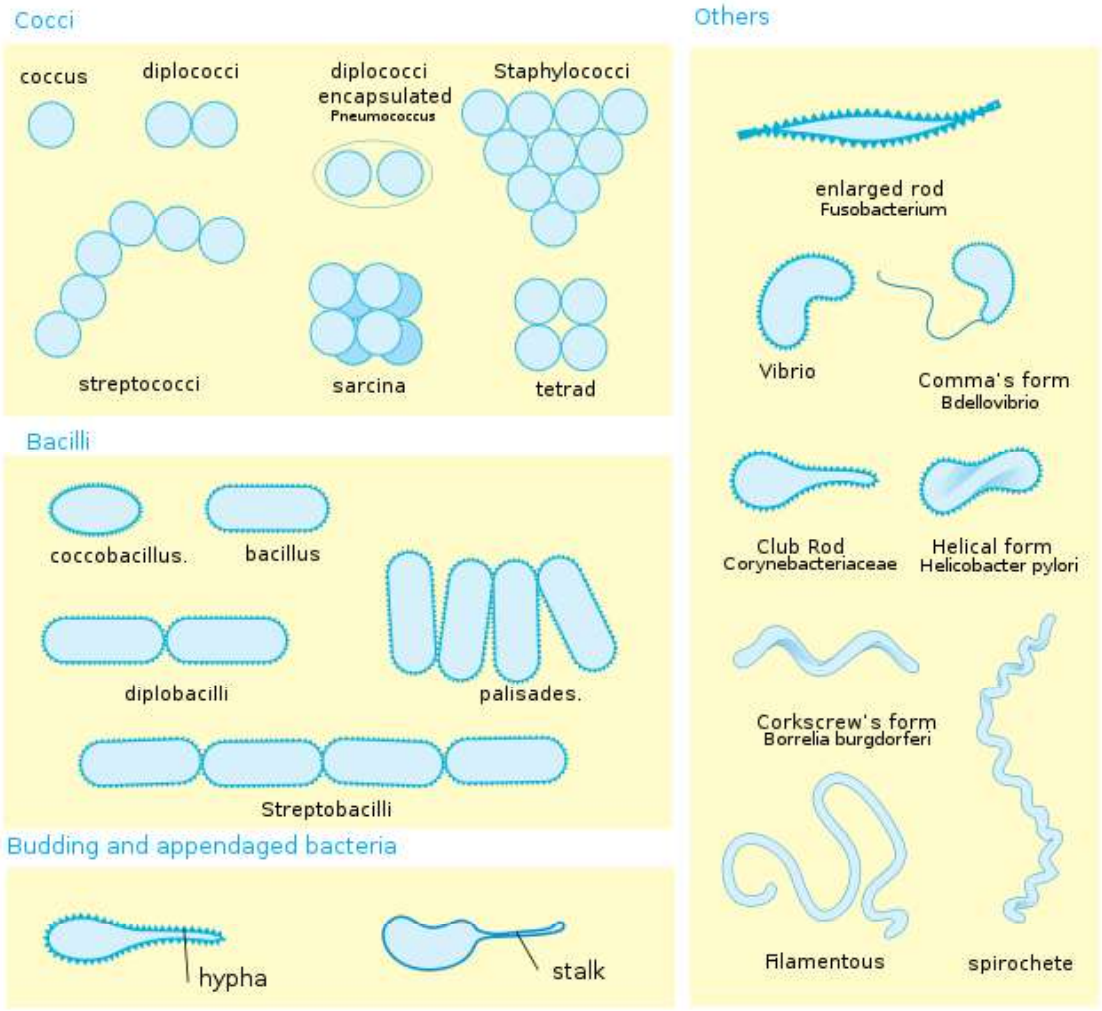
The Deinococcus-Thermus bacteria have Gram-positive stains, although they are structurally similar to Gram-negative bacteria.

Pathogenesis

Most pathogenic in humans are Gram-positive organisms. Classically, six Gram-positive genera are typically pathogenic in humans. Two of these, *Streptococcus* and *Staphylococcus*, are cocci (sphere-shaped bacteria). The remaining organisms are bacilli (rod-shaped bacteria) and can be subdivided based on their ability to form spores. The non-spore formers are *Corynebacterium* and *Listeria* (a coccobacillus), while *Bacillus* and *Clostridium* produce spores. The spore-forming bacteria can again be divided based on their respiration: *Bacillus* is a facultative anaerobe, while *Clostridium* is an obligate anaerobe.

Chapter 3

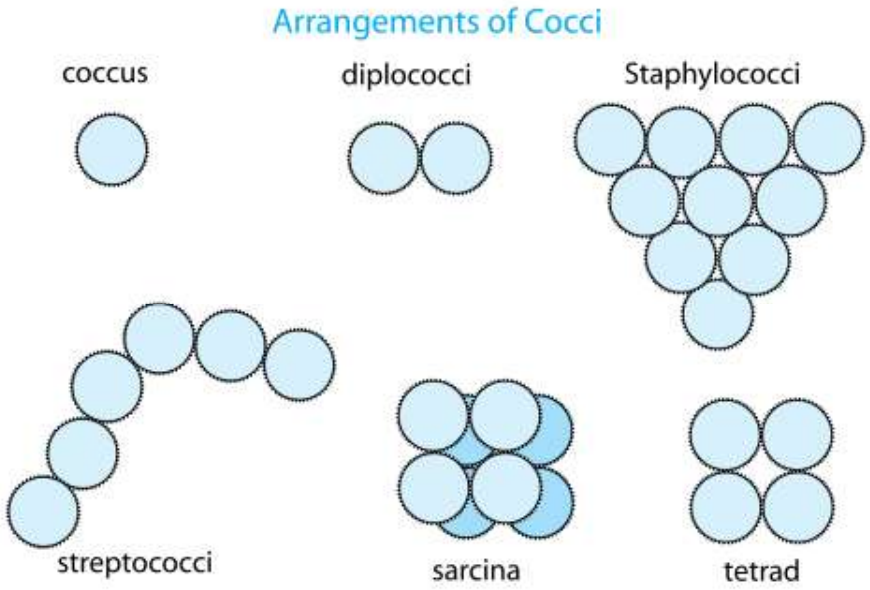
Bacterial Cellular Morphologies

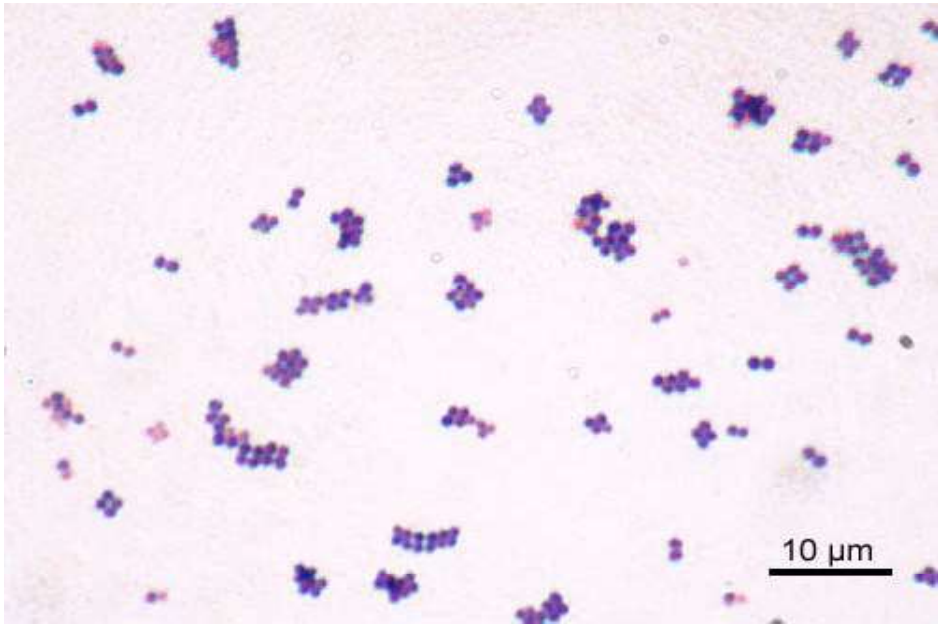


Bacteria display a large diversity of cell morphologies and arrangements

Bacteria are classified by direct examination with the light microscope through its morphology and aggregation.
The basic forms are spheres (coccus) and round-ended cylinders (bacillus). But there may be others such as helically-twisted cylinders (spirochetes), cylinders curved in one plane (Selenomonads) and unusual morphologies (square bacteria). They also conform diplos, tetrads, staphylos, streptos, palizadas etc.

Coccus





Staphylococcus bacteria

Cocci (singular - **coccus**, from the Latin *coccinus* (scarlet) and derived from the Greek *kokkos* (berry)) are any microorganism (usually bacteria) whose overall shape is spherical or nearly spherical. Describing a bacterium as a coccus, or sphere, distinguishes it from bacillus, or rod. This is the first of many taxonomic traits for identifying and classifying a bacterium according to binomial nomenclature.

Aggregations

Aggregations of coccoid bacteria often occur and these forms have specific names as well; listed here are the basic forms as well as representative bacterial genera:

- pairs, or *diplococci* (*Neisseria*)
- groups of four or eight known as tetrads or *sarcina* (*Micrococci*)
- bead-like chains, or *streptococci* (*Streptococcus*)
- grapelike clusters, or *staphylococci* (*Staphylococcus*)

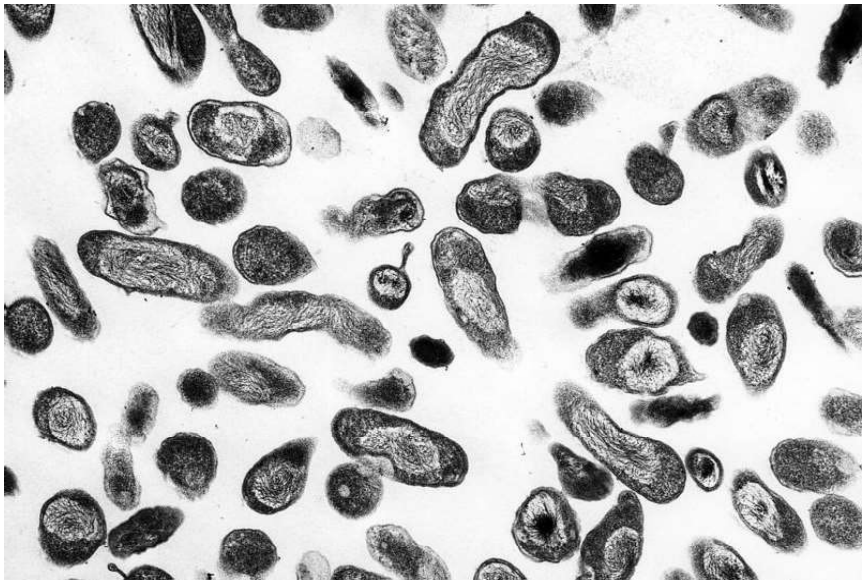
Diplococcus

A **diplococcus** (plural **diplococci**) is a round bacterium (a coccus) that typically occurs in pairs of two joined cells. Examples are *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Its name comes from diplo, meaning double, and coccus, meaning berry. This is because berries are round, like a diplococcus, and diplococci come in pairs of two.

In former times, a bacterial genus *Diplococcus* was recognized, but it is not used anymore.

Coccobacillus



Coxiella burnetii

A **coccobacillus** (plural *coccobacilli*) is a type of rod-shaped bacteria. The word *coccobacillus* reflects an intermediate shape between *coccus* (spherical) and *bacillus* (elongated). Coccobacilli rods are so short and wide that they resemble cocci. *Haemophilus influenzae* and *Chlamydia trachomatis* are coccobacilli. *Aggregatibacter actinomycetemcomitans* is a gram negative coccobacillus which is prevalent in subgingival plaques. *Acinetobacter* strains may grow on solid media as coccobacilli.

Coxiella burnetti is also a coccobacillus.

Clinical significance

Important human pathogens caused by coccoid bacteria include staphylococci infections, some types of food poisoning, some urinary tract infections, toxic shock syndrome, gonorrhea, as well as some forms of meningitis, throat infections, pneumonias, and sinusitis.

Bacillus

Although *Bacillus*, capitalized and italicized, specifically refers to the genus, the word **bacillus** may also be used to describe any rod-shaped bacterium, and in this sense, bacilli are found in many different taxonomic groups of bacteria. There is no connection between the shape of a bacterium and its colors in the Gram staining.

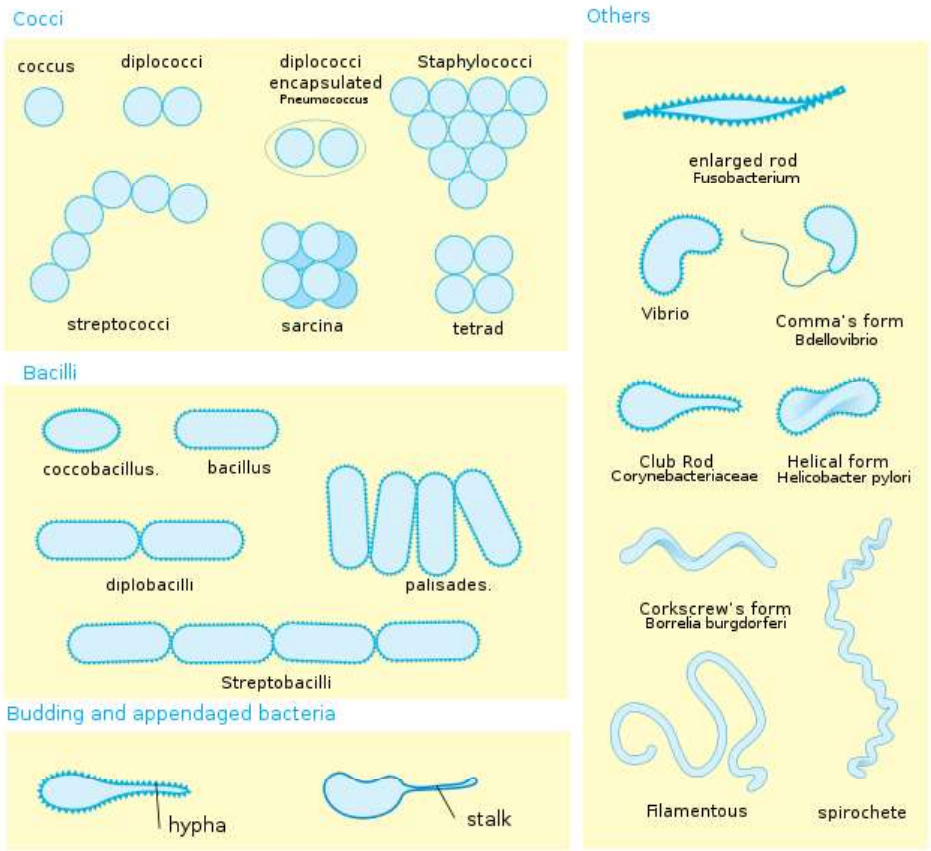
Bacilli are usually solitary, but can combine to form diplobacilli, streptobacilli, and palisades.

Chapter 4

Bacterial Cell Structure

Bacteria, despite their simplicity, contain a well developed cell structure which is responsible for many of their unique biological properties. Many structural features are unique to bacteria and are not found among archaea or eukaryotes. Because of the simplicity of bacteria relative to larger organisms and the ease with which they can be manipulated experimentally, the cell structure of bacteria has been well studied, revealing many biochemical principles that have been subsequently applied to other organisms.

Cell morphology



Bacteria come in a wide variety of shapes

Perhaps the most elemental structural property of bacteria is cell morphology (shape). Typical examples include:

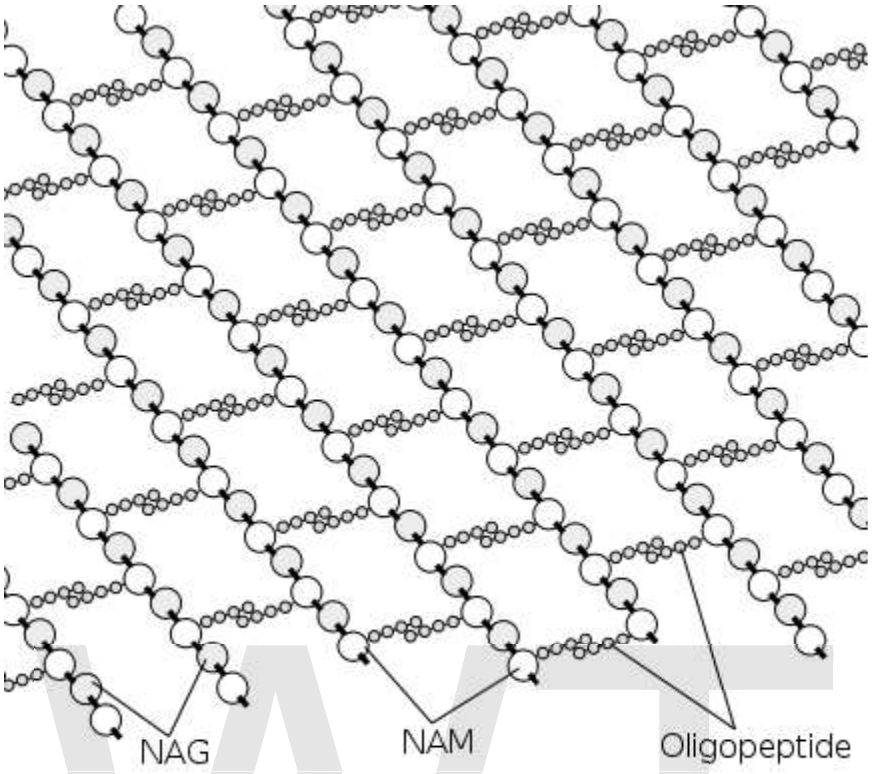
- coccus (spherical)
- bacillus (rod-like)
- spirillum (spiral)
- filamentous

Cell shape is generally characteristic of a given bacterial species, but can vary depending on growth conditions. Some bacteria have complex life cycles involving the production of stalks and appendages (e.g. *Caulobacter*) and some produce elaborate structures bearing reproductive spores (e.g. *Myxococcus*, *Streptomyces*). Bacteria generally form distinctive cell morphologies when examined by light microscopy and distinct colony morphologies when grown on Petri plates. These are often the first characteristics observed by a microbiologist to determine the identity of an unknown bacterial culture.

The importance of cell size

Perhaps the most obvious structural characteristic of bacteria is (with some exceptions) their small size. For example, *Escherichia coli* cells, an "average" sized bacterium, are about 2 micrometres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 μm^3. This corresponds to a wet mass of ca. 1 pg, assuming that the cell consists mostly of water. The dry mass of a single cell can be estimated as 20 % of the wet mass, amounting to 0.2 pg. About half of the dry mass of a bacterial cell consists of carbon, and also about half of it can be attributed to proteins. Therefore, a typical fully grown 1-liter culture of *Escherichia coli* (at an optical density of 1.0, corresponding to ca. 10^9 cells/ml) yields ca. 1 g wet cell mass.

Small size is extremely important because it allows for a large surface area-to-volume ratio which allows for rapid uptake and intracellular distribution of nutrients and excretion of wastes. At low surface area-to-volume ratios the diffusion of nutrients and waste products across the bacterial cell membrane limits the rate at which microbial metabolism can occur, making the cell less evolutionarily fit. The reason for the existence of large cells is unknown, although it is speculated that the increased cell volume is used primarily for storage of excess nutrients.



The structure of peptidoglycan.

As in other organisms, the bacterial cell wall provides structural integrity to the cell. In prokaryotes, the primary function of the cell wall is to protect the cell from internal turgor pressure caused by the much higher concentrations of proteins and other molecules inside the cell compared to its external environment. The bacterial cell wall differs from that of all other organisms by the presence of peptidoglycan (poly-*N*-acetylglucosamine and *N*-acetylmuramic acid), which is located immediately outside of the cytoplasmic membrane. Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape. It is relatively porous and is not considered to be a permeability barrier for small substrates. While all bacterial cell walls (with a few exceptions e.g. extracellular parasites such as *Mycoplasma*) contain peptidoglycan, not all cell walls have the same overall structures. Since the cell wall is required for bacterial survival, but is absent in eukaryotes, several antibiotics (penicillins and cephalosporins) stop bacterial infections by interfering with cell wall synthesis, while having no effects on human cells.

There are two main types of bacterial cell walls, Gram positive and Gram negative, which are differentiated by their Gram staining characteristics. For both Gram-positive and Gram-negative bacteria, particles of approximately 2 nm can pass through the peptidoglycan.

The Gram positive cell wall

Peptidoglycans(mucopeptides, glycopeptides, mureins) are the structural elements of almost all bacterial cell walls. They constitute almost 95% of the cell wall in some Gram positive bacteria and as little as 5-10% of the cell wall in Gram negative bacteria. Peptidoglycans are made up of a polysaccharide backbone consisting of alternating muramic acid(MA) and glucose amine(GA) residues in equal amounts. The cell wall of some Gram positive bacteria is completely dissolved by lysozyme, as this enzyme attacks the bonds between GA and MA. In other Gram positive bacteria, e.g. Staphylococcus aureus, the walls are resistant to the action of lysozyme. They have O-acetyl groups on carbon-6 of some MA residues. The matrix substances in the walls of Gram positive bacteria may be polysaccharides or teichoic acids. The latter are very widespread, but have been found only in Gram positive bacteria. There are two main types of teichoic acid: ribitol teichoic acids and glycerol teichoic acids. The latter one is more widespread. These acids are polymers of ribitol phosphate and glycerol phosphate, respectively, and only one type is found in the wall of any particular strain of bacteria. Teichoic acids form receptor sites for bacteriophages, and at least some of them are located on the surface of many Gram positive bacteria.

The Gram negative cell wall

Unlike the Gram positive cell wall, the Gram negative cell wall contains a **thin** peptidoglycan layer adjacent to the cytoplasmic membrane. This is responsible for the cell wall's inability to retain the crystal violet stain upon decolourisation with ethanol during Gram staining. In addition to the peptidoglycan layer, the Gram negative cell wall also contains an outer membrane composed by phospholipids and lipopolysaccharides, which face into the external environment. As the lipopolysaccharides are highly-charged, the Gram negative cell wall has an overall negative charge. The chemical structure of the outer membrane lipopolysaccharides is often unique to specific bacterial strains (i.e. sub-species) and is responsible for many of the antigenic properties of these strains.

The bacterial cytoplasmic membrane

The bacterial cytoplasmic membrane is composed of a phospholipid bilayer and thus has all of the general functions of a cell membrane such as acting as a permeability barrier for most molecules and serving as the location for the transport of molecules into the cell. In addition to these functions, prokaryotic membranes also function in energy conservation as the location about which a proton motive force is generated. Unlike eukaryotes, bacterial membranes (with some exceptions e.g. *Mycoplasma* and methanotrophs) generally do not contain sterols. However, many microbes do contain structurally related compounds called hopanoids which likely fulfill the same function. Unlike eukaryotes, bacteria can have a wide variety of fatty acids within their membranes. Along with typical saturated and unsaturated fatty acids, bacteria can contain fatty acids with additional methyl, hydroxy or even cyclic groups. The relative proportions of these fatty acids can be modulated by the bacterium to maintain the optimum fluidity of the membrane (e.g. following temperature change).

As a phospholipid bilayer, the lipid portion of the outer membrane is impermeable to charged molecules. However, channels called porins are present in the outer membrane that allow for passive transport of many ions, sugars and amino acids across the outer membrane. These molecules are therefore present in the periplasm, the region between the cytoplasmic and outer membranes. The periplasm contains the peptidoglycan layer and many proteins responsible for substrate binding or hydrolysis and reception of extracellular signals. The periplasm it is thought to exist as a gel-like state rather than a liquid due to the high concentration of proteins and peptidoglycan found within it. Because of its location between the cytoplasmic and outer membranes, signals received and substrates bound are available to be transported across the cytoplasmic membrane using transport and signalling proteins imbedded there.

Other bacterial surface structures

Fimbrae and Pili

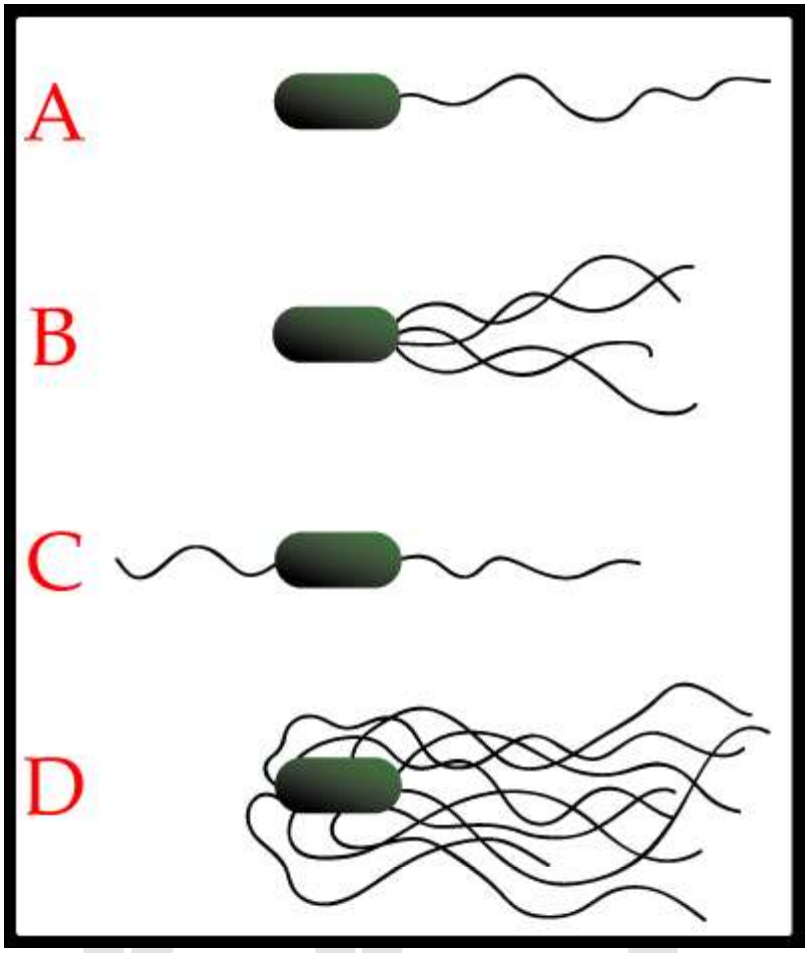
Fimbrae are protein tubes that extend out from the outer membrane in many members of the Proteobacteria. They are generally short in length and present in high numbers about the entire bacterial cell surface. Fimbrae usually function to facilitate the attachment of a bacterium to a surface (e.g. to form a biofilm) or to other cells (e.g. animal cells during pathogenesis)). A few organisms (e.g. *Myxococcus*) use fimbrae for motility to facilitate the assembly of multicellular structures such as fruiting bodies. Pili are similar in structure to fimbrae but are much longer and present on the bacterial cell in low numbers. Pili are involved in the process of bacterial conjugation. Non-sex pili also aid bacteria in gripping surfaces.

S-layers

An S-layer (surface layer) is a cell surface protein layer found in many different bacteria and in some archaea, where it serves as the cell wall. All S-layers are made up of a two-dimensional array of proteins and have a crystalline appearance, the symmetry of which differs between species. The exact function of S-layers is unknown, but it has been suggested that they act as a partial permeability barrier for large substrates. For example, an S-layer could conceivably keep extracellular proteins near the cell membrane by preventing their diffusion away from the cell. In some pathogenic species, an S-layer may help to facilitate survival within the host by conferring protection against host defence mechanisms.

Capsules and Slime Layers

Many bacteria secrete extracellular polymers outside of their cell walls. These polymers are usually composed of polysaccharides and sometimes protein. Capsules are relatively impermeable structures that cannot be stained with dyes such as India ink. They are structures that help protect bacteria from phagocytosis and desiccation. Slime layer is involved in attachment of bacteria to other cells or inanimate surfaces to form biofilms. Slime layers can also be used as a food reserve for the cell.



A-Monotrichous; B-Lophotrichous; C-Amphitrichous; D-Peritrichous;

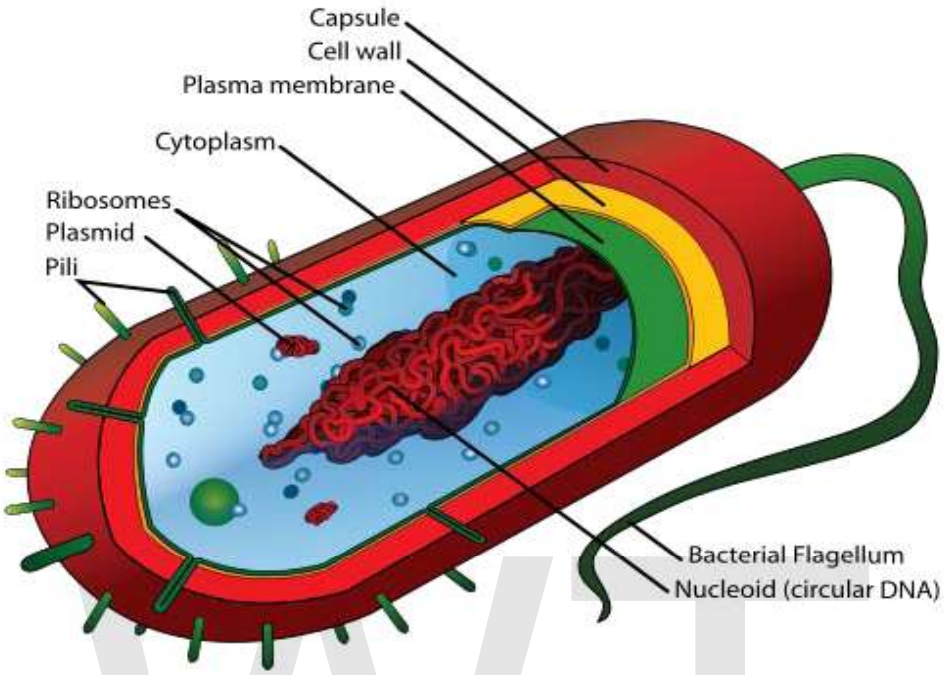
Flagella

Perhaps the most recognizable extracellular bacterial cell structures are flagella. Flagella are whip-like structures protruding from the bacterial cell wall and are responsible for bacterial motility (i.e. movement). The arrangement of flagella about the bacterial cell is unique to the species observed. Common forms include:

- Peritrichous - Multiple flagella found at several locations about the cell
- Polar - Single flagellum found at one of the cell poles
- Lophotrichous - A tuft of flagella found at one cell pole

Flagella are complex structures that are composed of many different proteins. These include flagellin, which makes up the whip-like tube and a protein complex that spans the cell wall and cell membrane to form a motor that causes the flagellum to rotate. This rotation is normally driven by proton motive force and are found in the body of the cell.

Intracellular bacterial cell structures



Cell structure of a Gram positive prokaryote

In comparison to eukaryotes, the intracellular features of the bacterial cell are extremely simple. Bacteria do not contain organelles in the same sense as eukaryotes. Instead, the chromosome and perhaps ribosomes are the only easily observable intracellular structures found in all bacteria. There do exist, however, specialized groups of bacteria that contain more complex intracellular structures, some of which are discussed below.

The bacterial chromosome and plasmids

Unlike eukaryotes, the bacterial chromosome is not enclosed inside of a membrane-bound nucleus but instead resides inside the bacterial cytoplasm. This means that the transfer of cellular information through the processes of translation, transcription and DNA replication all occur within the same compartment and can interact with other cytoplasmic structures, most notably ribosomes. The bacterial chromosome is not packaged using histones to form chromatin as in eukaryotes but instead exists as a highly compact supercoiled structure, the precise nature of which remains unclear. Most bacterial chromosomes are circular although some examples of linear chromosomes exist (e.g. *Borrelia burgdorferi*). Along with chromosomal DNA, most bacteria also contain small independent pieces of DNA called plasmids that often encode for traits that are advantageous but not essential to their bacterial host. Plasmids can be easily gained or lost by a bacterium and can be transferred between bacteria as a form of horizontal gene transfer.

Ribosomes and other multiprotein complexes

In most bacteria the most numerous intracellular structure is the ribosome, the site of protein synthesis in all living organisms. All prokaryotes have 70S (where S=Svedberg units) ribosomes while eukaryotes contain larger 80S ribosomes in their cytosol. The 70S ribosome is made up of a 50S and 30S subunits. The 50S subunit contains the 23S and 5S rRNA while the 30S subunit contains the 16S rRNA. These rRNA molecules differ in size in eukaryotes and are complexed with a large number of ribosomal proteins, the number and type of which can vary slightly between organisms. While the ribosome is the most commonly observed intracellular multiprotein complex in bacteria other large complexes do occur and can sometimes be seen using microscopy.

Intracellular membranes

While not typical of all bacteria some microbes contain intracellular membranes in addition to (or as extensions of) their cytoplasmic membranes. An early idea was that bacteria might contain membrane folds termed mesosomes, but these were later shown to be artifacts produced by the chemicals used to prepare the cells for electron microscopy. Examples of bacteria containing intracellular membranes are phototrophs, nitrifying bacteria and methane-oxidising bacteria. Intracellular membranes are also found in bacteria belonging to the poorly studied Planctomycetes group, although these membranes more closely resemble organellar membranes in eukaryotes and are currently of unknown function.

Cytoskeleton

The prokaryotic cytoskeleton is the collective name for all structural filaments in prokaryotes. It was once thought that prokaryotic cells did not possess cytoskeletons, but recent advances in visualization technology and structure determination have shown that filaments indeed exist in these cells. In fact, homologues for all major cytoskeletal proteins in eukaryotes have been found in prokaryotes. Cytoskeletal elements play essential roles in cell division, protection, shape determination, and polarity determination in various prokaryotes.

Nutrient storage structures

Most bacteria do not live in environments that contain large amounts of nutrients at all times. To accommodate these transient levels of nutrients bacteria contain several different methods of nutrient storage in times of plenty for use in times of want. For example, many bacteria store excess carbon in the form of polyhydroxyalkanoates or glycogen. Some microbes store soluble nutrients such as nitrate in vacuoles. Sulfur is most often stored as elemental (S^0) granules which can be deposited either intra- or extracellularly. Sulfur granules are especially common in bacteria that use hydrogen sulfide as an electron source. Most of the above mentioned examples can be viewed using a microscope and are surrounded by a thin nonunit membrane to separate them from the cytoplasm.

Gas vesicles

Gas vesicles are spindle-shaped structures found in some planktonic bacteria that provides buoyancy to these cells by decreasing their overall cell density. They are made up of a protein coat that is very impermeable to solvents such as water but permeable to most gases. By adjusting the amount of gas present in their gas vesicles bacteria can increase or decrease their overall cell density and thereby move up or down within the water column to maintain their position in an environment optimal for growth.

Carboxysomes

Carboxysomes are intracellular structures found in many autotrophic bacteria such as Cyanobacteria, Knallgasbacteria, Nitroso- and Nitrobacteria. They are proteinaceous structures resembling phage heads in their morphology and contain the enzymes of carbon dioxide fixation in these organisms (especially ribulose bisphosphate carboxylase/oxygenase, RuBisCO, and carbonic anhydrase). It is thought that the high local concentration of the enzymes along with the fast conversion of bicarbonate to carbon dioxide by carbonic anhydrase allows faster and more efficient carbon dioxide fixation than possible inside the cytoplasm. Similar structures are known to harbor the coenzyme B12-containing glycerol dehydratase, the key enzyme of glycerol fermentation to 1,3-propanediol, in some Enterobacteriaceae (e. g. Salmonella).

Magnetosomes

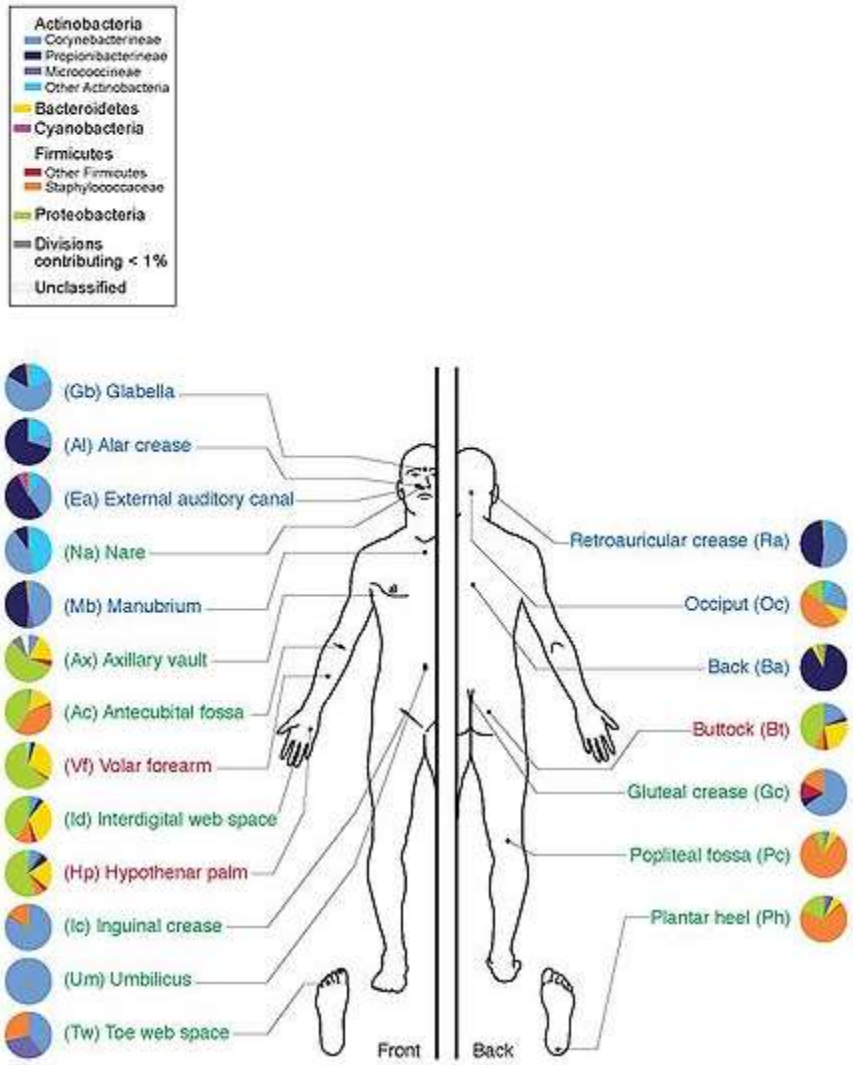
Magnetosomes are intracellular organelles found in magnetotactic bacteria that allow them to sense and align themselves along a magnetic field (magnetotaxis). The ecological role of magnetotaxis is unknown but it is hypothesized to be involved in the determination of optimal oxygen concentrations. Magnetosomes are composed of the mineral magnetite or greigite and are surrounded by a lipid bilayer membrane. The morphology of magnetosomes is species-specific.

Endospores

Perhaps the most well known bacterial adaptation to stress is the formation of endospores. Endospores are bacterial survival structures that are highly resistant to many different types of chemical and environmental stresses and therefore enable the survival of bacteria in environments that would be lethal for these cells in their normal vegetative form. It has been proposed that endospore formation has allowed for the survival of some bacteria for hundreds of millions of years (e.g. in salt crystals) although these publications have been questioned. Endospore formation is limited to several genera of Gram-positive bacteria such as *Bacillus* and *Clostridium*. It differs from reproductive spores in that only one spore is formed per cell resulting in no net gain in cell number upon endospore germination. The location of an endospore within a cell is species-specific and can be used to determine the identity of a bacterium.

Chapter 5

Skin Flora



Depiction of the human body and bacteria that predominate

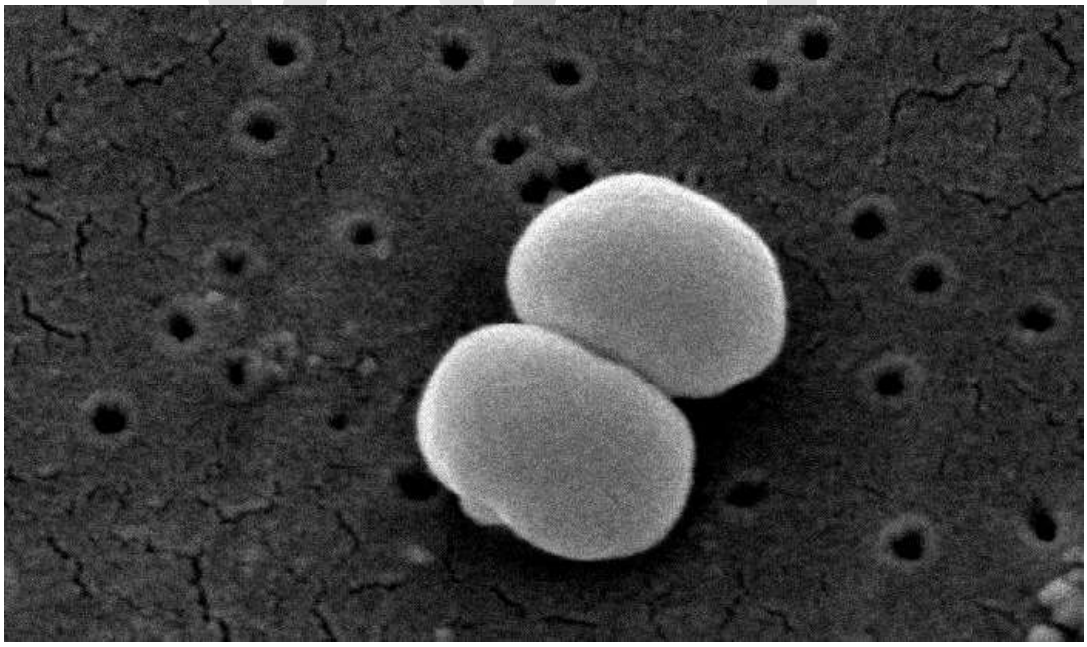
The **skin flora** are the microorganisms which reside on the skin. Most research has been upon those that reside upon the 2 square meters of human skin. Many of them are bacteria of which there are around 1000 species upon human skin from 19 phyla. The total number of bacteria on an average human has been estimated at 10^{12} (1,000,000,000,000). Most are found in the superficial layers of the epidermis and the upper parts of hair follicles.

Skin flora are usually non-pathogenic, and either commensals (are not harmful to their host) or mutualistic (offer a benefit). The benefits bacteria can offer include preventing transient pathogenic organisms from colonizing the skin surface, either by competing for nutrients, secreting chemicals against them, or stimulating the skin's immune system. However, resident microbes can cause skin diseases and enter the blood system creating life-threatening diseases particularly in immunosuppressed people. Hygiene to control such flora is important in preventing the transmission of antibiotic resistant hospital-acquired infections.

A major nonhuman skin flora is *Batrachochytrium dendrobatidis*, a chytrid and non-hyphal zoosporic fungus that causes chytridiomycosis, an infectious disease thought to be responsible for the decline in amphibian populations.

Species variety

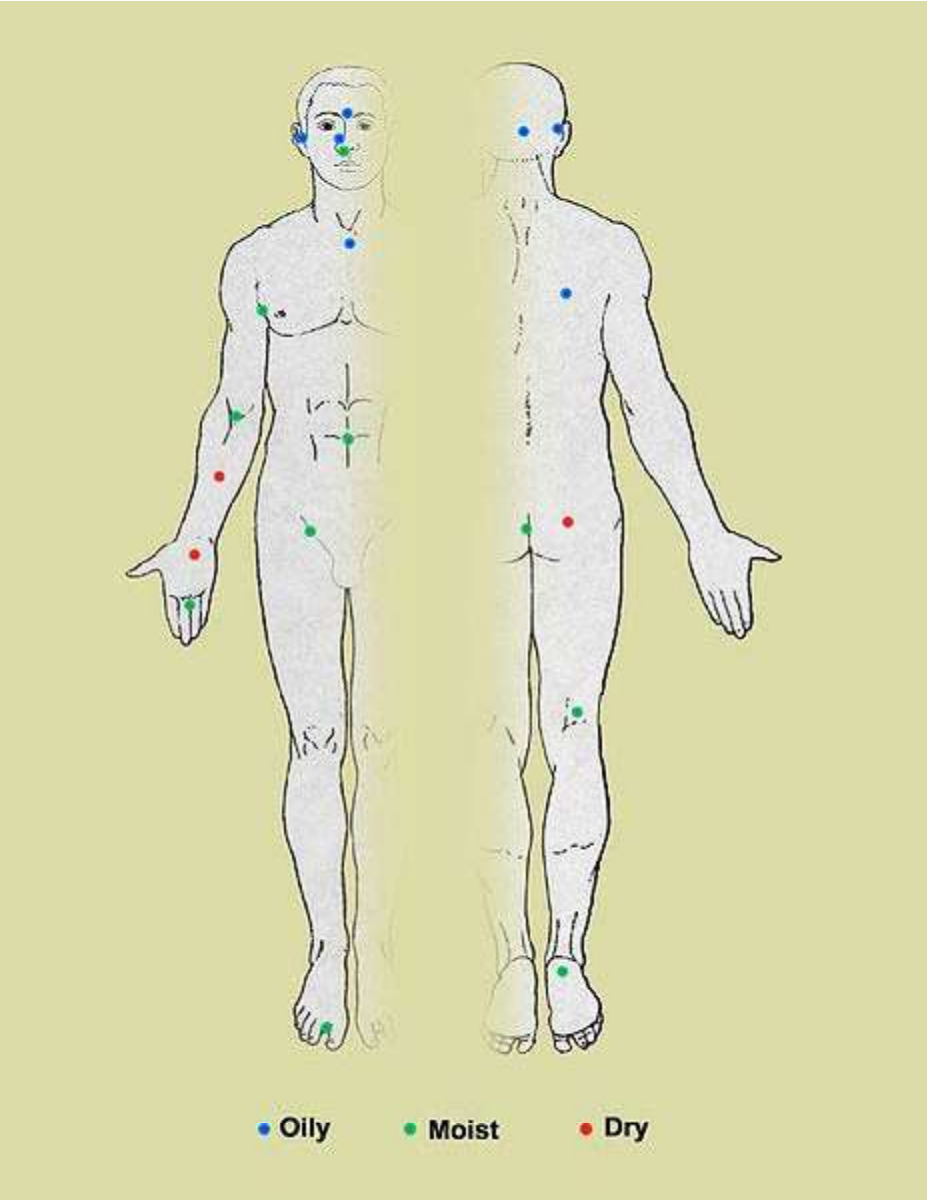
Bacteria



Scanning electron microscope image of *Staphylococcus epidermidis* one of roughly a thousand bacteria species present on human skin. Though usually not pathogenic, it can cause skin infections and even life threatening illnesses in those that are immunocompromised.

The estimate of the number of species present on skin bacteria has been radically changed by the use of 16S ribosomal RNA to identify bacterial species present on skin samples direct from their genetic material. Previously such identification had depended upon microbiological culture upon which many varieties of bacteria did not grow and so were hidden to science.

Staphylococcus epidermidis and *Staphylococcus aureus* were thought from cultural based research to be dominant. However 16S ribosomal RNA research finds that while common, these species make up only 5% of skin bacteria. However, skin variety provides a rich and diverse habit for bacteria. Most come from four phyla: Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%), and Bacteroidetes (6.3%).



Ecology of the 20 sites on the skin studied in the Human Microbiome {Project

There are three main ecological areas: moist, dry and sebaceous. *Propionibacteria* and *Staphylococci* species were the main species in sebaceous areas. In moist places on the body *Corynebacteria* together with *Staphylococci* dominate. In dry areas, there is a mixture of species but b-Proteobacteria and Flavobacteriales are dominant. Ecologically, sebaceous areas had greater species richness than moist and dry one. The areas with least similarity between people in species were the spaces between fingers, the spaces between toes, axillae, and umbilical cord stump. Most similarly were beside the nostril, nares (inside the nostril), and on the back.

Frequency of the best studied skin microbes

Organism	observations
Staphylococcus epidermidis	Common, occasionally pathogenic
Staphylococcus aureus	Infrequent, usually pathogenic
Staphylococcus warneri	Infrequent, occasionally pathogenic
Streptococcus pyogenes	Infrequent, usually pathogenic
Streptococcus mitis	Frequent, occasionally pathogenic
Propionibacterium acnes	Frequent, occasionally pathogenic
Corynebacterium spp.	Frequent, occasionally pathogenic
Acinetobacter johnsonii	Frequent, occasionally pathogenic
Pseudomonas aeruginosa	Infrequent, occasionally pathogenic

Fungal

A study of the area between toes in 100 young adults found 14 different genera of fungi. These include yeasts such as *Candida albicans*, *Rhodotorula rubra*, *Torulopsis* and *Trichosporon cutaneum*, dermatophytes (skin living fungi) such as *Microsporum gypseum*, and *Trichophyton rubrum* and nondermatophyte fungi (opportunistic fungi that can live in skin) such as *Rhizopus stolonifer*, *Trichosporon cutaneum*, *Fusarium*, *Scopulariopsis brevicaulis*, *Curvularia*, *Alternaria alternata*, *Paecilomyces*, *Aspergillus flavus* and *Penicillium* species.

Relationship to host

Skin microflora can be commensals, mutualistic or pathogens. Often they can be all three depending upon the strength of the person's immune system. Research upon the immune system in the gut and lungs has shown that microflora aids immunity development: however such research has only started upon whether this is the case with the skin. *Pseudomonas aeruginosa* is an example of a mutualistic bacterium that can turn into a pathogen and cause disease: if it gains entry into the blood system it can result in inflections in bone, joint, gastrointestinal, and respiratory systems. It can also cause dermatitis. However, Pseudomonas aeruginosa produces antimicrobial substances such as pseudomonic acid that are exploited commercially such as Mupirocin. This works against

staphylococcal and streptococcal infections. Pseudomonas aeruginosa also produces substances that inhibit the growth of fungus species such as *Candida krusei*, *Candida albicans*, *Torulopsis glabrata*, *Saccharomyces cerevisiae* and *Aspergillus fumigatus*. It can also inhibit the growth of *Helicobacter pylori*. So important is its antimicrobial actions that it has been noted that "removing *P. aeruginosa* from the skin, through use of oral or topical antibiotics, may inversely allow for aberrant yeast colonization and infection."

Another aspect of bacteria is the generation of body odor. Sweat is odorless but *Propionibacteria* in adolescent and adult sebaceous glands can turn its amino acids into propionic acid. *Staphylococcus epidermidis* create the other source of body odor: isovaleric acid (3-methyl butanoic acid). In addition to these, strong foot odor is due to *Bacillus subtilis*.

Skin defenses

Antimicrobial peptides

The skin creates antimicrobial peptides such as cathelicidins that control the proliferation of skin microbes. Cathelicidins not only reduce microbe numbers directly but also cause the secretion of cytokine release which induces inflammation, angiogenesis, and reepithelialization. Conditions such as atopic dermatitis have been linked to the suppression in cathelicidin production. In rosacea abnormal processing of cathelicidin cause inflammation. Psoriasis has been linked to self-DNA created from cathelicidin peptides that causes autoinflammation. A major factor controlling cathelicidin is vitamin D_3.

Acidity

The superficial layers of the skin are naturally acidic (pH 4-4.5) due to lactic acid in sweat and produced by skin bacteria. At this pH mutualistic flora such as *Staphylococci*, *Micrococci*, *Corynebacterium* and *Propionibacteria* grow but not transient bacteria such as Gram negative bacteria like *Escherichia* and *Pseudomonas* or Gram positive ones such as *Staphylococcus aureus* or *Candida albicans*. Another factor effecting the growth of pathological bacteria is that the antimicrobial substances secreted by the skin are enhanced in acidic conditions. In alkaline conditions, bacteria cease to be attached to the skin and are more readily shed. It has been observed that the skin also swells under alkaline conditions and opens up allowing move to the surface.

Immune system

If activated, the immune system in the skin produces cell-mediated immunity against microbes such as dermatophytes (skin fungi). One reaction is to increase stratum corneum turnover and so shed the fungus from the skin surface. Skin fungi such as *Trichophyton rubrum* have evolved to create substances that limit the immune response

to them. The shedding of skin is a general means to control the build up flora upon the skin surface.

Clinical

Skin diseases

Microorganisms play a role in noninfectious skin diseases such as atopic dermatitis, rosacea, psoriasis, and acne Damaged skin can cause nonpathogenic bacteria to become pathogenic.

Infected devices

Skin microbes are source of infected medical devices such as catheters.

Hygiene

Contagion

Skin flora do not readily pass between people: 30 seconds of moderate friction and dry hand contact results in a transfer of only 0.07% of natural hand flora from naked with a greater percentage from gloves.

Removal

The most effective (60 to 80% reduction) antimicrobial washing is with ethanol, isopropanol, and n-propanol. Viruses are most affected by high (95%) concentrations of ethanol, while bacteria are more affected by n-propanol.

Unmedicated soaps is not very effective as illustrated by the following data. Health care workers washed their hands once in nonmedicated liquid soap for 30 seconds. The students/technicians for 20 times.

Skin flora upon two hospital groups in colony-forming units per mL.

group and hand skin condition	unwashed	washed
Health care workers healthy	3.47	3.15
Health care workers damaged	3.33	3.29
Students/technicians healthy	4.39	3.54
Students/technicians damaged	4.58	4.43

An important use of hand washing is to prevent the transmission of antibiotic resistant skin flora that cause hospital-acquired infections such as Methicillin-resistant *Staphylococcus aureus*. While such flora have become antibiotic resistant to due

antibiotics there is no evidence that recommended antiseptics or disinfectants selects for antibiotic-resistant organisms when used in hand washing. However, many strains of organisms are resistant to some of the substances used in antibacterial soaps such as Triclosan.

One survey of bar soaps in dentist clinics found they all had their own flora and on average from two to five different genera of microorganisms with those used most more likely to have more species varieties. Another survey of bar soaps in public toilets found even more flora. Another study found that very dry soaps are not infected while all are that rest in pools of water. However, research upon soap that was specially infected found that soap flora do not transmit to the hands.

Damaged skin

Washing skin repeatedly can damage the protective external layer and cause transepidermal loss of water. This can be seen in roughness characterized by scaling and dryness, itchiness, dermatitis provoked by microorganisms and allergens penetrating the corneal layer and redness. Wearing gloves can cause further problems since it produces a humid environment favoring the growth of microbes and also contains irritants such as latex and talcum powder.

Hand washing can damage skin because the stratum corneum top layer of skin consists of 15 to 20 layers of keratin disks, corneocytes, each of which is each surrounded by a thin film of skin lipids which can be removed by alcohols and detergents.

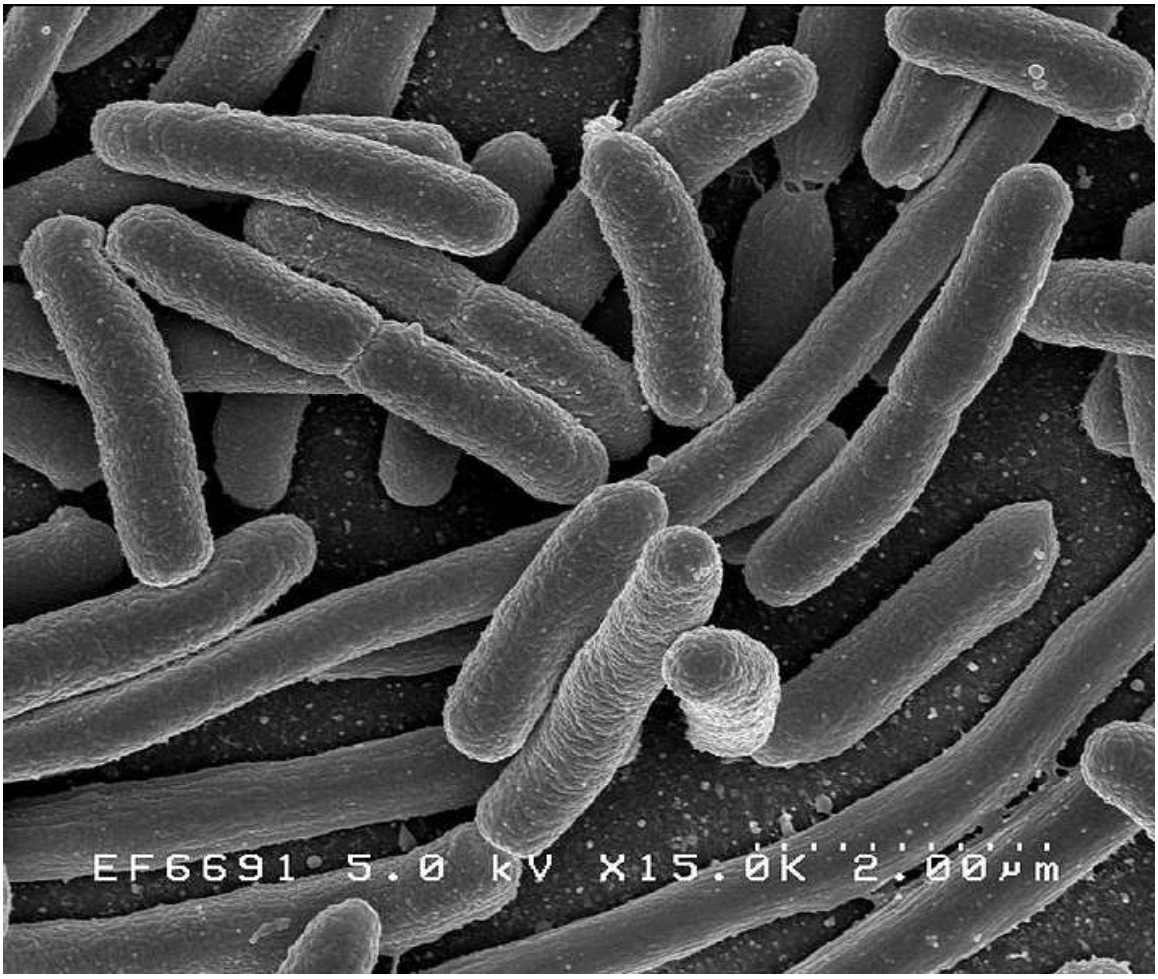
Damaged skin defined by extensive cracking of skin surface, widespread reddening or occasional bleeding has also been found to be more frequently colonized by *Staphylococcus hominis* and these were more likely to methicillin resistant. Though not related to greater antibiotic resistance, damaged skin was also more like to be colonized by *Staphylococcus aureus*, gram-negative bacteria, *Enterococci* and *Candida*.

Comparison with other flora

The skin flora is different from that of the gut which is predominately Firmicutes and Bacteroidetes. There is also low level of variation between people that is not found in gut studies. Both gut and skin flora however lack the diversity found in soil flora.

Chapter 6

Gut Flora



Escherichia coli, one of the many species of bacteria present in the human gut

Gut flora consists of microorganisms that live in the digestive tracts of animals and is the largest reservoir of human flora. *Gut* (the adjective) is synonymous with intestinal, and *flora* with microbiota and microflora.

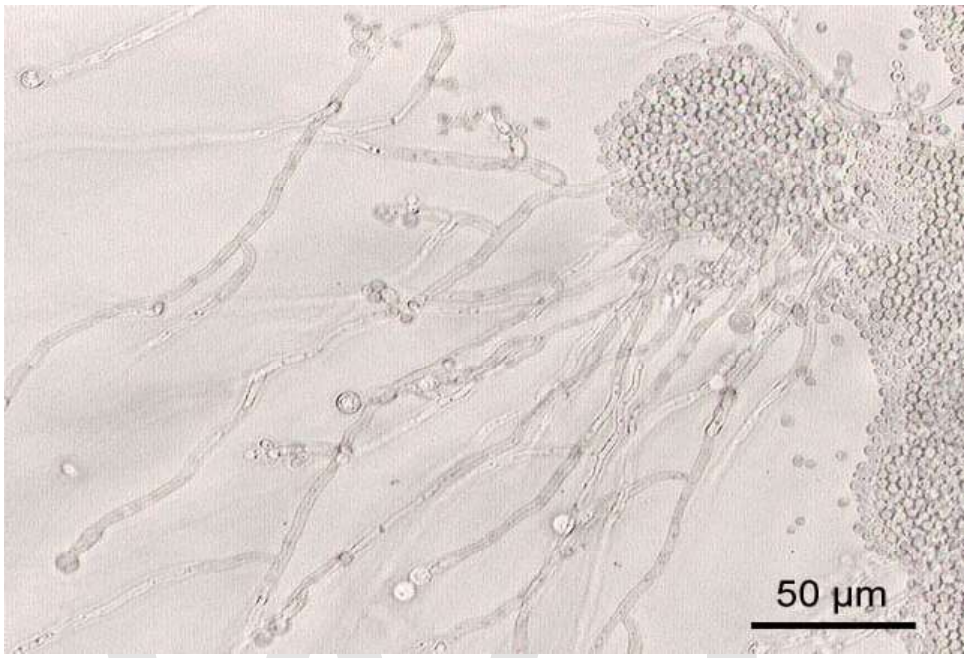
The human body, consisting of about 100 trillion cells, carries about ten times as many microorganisms in the intestines. The metabolic activities performed by these bacteria resemble those of an organ, leading some to liken gut bacteria to a "forgotten" organ. It is estimated that these gut flora have around 100 times as many genes in aggregate as there are in the human genome.

Bacteria make up most of the flora in the colon and up to 60% of the dry mass of feces. Somewhere between 300 and 1000 different species live in the gut, with most estimates at about 500. However, it is probable that 99% of the bacteria come from about 30 or 40 species. Fungi and protozoa also make up a part of the gut flora, but little is known about their activities.

Research suggests that the relationship between gut flora and humans is not merely commensal (a non-harmful coexistence), but rather a symbiotic relationship. Though people can survive without gut flora, the microorganisms perform a host of useful functions, such as fermenting unused energy substrates, training the immune system, preventing growth of harmful, pathogenic bacteria, regulating the development of the gut, producing vitamins for the host (such as biotin and vitamin K), and producing hormones to direct the host to store fats. However, in certain conditions, some species are thought to be capable of causing disease by producing infection or increasing cancer risk for the host.

Over 99% of the bacteria in the gut are anaerobes, but in the cecum, aerobic bacteria reach high densities.

Types



Candida albicans, a dimorphic fungus that grows as a yeast in the gut

Not all the species in the gut have been identified because most cannot be cultured, and identification is difficult. Populations of species vary widely among different individuals but stay fairly constant within an individual over time, even though some alterations may occur with changes in lifestyle, diet and age. An effort to describe the microflora of the gut and other body locations better has been initiated. In 2009, scientists from INRA (France) highlighted the existence of a small number of species shared by all individuals constituting the human intestinal microbiota phylogenetic core.

Most bacteria belong to the genera *Bacteroides*, *Clostridium*, *Fusobacterium*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, and *Bifidobacterium*. Other genera, such as *Escherichia* and *Lactobacillus*, are present to a lesser extent. Species from the genus *Bacteroides* alone constitute about 30% of all bacteria in the gut, suggesting that this genus is especially important in the functioning of the host.

The currently known genera of fungi of the gut flora include *Candida*, *Saccharomyces*, *Aspergillus*, and *Penicillium*.

Acquisition of gut flora in human infants

The gastrointestinal tract of a normal fetus is sterile. During birth and rapidly thereafter, bacteria from the mother and the surrounding environment colonize the infant's gut. Immediately after vaginal delivery, babies may have bacterial strains derived from the mothers' feces in the upper gastrointestinal tract. Infants born by caesarean section may

also be exposed to their mothers' microflora, but the initial exposure is most likely to be from the surrounding environment such as the air, other infants, and the nursing staff, which serve as vectors for transfer. The primary gut flora in infants born by caesarean delivery may be disturbed for up to six months after birth, whereas vaginally born infants take up to one month for their intestinal microflora to be well established. After birth, environmental, oral and cutaneous bacteria are readily transferred from the mother to the infant through suckling, kissing, and caressing. All infants are initially colonized by large numbers of *E. coli* and streptococci. Within a few days, bacterial numbers reach 10^8 to 10^{10} per gram of feces. During the first week of life, these bacteria create a reducing environment favorable for the subsequent bacterial succession of strict anaerobic species mainly belonging to the genera *Bifidobacterium*, *Bacteroides*, *Clostridium*, and *Ruminococcus*. Breast-fed babies become dominated by bifidobacteria, possibly due to the contents of bifidobacterial growth factors in breast milk. In contrast, the microbiota of formula-fed infants is more diverse, with high numbers of *Enterobacteriaceae*, enterococci, bifidobacteria, *Bacteroides*, and clostridia. After the introduction of solid food and weaning, the microflora of breast-fed infants become similar to that of formula-fed infants. By the second year of life, the fecal microflora resemble that of adults.

Functions

Bacteria in the gut fulfill a host of useful functions for humans, including digestion of unutilized energy substrates, stimulating cell growth, repressing the growth of harmful microorganisms, training the immune system to respond only to pathogens, and defending against some diseases.

Carbohydrate fermentation and absorption

Without gut flora, the human body would be unable to utilize some of the undigested carbohydrates it consumes, because some types of gut flora have enzymes that human cells lack for breaking down certain polysaccharides. Rodents raised in a sterile environment and lacking in gut flora need to eat 30% more calories just to remain the same weight as their normal counterparts. Carbohydrates that humans cannot digest without bacterial help include certain starches, fiber, oligosaccharides and sugars that the body failed to digest and absorb like lactose in the case of lactose intolerance and sugar alcohols, mucus produced by the gut, and proteins. A further result is flatulence, specifically the metabolism of oligosaccharides (notably from beans) by *Methanobrevibacter smithii*.

Bacteria turn carbohydrates they ferment into short chain fatty acids, or SCFAs, by a form of fermentation called saccharolytic fermentation. Products include acetic acid, propionic acid and butyric acid. These materials can be used by host cells, providing a major source of useful energy and nutrients for humans, as well as helping the body to absorb essential dietary minerals such as calcium, magnesium and iron. Gases and organic acids, such as lactic acid, are also produced by saccharolytic fermentation. Acetic acid is used by muscle, propionic acid helps the liver produce ATP, and butyric acid provides energy to gut cells and may prevent cancer. Evidence also indicates that bacteria

enhance the absorption and storage of lipids and produce and then facilitate the body to absorb needed vitamins like vitamin K.

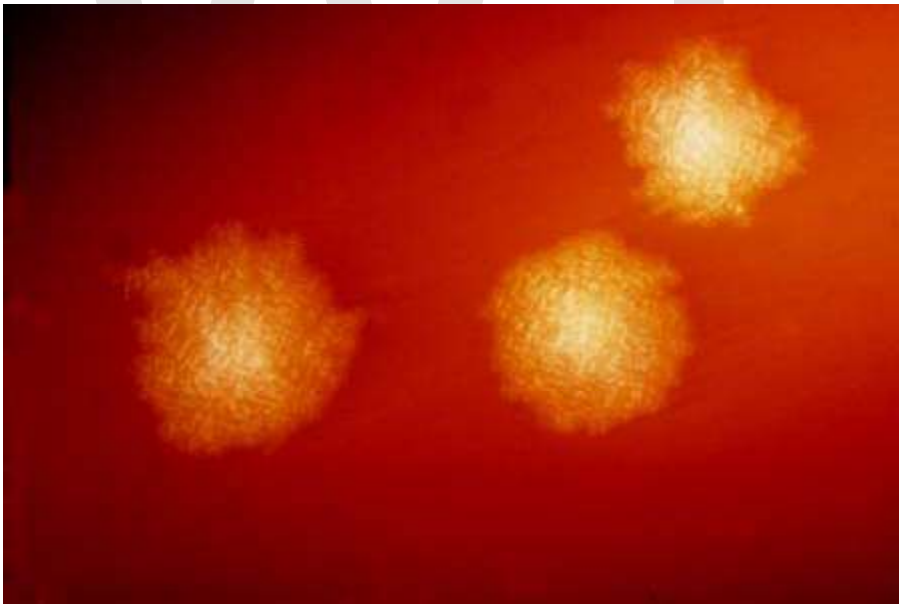
Another, less favorable type of fermentation, proteolytic fermentation, breaks down proteins like enzymes, dead hosts and bacterial cells, and collagen and elastin found in food, and can produce toxins and carcinogens in addition to SCFAs. Thus, a diet lower in protein reduces exposure to toxins.

Beneficial flora increase the gut's absorption of water, reduce counts of damaging bacteria, increase growth of human gut cells, and stimulate growth of indigenous bacteria.

Trophic effects

Another benefit of SCFAs is that they increase growth of intestinal epithelial cells and control their proliferation and differentiation. They may also cause lymphoid tissue near the gut to grow. Bacterial cells also alter intestinal growth by changing the expression of cell surface proteins such as sodium/glucose transporters. In addition, changes they make to cells may prevent injury to the gut mucosa from occurring.

Repression of pathogenic microbial growth



C. difficile colonies on a blood agar plate. The overgrowth of *C. difficile* in the gut can be harmful to the host.

Another important role of helpful gut flora is that they prevent species that would harm the host from colonizing the gut, an activity termed the "barrier effect". Harmful yeasts and bacterial species such as *Clostridium difficile* (the overgrowth of which can cause pseudomembranous colitis) are unable to grow excessively due to competition from

helpful gut flora species adhering to the mucosal lining of the intestine, thus animals without gut flora are infected very easily. The barrier effect protects humans from both invading species and species normally present in the gut at low numbers, whose growth is usually inhibited by the gut flora.

Helpful bacteria prevent the growth of pathogenic species by competing for nutrition and attachment sites to the epithelium of the colon. Symbiotic bacteria are more at home in this ecological niche and are thus more successful in the competition. Indigenous gut floras also produce bacteriocins, which are proteinaceous toxins that inhibit growth of similar bacterial strains, substances that kill harmful microbes and the levels of which can be regulated by enzymes produced by the host.

The process of fermentation, since it produces lactic acid and different fatty acids, also serves to lower the pH in the colon, preventing the proliferation of harmful species of bacteria and facilitating that of helpful species. The pH may also enhance the excretion of carcinogens.

Immunity

Gut flora have a continuous and dynamic effect on the host's gut and systemic immune systems. The bacteria are key in promoting the early development of the gut's mucosal immune system both in terms of its physical components and function and continue to play a role later in life in its operation. The bacteria stimulate the lymphoid tissue associated with the gut mucosa to produce antibodies to pathogens. The immune system recognizes and fights harmful bacteria, but leaves the helpful species alone, a tolerance developed in infancy.

As soon as an infant is born, bacteria begin colonizing its digestive tract. The first bacteria to settle in are able to affect the immune response, making it more favorable to their own survival and less so to competing species; thus the first bacteria to colonize the gut are important in determining the person's lifelong gut flora makeup. However, there is a shift at the time of weaning from predominantly facultative aerobic species, such as *Streptococci* and *Escherichia coli*, to mostly obligate anaerobic species.

Recent findings have shown that gut bacteria play a role in the expression of toll-like receptors (TLRs) in the intestines, molecules that help the host repair damage due to injury. TLRs cause parts of the immune system to repair injury caused, for example, by radiation. TLRs are one of the two classes of pattern-recognition receptors (PRR) that provide the intestine the ability to discriminate between the pathogenic and commensal bacteria. These PRRs identify the pathogens that have crossed the mucosal barriers and trigger a set of responses that take action against the pathogen, which involve three main immunosensory cells: surface enterocytes, M cells and dendritic cells.

The other class of PRRs are known as the nucleotide-binding oligomerization domain/caspase recruitment domain isoforms (NOD/CARD), which are cytoplasmic proteins that recognize endogenous or microbial molecules or stress responses and forms

oligomers that activate inflammatory caspases. This would result in the cleavage and activation of important inflammatory cytokines and/or activate NF-κB signaling pathway to induce the production of inflammatory molecules.

Bacteria can influence the phenomenon known as oral tolerance, in which the immune system is less sensitive to an antigen (including those produced by gut bacteria) once it has been ingested. This tolerance, mediated in part by the gastrointestinal immune system and in part by the liver, can reduce an overreactive immune response like those found in allergies and auto-immune disease.

Some species of gut flora, such as some of those in the *Bacteroides* genus, are able to change their surface receptors to mimic those of host cells in order to evade immune response. Bacteria with neutral and harmful effects on the host can also use these types of strategies. The host immune system has also adapted to this activity, preventing overgrowth of harmful species.

Metabolic function

The resident gut microflora positively control the intestinal epithelial cell differentiation and proliferation through the production of short-chain fatty acids. They also mediate other metabolic effects such as the syntheses of vitamins like biotin and folate, as well as absorption of ions including magnesium, calcium and iron.

The gut flora plays a major role in metabolizing dietary carcinogens, the microcomponents and the macrocomponents. The microcomponents are genotoxic, and the major focus is on recent advances in heterocyclic amines (HCAs), which are produced by cooking proteinaceous food, such as meat and fish, which can then induce tumors in organs like the breast, colon and prostate. HCAs are naturally occurring; therefore, the complete avoidance of them is impractical, which is why the metabolic function of gut flora of such components is of great importance to our body, as this would help in prevention of such tumors that are difficult to avoid. The macrocomponents consists of the excessive intake of fat and sodium chloride, which can later promote tumors, such as in breasts and colons, from fat and gastric carcinogenesis from sodium chloride.

Preventing allergy

Bacteria are also implicated in preventing allergies, an overreaction of the immune system to non-harmful antigens. Studies on the gut flora of infants and young children have shown that those who have or later develop allergies have different compositions of gut flora from those without allergies, with higher chances of having the harmful species *C. difficile* and *S. aureus* and lower prevalence of *Bacteroides* and *Bifidobacteria*. One explanation is that since helpful gut flora stimulate the immune system and "train" it to respond properly to antigens, a lack of these bacteria in early life leads to an inadequately trained immune system that overreacts to antigens. On the other hand, the differences in flora could be a result, not a cause, of the allergies.

Preventing inflammatory bowel disease

Another indicator that bacteria help train the immune system is the epidemiology of Inflammatory Bowel Disease, or IBD, such as Crohn's Disease (CD). Some authors suggest that SCFAs prevent IBD. In addition, some forms of bacteria can prevent inflammation. The incidence and prevalence of IBD is high in industrialized countries with a high standard of living and low in less economically developed countries, having increased in developed countries throughout the twentieth century. The disease is also linked to good hygiene in youth; lack of breastfeeding; and consumption of large amounts of sucrose and animal fat. Its incidence is inversely linked with poor sanitation during the first years of life and consumption of fruits, vegetables, and unprocessed foods. Also, the use of antibiotics, which kill native gut flora and harmful infectious pathogens alike, especially during childhood, is associated with inflammatory bowel disease. On the other hand, using probiotics, bacteria consumed as part of the diet that impart health benefits (aside from just nutrition), helps treat IBD.

Alterations in flora balance

Effects of antibiotic use

Altering the numbers of gut bacteria, for example by taking broad-spectrum antibiotics, may affect the host's health and ability to digest food. People may take the drugs to cure bacterial illnesses or may unintentionally consume significant amounts of antibiotics by eating the meat of animals to which they were fed. Antibiotics can cause antibiotic-associated diarrhea (AAD) by irritating the bowel directly, changing the levels of gut flora, or allowing pathogenic bacteria to grow. Another harmful effect of antibiotics is the increase in numbers of antibiotic-resistant bacteria found after their use, which, when they invade the host, cause illnesses that are difficult to treat with antibiotics.

Changing the numbers and species of gut flora can reduce the body's ability to ferment carbohydrates and metabolize bile acids and may cause diarrhea. Carbohydrates that are not broken down may absorb too much water and cause runny stools, or lack of SCFAs produced by gut flora could cause the diarrhea.

A reduction in levels of native bacterial species also disrupts their ability to inhibit the growth of harmful species such as *C. difficile* and *Salmonella kedougou*, and these species can get out of hand, though their overgrowth may be incidental and not be the true cause of diarrhea.

Gut flora composition also changes in severe illnesses, due not only to antibiotic use but also to such factors as ischemia of the gut, failure to eat, and immune compromise. Negative effects from this have led to interest in selective digestive tract decontamination (SDD), a treatment to kill only pathogenic bacteria and allow the re-establishment of healthy ones.

Pharmabiotics

Pharmabiotics is a generic term to encompass any form of therapeutic exploitation of the commensal flora, including the use of live probiotic bacteria, probiotic-derived biologically active metabolites, prebiotics, synbiotics or genetically modified commensal bacteria. Since the lack of gut flora can have such harmful health effects, the use of probiotics has anti-inflammatory effects in the gut and may be useful for improving health. Prebiotics are dietary components that can help foster the growth of micro-organisms in the gut, which may lead to better health. There is evidence supporting a therapeutic role for probiotic strategies for treating mucosal inflammatory disorders such as IBD, atopy, infection, diarrhoea, cancer and arthritis.

Role in disease

Bacteria in the digestive tract have pathogenic properties in addition to their health-inducing ones: they can produce toxins and carcinogens and have been implicated in such conditions as multisystem organ failure, sepsis, colon cancer, and IBD. A major factor in health is the balance of bacterial numbers; if the numbers grow too high or low, it will result in harm to the host. The host has enzymes to regulate this balance.

Cancer

Some genera of bacteria, such as *Bacteroides* and *Clostridium*, have been associated with an increase in tumor growth rate, while other genera, such as *Lactobacillus* and *Bifidobacteria*, are known to prevent tumor formation.

Translocation

Helpful bacteria can be very harmful to the host if they get outside of the intestinal tract. Translocation, which occurs when bacteria leave the gut through its mucosal lining, the border between the lumen of the gut and the inside of the body, can occur in a number of different diseases. It can be caused by too much growth of bacteria in the small intestine, reduced immunity of the host, or increased gut lining permeability. The gut can become more permeable in diseases like cirrhosis, which is damaging due in part to the activity of gut flora.

If the gut is perforated, bacteria can invade the body, causing a potentially fatal infection. Aerobic bacteria can make an infection worse by using up all available oxygen and creating an environment favorable to anaerobes.

Inflammatory bowel disease

Some suspect that IBD is due to a reduction in immune tolerance and subsequent overreaction of the host's immune system to harmful or non-harmful bacteria. IBD may be caused by the entire gut flora together or some specific types.

It has been noted that though Ulcerative Colitis and Crohn's disease (two types of IBD) probably have genetic components, they are not inherited in a Mendelian fashion and are thus probably due to a complex set of factors rather than solely to a gene. Though neither bacterial colonization nor genetics is sufficient to cause the disease, bacteria probably play a role in these disorders.

Some suspect that inflammation in IBD is due to increased permeability of the inner lining of the colon, which may allow bacteria to invade the tissues and cause an immune reaction that leads to prolonged inflammation. Tissue damage in IBD results from the immunological misperception of danger within the naturally occurring flora or due to failure of normal tolerance to pathogenic bacteria. It is still unclear whether the inflammation that occurs is due to a specific subset of intestinal microbes or due to a problem with the tolerance of commensal gut flora. Abnormal tight junctions, which are supposed to prevent permeability, have been found in cells of patients with IBD. Because of the potentially harmful role of these bacteria, antibiotics are frequently prescribed to treat Crohn’s disease. However, inflammation could occur first and cause the increased intestinal permeability found in diseases such as Crohn's, so the causative role of bacteria is not clear. Conventional therapies for IBD primarily target the mucosal inflammatory responses by using pharmabiotics.

Colitis

It has been suggested that commensal bacteria are responsible for the development of colitis, since mice raised in a sterile environment do not get the disease. However, while some bacterial strains such as *C. difficile* and even normal gut bacteria cause colitis, others prevent the disease in mice.

Obesity

It is known from experiments on mice that obese mice lacking leptin, a lipid metabolism regulator (ob/ob mice), have a distinct gut flora compared to (normal) lean mice, reflected in a change in the ratio between bacteria from the divisions Bacteroidetes and Firmicutes, which is shifted towards fewer Bacteroidetes and more Firmicutes in obese mice.

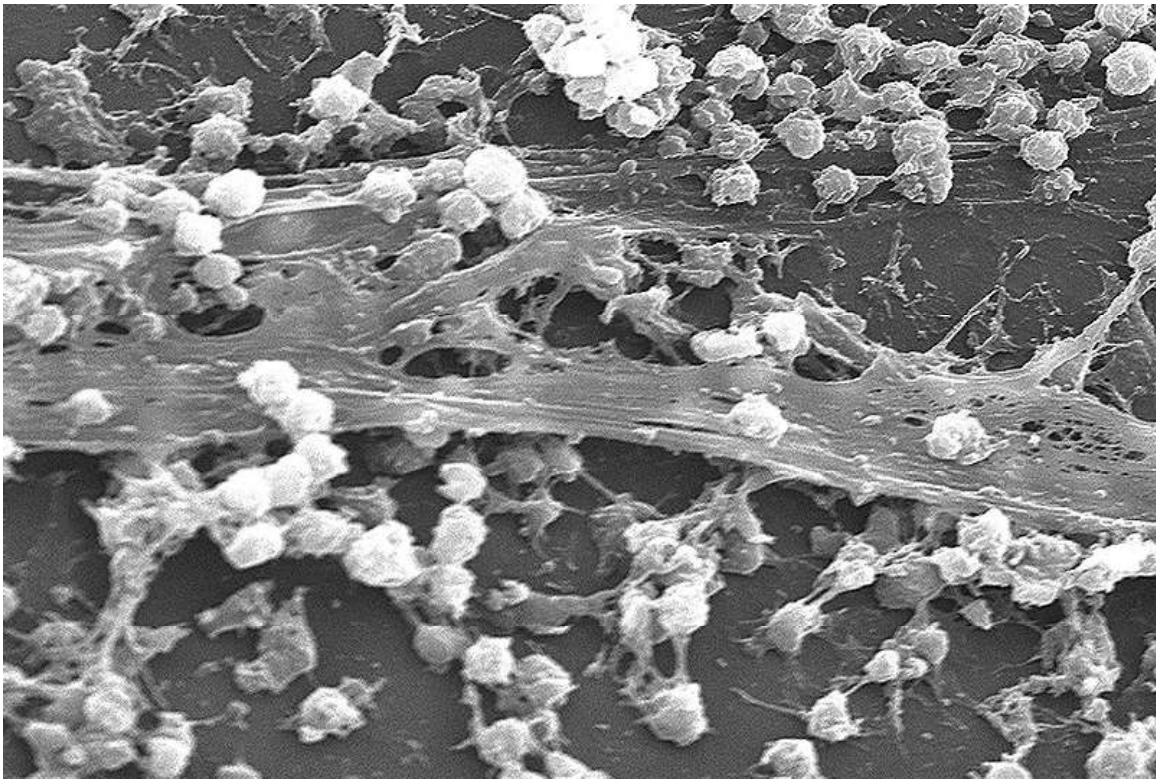
The microbes occupying the human gut are also in direct relation to obesity. A shift in the ratio between bacterial divisions Firmicutes and Bacteroidetes can be observed in lean and obese individuals—in the latter, a shift towards Firmicutes can be observed. The ratio between Firmicutes and Bacteroidetes dynamically reflects the overall weight condition of an individual, shifting towards Bacteroidetes if an obese individual loses weight.

The mutual influence of gut flora composition and weight condition is connected to differences in the energy-reabsorbing potential of different ratios of Firmicutes and Bacteroidetes, especially in the digestion of fatty acids and dietary polysaccharides, as shown by experiments wherein the (caecum) gut flora of obese mice were transplanted

into germ-free recipient mice, leading to an increase in weight despite a decrease in food consumption.

Chapter 7

Biofilm



Staphylococcus aureus biofilm on an indwelling catheter

A **biofilm** is an aggregate of microorganisms in which cells adhere to each other and/or to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS, which is also referred to as *slime* (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are

physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.

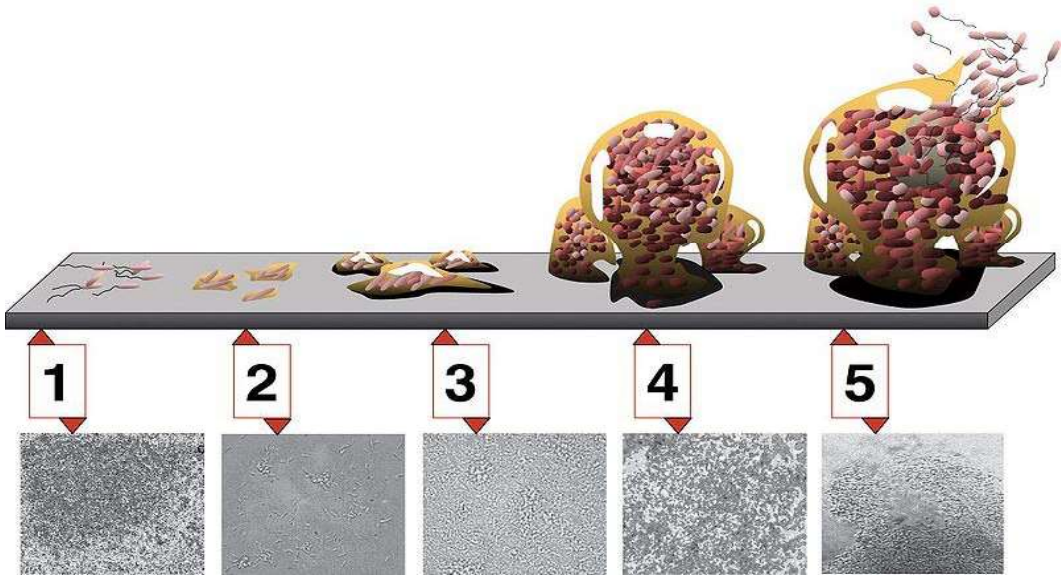
Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or in some cases, by exposure of planktonic cells to sub-inhibitory concentrations of antibiotics. When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated.

Formation

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible adhesion via van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.

The first colonists facilitate the arrival of other cells by providing more diverse adhesion sites and beginning to build the matrix that holds the biofilm together. Some species are not able to attach to a surface on their own but are often able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able to communicate via quorum sensing using such products as AHL. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. The final stage of biofilm formation is known as development, and is the stage in which the biofilm is established and may only change in shape and size. The development of a biofilm may allow for an aggregate cell colony (or colonies) to be increasingly antibiotic resistant.

Development



Five stages of biofilm development. Each stage of development in the diagram is paired with a photomicrograph of a developing *P. aeruginosa* biofilm. All photomicrographs are shown to same scale.

There are five stages of biofilm development:

1. Initial attachment
2. Irreversible attachment
3. Maturation I
4. Maturation II
5. Dispersion

Dispersal

Dispersal of cells from the biofilm colony is an essential stage of the biofilm lifecycle. Dispersal enables biofilms to spread and colonize new surfaces. Enzymes that degrade the biofilm extracellular matrix, such as dispersin B and deoxyribonuclease, may play a role in biofilm dispersal. Biofilm matrix degrading enzymes may be useful as anti-biofilm agents. Recent evidence has shown that a fatty acid messenger, cis-2-decenoic acid, is capable of inducing dispersion and inhibiting growth of biofilm colonies. Secreted by *Pseudomonas aeruginosa*, this compound induces dispersion in several species of bacteria and the yeast *Candida albicans*

Properties

Biofilms are usually found on solid substrates submerged in or exposed to an aqueous solution, although they can form as floating mats on liquid surfaces and also on the surface of leaves, particularly in high humidity climates. Given sufficient resources for growth, a biofilm will quickly grow to be macroscopic. Biofilms can contain many different types of microorganism, e.g. bacteria, archaea, protozoa, fungi and algae; each group performing specialized metabolic functions. However, some organisms will form monospecies films under certain conditions.

Extracellular matrix

The biofilm is held together and protected by a matrix of excreted polymeric compounds called EPS. EPS is an abbreviation for either extracellular polymeric substance or exopolysaccharide. This matrix protects the cells within it and facilitates communication among them through biochemical signals. Some biofilms have been found to contain water channels that help distribute nutrients and signalling molecules. This matrix is strong enough that under certain conditions, biofilms can become fossilized.

Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and

the outer layer of cells protect the interior of the community. In some cases antibiotic resistance can be increased a thousandfold. Lateral gene transfer is greatly facilitated in biofilms and leads to a more stable biofilm structure.

The concept that biofilms are more resistant to antimicrobials is not completely accurate. For instance the biofilm form of *Pseudomonas aeruginosa* has no greater resistance to antimicrobials, when compared to stationary phase planktonic cells, although, when the biofilm is compared to logarithmic phase planktonic cells, the biofilm does have greater resistance to antimicrobials. This resistance to antibiotics in both stationary phase cells and biofilms may be due to the presence of persister cells.

Examples

Biofilm in Yellowstone National Park. Longest raised mat area is about half a meter long.

Biofilms are ubiquitous. Nearly every species of microorganism, not only bacteria and archaea, have mechanisms by which they can adhere to surfaces and to each other. Biofilms will form on virtually every non-shedding surface in a non-sterile aqueous environment.

- Biofilms can be found on rocks and pebbles at the bottom of most streams or rivers and often form on the surface of stagnant pools of water. In fact, biofilms are important components of food chains in rivers and streams and are grazed by the aquatic invertebrates upon which many fish feed.

- Biofilms can grow in the most extreme environments: from, for example, the extremely hot, briny waters of hot springs ranging from very acidic to very alkaline, to frozen glaciers.

- In the human environment, biofilms can grow in showers very easily since they provide a moist and warm environment for the biofilm to thrive. Biofilms can form inside water and sewage pipes and cause clogging and corrosion. Biofilms on floors and counters can make sanitation difficult in food preparation areas.

- Biofilms in cooling- or heating-water systems are known to reduce heat transfer.

- Biofilms in marine engineering systems, such as pipelines of the offshore oil and gas industry, can lead to substantial corrosion problems. Corrosion is mainly due to abiotic factors; however, at least 20% is caused by microorganisms that are attached to the metal subsurface (i.e., microbially-influenced corrosion).

- Bacterial adhesion to boat hulls serves as the foundation for biofouling of seagoing vessels. Once a film of bacteria forms, it is easier for other marine organisms such as barnacles to attach. Such fouling can reduce maximum vessel speed by up to 20%, prolonging voyages and consuming fuel. Time in dry dock for refitting and repainting reduces the productivity of shipping assets, and the useful life of ships is also reduced due to corrosion and mechanical removal (scraping) of marine organisms from ships' hulls.

- Biofilms can also be harnessed for constructive purposes. For example, many sewage treatment plants include a treatment stage in which waste water passes over biofilms grown on filters, which extract and digest organic compounds. In such biofilms, bacteria are mainly responsible for removal of organic matter (BOD), while protozoa and rotifers are mainly responsible for removal of suspended solids (SS), including pathogens and other microorganisms. Slow sand filters rely on biofilm development in the same way to filter surface water from lake, spring or river sources for drinking purposes. What we regard as clean water is a waste material to these microcellular organisms since they are unable to extract any further nutrition from the purified water.

- Biofilms can help eliminate petroleum oil from contaminated oceans or marine systems. The oil is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently-discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB).

- Stromatolites are layered accretionary structures formed in shallow water by the trapping, binding and cementation of sedimentary grains by microbial biofilms, especially of cyanobacteria. Stromatolites include some of the most ancient records of life on Earth, and are still forming today.

- Biofilms are present on the teeth of most animals as dental plaque, where they may cause tooth decay and gum disease.

- Biofilms are found on the surface of and inside plants. They can either contribute to crop disease or, as in the case of nitrogen-fixing Rhizobium on roots, exist symbiotically with the plant. Examples of crop diseases related to biofilms include Citrus Canker, Pierce's Disease of grapes, and Bacterial Spot of plants such as peppers and tomatoes.

Biofilms and infectious diseases

Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections. Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle-ear infections, formation of dental plaque, gingivitis, coating contact lenses, and less common but more lethal processes such as endocarditis, infections in cystic fibrosis, and infections of permanent indwelling devices such as joint prostheses and heart valves. More recently it has been noted that bacterial biofilms may impair cutaneous wound healing and reduce topical antibacterial efficiency in healing or treating infected skin wounds.

It has recently been shown that biofilms are present on the removed tissue of 80% of patients undergoing surgery for chronic sinusitis. The patients with biofilms were shown to have been denuded of cilia and goblet cells, unlike the controls without biofilms who had normal cilia and goblet cell morphology. Biofilms were also found on samples from two of 10 healthy controls mentioned. The species of bacteria from interoperative cultures did not correspond to the bacteria species in the biofilm on the respective patient's tissue. In other words, the cultures were negative though the bacteria were present.

Biofilms can also be formed on the inert surfaces of implanted devices such as catheters, prosthetic cardiac valves and intrauterine devices.

New staining techniques are being developed to differentiate bacterial cells growing in living animals, e.g. from tissues with allergy-inflammations .

Pseudomonas aeruginosa biofilms

The achievements of medical care in industrialised societies are markedly impaired due to chronic opportunistic infections that have become increasingly apparent in immunocompromised patients and the aging population. Chronic infections remain a major challenge for the medical profession and are of great economic relevance because traditional antibiotic therapy is usually not sufficient to eradicate these infections. One major reason for persistence seems to be the capability of the bacteria to grow within biofilms that protects them from adverse environmental factors. *Pseudomonas aeruginosa* is not only an important opportunistic pathogen and causative agent of

emerging nosocomial infections but can also be considered a model organism for the study of diverse bacterial mechanisms that contribute to bacterial persistence. In this context the elucidation of the molecular mechanisms responsible for the switch from planktonic growth to a biofilm phenotype and the role of inter-bacterial communication in persistent disease should provide new insights in *P. aeruginosa* pathogenicity, contribute to a better clinical management of chronically infected patients and should lead to the identification of new drug targets for the development of alternative anti-infective treatment strategies.

Dental plaque

Dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly *Streptococcus mutans* and *Streptococcus sanguinis*), salivary polymers and bacterial extracellular products. Plaque is a biofilm on the surfaces of the teeth. This accumulation of microorganisms subject the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease.

Legionellosis

Legionella bacteria are known to grow under certain conditions in biofilms, in which they are protected against disinfectants. Workers in cooling towers, persons working in air conditioned rooms and people taking a shower are exposed to Legionella by inhalation when the systems are not well designed, constructed, or maintained.

Neisseria gonorrhoeae biofilms

Neisseria gonorrhoeae is an exclusive human pathogen. Recent studies have demonstrated that it utilizes two distinct mechanisms for entry into human urethral and cervical epithelial cells involving different bacterial surface ligands and host receptors. In addition it has been demonstrated that the gonococcus can form biofilms on glass surfaces and over human cells. There is evidence for formation of gonococcal biofilms on human cervical epithelial cells during natural disease and that outer membrane blebbing by the gonococcus is crucial in biofilm formation over human cervical epithelial cells.

Molecular genetics

Technological progress in microscopy, molecular genetics and genome analysis has significantly advanced our understanding of the structural and molecular aspects of biofilms, especially of extensively studied model organisms such as *Pseudomonas aeruginosa*. Biofilm development can be divided into several key steps including attachment, microcolony formation, biofilm maturation and dispersion; and in each step bacteria may recruit different components and molecules including flagellae, type IV pili, DNA and exopolysaccharides. The rapid progress in biofilm research has also unveiled several genetic regulation mechanisms implicated in biofilm regulation such as quorum sensing and the novel secondary messenger cyclic-di-GMP. Understanding the molecular

mechanisms of biofilm formation has facilitated the exploration of novel strategies to control bacterial biofilms.

Chapter 8

Exotoxin

An **exotoxin** is a toxin excreted by a microorganism, including bacteria, fungi, algae, and protozoa. An exotoxin can cause damage to the host by destroying cells or disrupting normal cellular metabolism. They are highly potent and can cause major damage to the host. Exotoxins may be secreted, or, similar to endotoxins, may be released during lysis of the cell.

Most exotoxins can be destroyed by heating. They may exert their effect locally or produce systemic effects. Well-known exotoxins include the botulinum toxin produced by *Clostridium botulinum* and the *Corynebacterium diphtheriae* exotoxin, which is produced during life-threatening symptoms of diphtheria.

Exotoxins are susceptible to antibodies produced by the immune system, but many exotoxins are so toxic that they may be fatal to the host before the immune system has a chance to mount defenses against it.

Types

Many exotoxins have been categorized by their mode of action on target cells.

This classification, while fairly exhaustive, is not the only system used. Other systems for classifying or identifying toxins include:

- By organism generating the toxin
- By organism susceptible to the toxin
- By tissue target type susceptible to the toxin (neurotoxins affect the nervous system, cardiotoxins affect the heart, etc.)
- By structure (for example, AB5 toxin)

- By the ability of the toxin to endure in hostile environments, such as heat, dryness, radiation, or salinity. In this context, "labile" implies susceptibility, and "stable" implies a lack of susceptibility.
- By a letter, such as "A", "B", or "C", to communicate the order in which they were identified.

The same exotoxin may have different names, depending of the field of research.

Type I: cell surface-active

Type I toxins bind to a receptor on the cell surface and stimulate intracellular signaling pathways. Two examples are described below.

Superantigens

Superantigens are produced by several bacteria. The best-characterized superantigens are those produced by the strains of *Staphylococcus aureus* and *Streptococcus pyogenes* that cause toxic shock syndrome. Superantigens bridge the MHC class II protein on antigen-presenting cells with the T cell receptor on the surface of T cells with a particular Vβ chain. As a consequence, up to 20% of all T cells are activated, leading to massive secretion of proinflammatory cytokines, which produce the symptoms of toxic shock.

Heat-stable enterotoxins

Some strains of *E. coli* produce heat-stable enterotoxins (ST), which are small peptides that are able to withstand heat treatment at 100^{o}C. Different STs recognize distinct receptors on the cell surface and thereby affect different intracellular signaling pathways. For example, STa enterotoxins bind and activate membrane-bound guanylate cyclase, which leads to the intracellular accumulation of cyclic GMP and downstream effects on several signaling pathways. These events lead to the loss of electrolytes and water from intestinal cells.

Type II: membrane damaging

Membrane-damaging toxins exhibit hemolysin or cytolysin activity *in vitro*. However, induction of cell lysis may not be the primary function of the toxins during infection. At low concentrations of toxin, more subtle effects such as modulation of host cell signal transduction may be observed in the absence of cell lysis. Membrane-damaging toxins can be divided into two categories, the channel-forming toxins and toxins that function as enzymes that act on the membrane.

Channel-forming toxins

Most channel-forming toxins, which form pores in the target cell membrane, can be classified into two families, the cholesterol-dependent toxins and the RTX toxins.

- **Cholesterol-dependent cytolysins**

Formation of pores by cholesterol-dependent cytolysins (CDC) such as perfringolysin O of *Clostridium perfringens* requires the presence of cholesterol in the target cell. The size of the pores formed by members of this family is extremely large: 25-30 nm in diameter. A conserved 11-amino acid sequence is found at the C-terminus of all family members. Moreover, all CDCs are secreted by the type II secretion system. The exception is pneumolysin, which is released from the cytoplasm of *Streptococcus pneumoniae* when the bacteria lyse. Pneumolysin, *Clostridium perfringens* perfringolysin, and *Listeria monocytogenes* listeriolysin O cause specific modifications of histones in the host cell nucleus, resulting in down-regulation of several genes encoding proteins involved in the inflammatory response. Histone modification does not involve the pore-forming activity of the CDCs.

- **RTX toxins**

RTX (*r*epeats in *tox*in) cytolysins can be identified by the presence of a specific tandemly-repeated nine-amino acid residue sequence in the protein. The prototype RTX member is the HlyA hemolysin of *E. coli*. RTX is also found in *Legionella pneumophila*.

Enzymatically Active Toxins

One example is the α toxin of *C. perfringens*, which causes gas gangrene; α toxin has phospholipase activity.

Type III: intracellular

Type III exotoxins can be classified by their mode of entry into the cell, or by their mechanism once inside.

By mode of entry

Intracellular toxins must be able to gain access to the cytoplasm of the target cell to exert their effects.

- Some bacteria deliver toxins directly from their cytoplasm to the cytoplasm of the target cell through a needle-like structure. The effector proteins injected by the type III secretion apparatus of *Yersinia* into target cells are one example.

- Another group of intracellular toxins is the AB toxins. The 'B'-subunit (***b****inding*) attaches to target regions on cell membranes, the 'A'-subunit (***a****ctive*) enters through the membrane and possesses enzymatic function that affects internal cellular bio-mechanisms. A common example of this A-subunit activity is called ADP-ribosylation in which the A-subunit catalyzes the addition of an ADP-ribose group onto specific residues on a protein. The structure of these toxins allows for the development of specific vaccines and treatments. Certain compounds can be

attached to the B unit, which is not, in general, harmful, which the body learns to recognize, and which elicits an immune response. This allows the body to detect the harmful toxin if it is encountered later, and to eliminate it before it can cause harm to the host. Toxins of this type include cholera toxin, pertussis toxin, Shiga toxin and heat-liable enterotoxin from *E. coli*.

By mechanism

Once in the cell, many of the exotoxins act at the eukaryotic ribosomes (especially 60S), as protein synthesis inhibitors. (Ribosome structure is one of the most important differences between eukaryotes and prokaryotes, and, in a sense, these exotoxins are the bacterial equivalent of antibiotics such as clindamycin.)

- Some exotoxins act directly at the ribosome to inhibit protein synthesis. An example is Shiga toxin.

- Other toxins act at elongation factor-2. In the case of the diphtheria toxin, EF2 is ADP-ribosylated and becomes unable to participate in protein elongation, and, so, the cell dies. Pseudomonas exotoxin has a similar action.

Other intracellular toxins do not directly inhibit protein synthesis.

- For example, Cholera toxin ADP-ribosylates, thereby activating tissue adenylate cyclase to increase the concentration of cAMP, which causes the movement of massive amounts of fluid and electrolytes from the lining of the small intestine and results in life-threatening diarrhea.

- Another example is Pertussis toxin.

Extracellular matrix damage

These "toxins" allow the further spread of bacteria and, as a consequence, deeper tissue infections. Examples are hyaluronidase and collagenase. These molecules, however, are enzymes that are secreted by a variety of organisms and are not usually considered toxins. They are often referred to as virulence factors, since they allow the organisms to move deeper into the hosts tissues.

Chapter 9

Coley's Toxins

Coley's Toxins (also called **Coley's toxin**, **Coley's vaccine**, **Coley vaccine** or **Mixed Bacterial Vaccine**) is a mixture consisting of killed bacteria of species *Streptococcus pyogenes* and *Serratia marcescens*, named after William Coley, a surgical oncologist who developed the mixture in the late 19th century as a treatment for cancer.

History

Observations of a relationship between infection and cancer regression date back to at least the 18th century. More specifically, observations of an apparent relationship between erysipelas and remission of cancer predate Coley. For example, Anton Chekhov, in his capacity as a physician, recorded such a relationship in 1884.

Coley started his investigations after the death of one of his first patients, Elizabeth Dashiell, from sarcoma. Dashiell was a close childhood friend of John D. Rockefeller, Jr., who later indicated that her death was what first motivated his subsequent funding of cancer research.

Frustrated by this case, Coley's subsequent research led him to find evidence of the apparent relationship between infection and cancer regression, which he published in 1891. His initial attempts at deliberate infection were mixed, but in 1893 he began combining *Streptococcus pyogenes* and *Serratia marcescens*, based upon research from G.H. Roger indicating that this combination led to greater virulence.

The so-called Coley's Toxins were used against different types of cancer from the year 1893 through the year 1963. From 1923 on, Parke-Davis was the only source of Coley's Toxins in the United States. In the wake of the thalidomide controversy and the Kefauver Harris Amendment of 1963, Coley's Toxins were assigned "new drug" status by the Food and Drug Administration (FDA), making it illegal to prescribe them outside of clinical trials. Since then, several small clinical trials have been conducted with mixed results.

Coley's Toxins were also produced by the small German pharmaceutical company *Südmedica* and sold under the trade name *Vaccineurin*. However, production ceased by 1990 because of a lack of re-approval by German Federal Institute for Drugs and Medical Devices.

Rationale

There are multiple rationales proposed for how Coley's Toxins affect the patient.

Macrophages

One rationale argues that macrophages are either in "repair mode", furthering the growing of cancer, or in "defense mode", destroying cancer. However, macrophages are in "defense mode" only if there is some recognized enemy. As cancer tissue is not recognized as enemy (but as normal body tissue), there is a need to bring more macrophages into "defense mode" by simulating an infection. The simulated infection results in a real fever. Unlike hyperthermia, real fever not only means heating of the body but also higher activity of the immune system. Thus, fever is seen as a precondition for a therapy using Coley's Toxins to succeed.

Tumor Necrosis Factor and Interleukin

One of the agents in Coley's Toxin that is thought to be biologically active is a lipopolysaccharide which causes fever. The resulting fever from the lipopolysaccaride is thought to increase lymphocyte activity and boosts tumor necrosis factor (TNF). Tsung and Norton in *Surgical Oncology* reported that the active agent was thought to be interleukin-12, rather than TNF.

Streptokinase

Another hypothesis argues that streptokinase (produced by bacteria of type "streptococcus" together with plasminogen from the patient) is the active agent of Coley's Toxins. This hypothesis is supported by the fact that streptokinase has been associated with successful treatment of thromboangiitis obliterans.

Anti-angiogenesis

In addition to the mechanisms above, Coley's Toxins might be antiangiogenic - suppressing the formation of new blood vessels which are vital to the growth of tumors., however, angiogenesis is not a biochemical cause by itself but needs external triggers.

Dendritic cells

A robust fever, which occurs in response to Coley Fluid, generates inflammatory factors with co-stimulatory activity, which activate resting dendritic cells (DC), leading to the activation of anergic T cells, maybe accomplished by a second process, where a possible physical damage of cancer cells leads to a sudden supply of cancer antigens to DC.,

PAMP

Recently (2008), an immunological explanation binding together immunological data with findings about spontaneous regression and epidemiological data indicating a lowered risk to develop cancer later after common infections, has been published. According to this hypothesis, pathogenic substances produced by bacteria, viruses, infectious fungi and other pathogens, but not human tissue, called 'pathogen associated molecular pattern' (PAMP) lead to activation and maturation of tumor-antigen loaded dendritic cells. One PAMP thought to play a major role is the unmethlyated CpG motif found in bacterial DNA. The CpG motif is recognized by toll like receptor 9 (TLR9) and can induce a strong TH1 response.

Availability

MBVax Bioscience, a Canadian Biotech company, produces Coley Fluid for research and clinical study. A private biotech company, Coley Pharmaceutical Group, has conducted clinical trials using genetic sequences which may have contributed to Coley's Toxin's effectiveness, and was acquired by Pfizer in January 2008. In addition, the Waisbren Clinic in Wisconsin reports they have used Coley's Toxin to treat patients since 1972. *Coley's Toxins* are generally not available where approval or licence is required. (In particular, this is the case at least in the United States as well as in Germany).

Germany

However, there are some specialized medical doctors at least in Germany, who still apply *Coley's Toxins* to patients. They can do so legally, because in Germany, unapproved medications may not be given away (or sold), but they may still be produced. Thus, these medical doctors go to special laboratories and produce *Coley's Toxins* there using their own hands. *Coley's Toxins* may still be applied by a licensed medical doctor, because (in Germany) there is the "*Therapiefreiheit*" ("therapy freedom"), the legal right of a physician to apply whichever therapy he/she believes to be appropriate, considering all his/her medical knowledge.

This kind of therapy is offered as *"Fiebertherapie"* (fever therapy). However, a fever therapy using *Coley's Toxins* - i.e. used by Dr. Josef Issels with *Vaccineurin* - should not be confused with hyperthermia therapy or thermotherapy, sometimes (falsely) denominated as "fever therapy" as well.

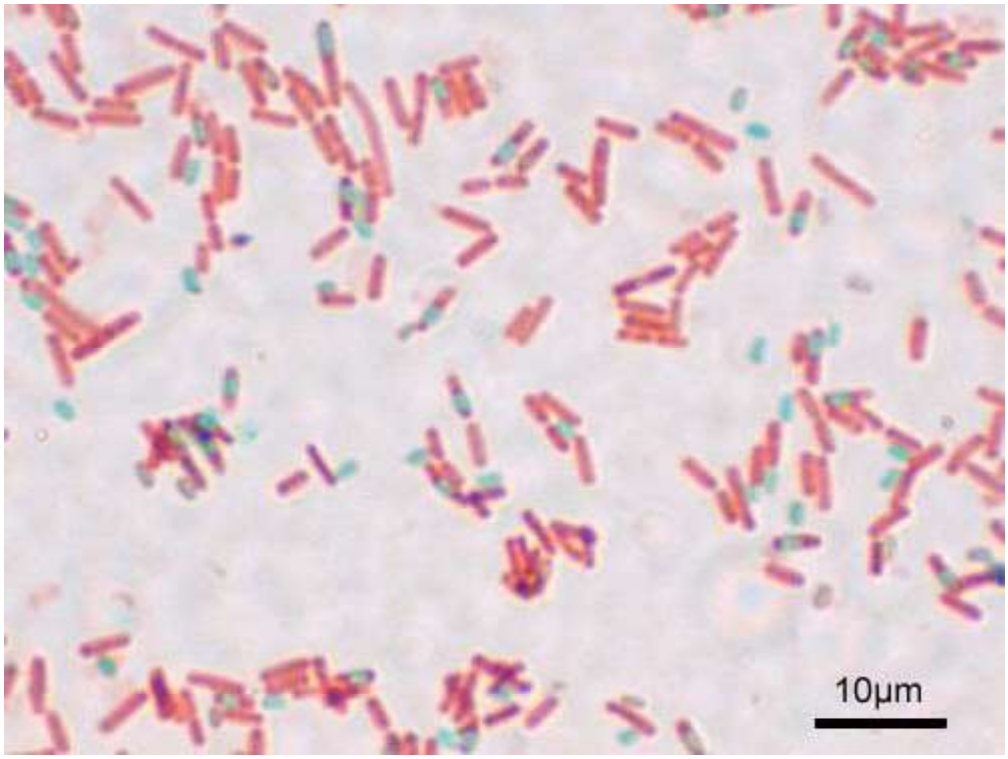
Name

There are several names for *Coley's Toxins* or *Coley's vaccine*. The reason may lie in the difficulty of classifying such a substance under the view of the established medicine:

- *Coley's vaccine* is not a vaccine in the usual sense, namely that it prevents an infection. Rather than that, it triggers infection-like reactions. However, *Coley's vaccine* may work like many ordinary vaccines: it induces an immune response, in this case against the cancer. In this sense, it predates current attempts to develop cancer vaccines.
- The term toxin is applied as Coley's Toxins contain both endotoxins and exotoxins.

Chapter 10

Endospore



A stained preparation of *Bacillus subtilis* showing endospores as green and the vegetative cell as red

An **endospore** is a dormant, tough, and temporarily non-reproductive structure produced by certain bacteria from the Firmicute phylum. The name "endospore" is suggestive of a spore or seedlike form (*endo* means within), but it is not a true spore (i.e. not an offspring). It is a stripped-down, dormant form to which the bacterium can reduce itself. The endospore becomes important when the bacterium is experiencing an environment that is deleterious to the usual vegetative state of the bacterium, such as in desiccating conditions. Endospores enable bacterium to survive periods of environmental stress lasting at least several thousand years, and revival of spores many millions of years old

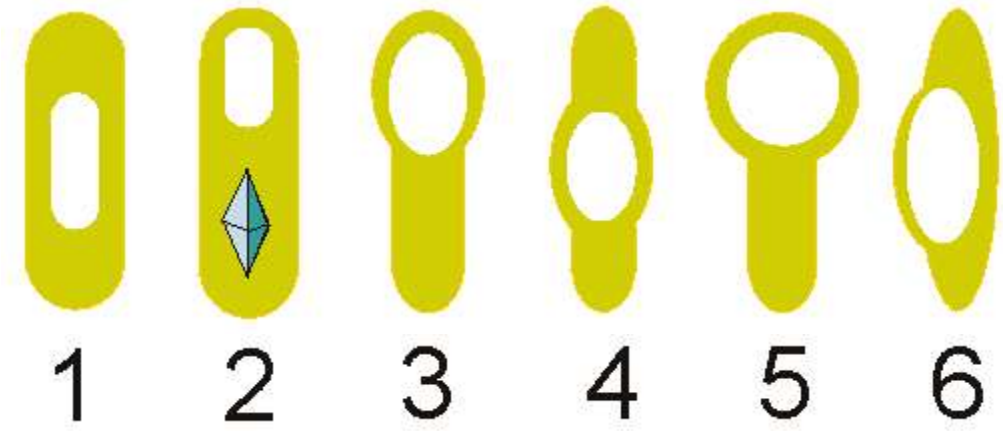
has been claimed. When the environment becomes more favorable, the endospore can reactivate itself to the vegetative state. Most types of bacteria cannot change to the endospore form, but examples include *Bacillus* and *Clostridium*.

The endospore consists of the bacterium's DNA and part of its cytoplasm, surrounded by a very tough outer coating.

Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants. Common anti-bacterial agents that work by destroying vegetative cell walls don't work on endospores. Endospores are commonly found in soil and water, where they may survive for long periods of time.

Some classes of bacteria can turn into exospores, also known as microbial cysts, instead of endospores. Exospores and endospores are two kinds of "hibernating" or dormant stages seen in some classes of microorganisms.

Structure



Variations in endospore morphology: (1, 4) central endospore; (2, 3, 5) terminal endospore; (6) lateral endospore

In contrast to eukaryotic spores, which are produced by many eukaryotes for reproductive purposes, bacteria will produce a single endospore internally. The spore is sometimes surrounded by a thin covering known as the *exosporium*, which overlies the *spore coat*. The spore coat, which acts like a sieve that excludes large toxic molecules like lysozyme, is resistant to many toxic molecules and may also contain enzymes that are involved in germination. The *cortex* lies beneath the spore coat and consists of peptidoglycan. The *core wall* lies beneath the cortex and surrounds the protoplast or *core* of the endospore. The core contains the spore chromosomal DNA is encased in chromatin-like proteins known as SASPs, that protect the spore DNA from UV radiation and heat. The core also contains normal cell structures, such as ribosomes and other enzymes, but is not metabolically active.

Up to 20% of the dry weight of the endospore consists of calcium dipicolinate within the core, which is thought to stabilize the DNA. Dipicolinic acid could be responsible for the heat resistance of the spore, and calcium may aid in resistance to heat and oxidizing agents. However, mutants resistant to heat but lacking dipicolinic acid have been isolated, suggesting other mechanisms contributing to heat resistance are at work.

Visualising endospores under the light microscope can be difficult due to the impermeability of the endospore wall to dyes and stains. While the rest of a bacterial cell may stain, the endospore is left colourless. To combat this, a special stain technique called a Moeller stain is used. That allows the endospore to show up as red, while the rest of the cell stains blue. Another staining technique for endospores is the Schaeffer-Fulton stain, which stains endospores green and bacterial bodies red. The arrangement of spore layers is as follows:

- Exosporium
- Spore coat
- Spore cortex
- Core wall

Location

The position of the endospore differs among bacterial species and is useful in identification. The main types within the cell are terminal, subterminal, and centrally placed endospores. Terminal endospores are seen at the poles of cells, whereas central endospores are more or less in the middle. Subterminal endospores are those between these two extremes, usually seen far enough towards the poles but close enough to the center so as not to be considered either terminal or central. Lateral endospores are seen occasionally.

Examples of bacteria having terminal endospores include *Clostridium tetani*, the pathogen that causes the disease tetanus. Bacteria having a centrally placed endospore include *Bacillus cereus*, and those having a subterminal endospore include *Bacillus subtilis*. Sometimes the endospore can be so large the cell can be distended around the endospore, this is typical of *Clostridium tetani*.

Formation and destruction

When a bacterium detects environmental conditions are becoming unfavourable it may start the process of endosporulation, which takes about eight hours. The DNA is replicated and a membrane wall known as a *spore septum* begins to form between it and the rest of the cell. The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around the DNA, and the developing structure is now known as a forespore. Calcium dipicolinate is incorporated into the forespore during this time. Next the peptidoglycan cortex forms between the two layers and the bacterium adds a spore coat to the outside of the forespore. Sporulation is now complete, and the mature endospore will be released when the surrounding vegetative cell is degraded.

Endospores are resistant to most agents that would normally kill the vegetative cells they formed from. Household cleaning products generally have no effect, nor do most alcohols, quaternary ammonium compounds or detergents. Alkylating agents however, such as ethylene oxide, are effective against endospores.

While resistant to extreme heat and radiation, endospores can be destroyed by burning or by autoclaving. Endospores are able to survive boiling at 100°C for hours, although the longer the number of hours the fewer that will survive. An indirect way to destroy them is to place them in an environment that reactivates them to their vegetative state. They will germinate within a day or two with the right environmental conditions, and then the vegetative cells can be straightforwardly destroyed. This indirect method is called Tyndallization. It was the usual method for a while in the late 19th century before the advent of inexpensive autoclaves. Prolonged exposure to ionising radiation, such as x-rays and gamma rays, will also kill most endospores.

Reactivation

Reactivation of the endospore occurs when conditions are more favourable and involves *activation*, *germination*, and *outgrowth*. Even if an endospore is located in plentiful nutrients, it may fail to germinate unless activation has taken place. This may be triggered by heating the endospore. Germination involves the dormant endospore starting metabolic activity and thus breaking hibernation. It is commonly characterised by rupture or absorption of the spore coat, swelling of the endospore, an increase in metabolic activity, and loss of resistance to environmental stress. Outgrowth follows germination and involves the core of the endospore manufacturing new chemical components and exiting the old spore coat to develop into a fully functional vegetative bacterial cell, which can divide to produce more cells.

Importance

As a simplified model for cellular differentiation, the molecular details of endospore formation have been extensively studied, specifically in the model organism *Bacillus subtilis*. These studies have contributed much to our understanding of the regulation of gene expression, transcription factors, and the sigma factor subunits of RNA polymerase.

Endospores of the bacterium *Bacillus anthracis* were used in the 2001 anthrax attacks. The powder found in contaminated postal letters was composed of extracellular anthrax endospores. Inhalation, ingestion or skin contamination of these endospores, which were technically incorrectly labelled as "spores", led to a number of deaths.

Geobacillus stearothermophilus endospores are used as biological indicators when an autoclave is used in sterilization procedures.

Endospore-forming bacteria

Examples of endospore-forming bacteria include the genera:

- *Acetonema*
- *Alkalibacillus*
- *Ammoniphilus*
- *Amphibacillus*
- *Anaerobacter*
- *Anaerospora*
- *Aneurinibacillus*
- *Anoxybacillus*
- *Bacillus*
- *Brevibacillus*
- *Caldanaerobacter*
- *Caloramator*
- *Caminicella*
- *Cerasibacillus*
- *Clostridium*
- *Clostridiisalibacter*
- *Cohnella*
- *Coxiella*
- *Dendrosporobacter*
- *Desulfotomaculum*
- *Desulfosporomusa*
- *Desulfosporosinus*
- *Desulfovirgula*
- *Desulfunispora*
- *Desulfurispora*
- *Filifactor*
- *Filobacillus*
- *Gelria*
- *Geobacillus*
- *Geosporobacter*
- *Gracilibacillus*
- *Halonatronum*
- *Heliobacterium*
- *Heliophilum*
- *Laceyella*
- *Lentibacillus*
- *Lysinibacillus*
- *Mahella*
- *Metabacterium*
- *Moorella*
- *Natroniella*
- *Oceanobacillus*
- *Orenia*
- *Ornithinibacillus*
- *Oxalophagus*
- *Oxobacter*

- *Paenibacillus*
- *Paraliobacillus*
- *Pelospora*
- *Pelotomaculum*
- *Piscibacillus*
- *Planifilum*
- *Pontibacillus*
- *Propionispora*
- *Salinibacillus*
- *Salsuginibacillus*
- *Seinonella*
- *Shimazuella*
- *Sporacetigenium*
- *Sporoanaerobacter*
- *Sporobacter*
- *Sporobacterium*
- *Sporohalobacter*
- *Sporolactobacillus*
- *Sporomusa*
- *Sporosarcina*
- *Sporotalea*
- *Sporotomaculum*
- *Syntrophomonas*
- *Syntrophospora*
- *Tenuibacillus*
- *Tepidibacter*
- *Terribacillus*
- *Thalassobacillus*
- *Thermoacetogenium*
- *Thermoactinomyces*
- *Thermoalkalibacillus*
- *Thermoanaerobacter*
- *Thermoanaeromonas*
- *Thermobacillus*
- *Thermoflavimicrobium*
- *Thermovenabulum*
- *Tuberibacillus*
- *Virgibacillus*
- *Vulcanobacillus*

Chapter 11

Fungus

Fungi Temporal range: Early Devonian–Recent€


Clockwise from top left: *Amanita muscaria*, a basidiomycete; *Sarcoscypha coccinea*, an ascomycete; bread covered in mold; a chytrid; a *Penicillium* conidiophore.

Scientific classification	
Domain:	Eukaryota
(unranked):	Opisthokonta
Kingdom:	**Fungi** (L., 1753) R.T. Moore, 1980

Subkingdoms/Phyla/Subphyla
Blastocladiomycota
Chytridiomycota
Glomeromycota

Microsporidia
Neocallimastigomycota

Dikarya (inc. Deuteromycota)

Ascomycota
Pezizomycotina
Saccharomycotina
Taphrinomycotina
Basidiomycota
Agaricomycotina
Pucciniomycotina
Ustilaginomycotina

Subphyla Incertae sedis

Entomophthoromycotina
Kickxellomycotina
Mucoromycotina
Zoopagomycotina

A **fungus** is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds (British English: moulds), as well as the more familiar mushrooms. These organisms are classified as a kingdom, **Fungi**, which is separate from plants, animals, and bacteria. One major difference is that fungal cells have cell walls that contain chitin, unlike the cell walls of plants, which contain cellulose. These and other differences show that the fungi form a single group of related organisms, named the *Eumycota* (*true fungi* or *Eumycetes*), that share a common ancestor (a *monophyletic group*). This fungal group is distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds). The discipline of biology devoted to the study of fungi is known as mycology, which is often regarded as a branch of botany, even though genetic studies have shown that fungi are more closely related to animals than to plants.

Abundant worldwide, most fungi are inconspicuous because of the small size of their structures, and their cryptic lifestyles in soil, on dead matter, and as symbionts of plants, animals, or other fungi. They may become noticeable when fruiting, either as mushrooms or molds. Fungi perform an essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange. They have long been used as a direct source of food, such as mushrooms and truffles, as a leavening agent for bread, and in fermentation of various food products, such as wine, beer, and soy sauce. Since the 1940s, fungi have been used for the production of antibiotics, and, more recently, various enzymes produced by fungi are used industrially and in detergents. Fungi are also used as biological pesticides to control weeds, plant diseases and insect pests. Many species produce bioactive compounds called mycotoxins, such as alkaloids and polyketides, that are toxic to animals including humans. The fruiting structures of a few species contain

psychotropic compounds and are consumed recreationally or in traditional spiritual ceremonies. Fungi can break down manufactured materials and buildings, and become significant pathogens of humans and other animals. Losses of crops due to fungal diseases (e.g. rice blast disease) or food spoilage can have a large impact on human food supplies and local economies.

The fungus kingdom encompasses an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies ranging from single-celled aquatic chytrids to large mushrooms. However, little is known of the true biodiversity of Kingdom Fungi, which has been estimated at around 1.5 million species, with about 5% of these having been formally classified. Ever since the pioneering 18th and 19th century taxonomical works of Carl Linnaeus, Christian Hendrik Persoon, and Elias Magnus Fries, fungi have been classified according to their morphology (e.g., characteristics such as spore color or microscopic features) or physiology. Advances in molecular genetics have opened the way for DNA analysis to be incorporated into taxonomy, which has sometimes challenged the historical groupings based on morphology and other traits. Phylogenetic studies published in the last decade have helped reshape the classification of Kingdom Fungi, which is divided into one subkingdom, seven phyla, and ten subphyla.

Etymology

The English word *fungus* is directly adopted from the Latin *fungus* (mushroom), used in the writings of Horace and Pliny. This in turn is derived from the Greek word *sphongos*/σφογγος ("sponge"), which refers to the macroscopic structures and morphology of mushrooms and molds; the root is also used in other languages, such as the German *Schwamm* ("sponge"), *Schimmel* ("mold"), and the French *champignon* and the Spanish *champiñon* (which both mean "mushroom"). The use of the word *mycology*, which is derived from the Greek *mykes*/μύκης (mushroom) and *logos*/λόγος (discourse), to denote the scientific study of fungi is thought to have originated in 1836 with English naturalist Miles Joseph Berkeley's publication *The English Flora of Sir James Edward Smith, Vol. 5.*

Characteristics

Before the introduction of molecular methods for phylogenetic analysis, taxonomists considered fungi to be members of the Plant Kingdom because of similarities in lifestyle: both fungi and plants are mainly immobile, and have similarities in general morphology and growth habitat. Like plants, fungi often grow in soil, and in the case of mushrooms form conspicuous fruiting bodies, which sometimes bear resemblance to plants such as mosses. The fungi are now considered a separate kingdom, distinct from both plants and animals, from which they appear to have diverged around one billion years ago. Some morphological, biochemical, and genetic features are shared with other organisms, while others are unique to the fungi, clearly separating them from the other kingdoms:

Shared features:

- With other eukaryotes: As other eukaryotes, fungal cells contain membrane-bound nuclei with chromosomes that contain DNA with noncoding regions called introns and coding regions called exons. In addition, fungi possess membrane-bound cytoplasmic organelles such as mitochondria, sterol-containing membranes, and ribosomes of the 80S type. They have a characteristic range of soluble carbohydrates and storage compounds, including sugar alcohols (e.g., mannitol), disaccharides, (e.g., trehalose), and polysaccharides (e.g., glycogen, which is also found in animals).
- With animals: Fungi lack chloroplasts and are heterotrophic organisms, requiring preformed organic compounds as energy sources.
- With plants: Fungi possess a cell wall and vacuoles. They reproduce by both sexual and asexual means, and like basal plant groups (such as ferns and mosses) produce spores. Similar to mosses and algae, fungi typically have haploid nuclei.
- With euglenoids and bacteria: Higher fungi, euglenoids, and some bacteria produce the amino acid L-lysine in specific biosynthesis steps, called the α-aminoadipate pathway.
- The cells of most fungi grow as tubular, elongated, and thread-like (filamentous) structures and are called hyphae, which may contain multiple nuclei and extend at their tips. Each tip contains a set of aggregated vesicles—cellular structures consisting of proteins, lipids, and other organic molecules—called Spitzenkörper. Both fungi and oomycetes grow as filamentous hyphal cells. In contrast, similar-looking organisms, such as filamentous green algae, grow by repeated cell division within a chain of cells.
- In common with some plant and animal species, more than 60 fungal species display the phenomenon of bioluminescence.

Unique features:

- Some species grow as single-celled yeasts that reproduce by budding or binary fission. Dimorphic fungi can switch between a yeast phase and a hyphal phase in response to environmental conditions.
- The fungal cell wall is composed of glucans and chitin; while the former compounds are also found in plants and the latter in the exoskeleton of arthropods, fungi are the only organisms that combine these two structural molecules in their cell wall. In contrast to plants and the oomycetes, fungal cell walls do not contain cellulose.

Omphalotus nidiformis, a bioluminescent mushroom

Most fungi lack an efficient system for long-distance transport of water and nutrients, such as the xylem and phloem in many plants. To overcome these limitations, some fungi, such as *Armillaria*, form rhizomorphs, that resemble and perform functions similar to the roots of plants. Another characteristic shared with plants includes a biosynthetic pathway for producing terpenes that uses mevalonic acid and pyrophosphate as chemical building blocks. However, plants have an additional terpene pathway in their chloroplasts, a structure fungi do not possess. Fungi produce several secondary metabolites that are similar or identical in structure to those made by plants. Many of the plant and fungal enzymes that make these compounds differ from each other in sequence and other characteristics, which indicates separate origins and evolution of these enzymes in the fungi and plants.

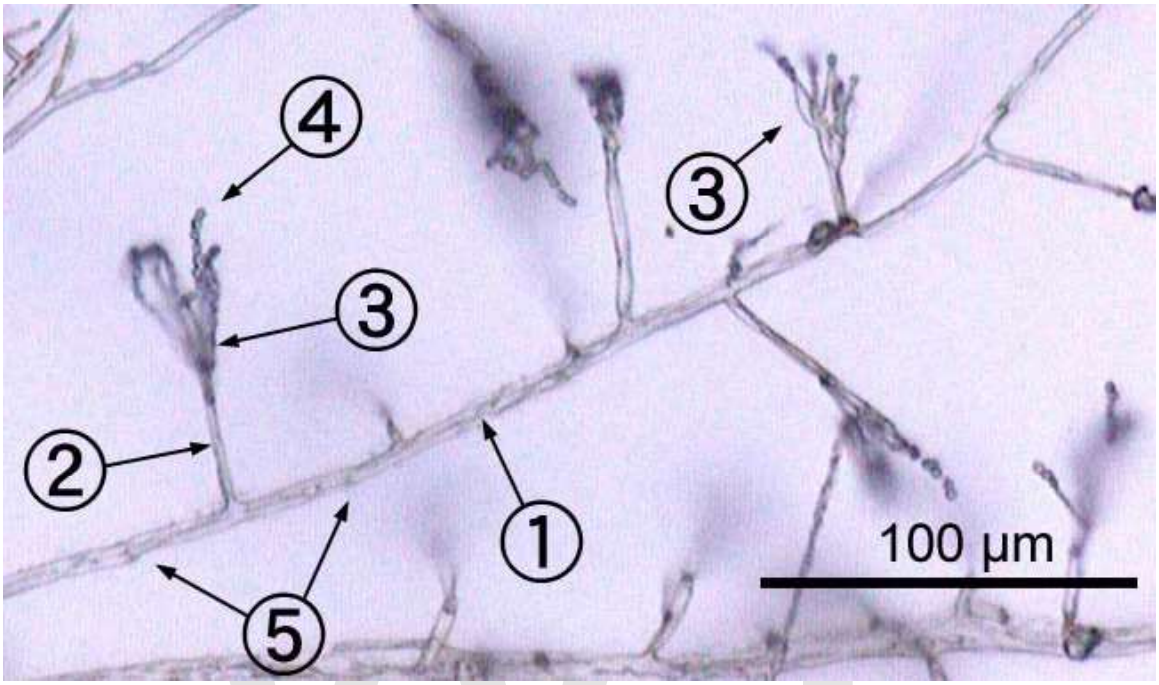
Diversity

Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments such as deserts or areas with high salt concentrations or ionizing radiation, as well as in deep sea sediments. Some can survive the intense UV and cosmic radiation encountered during space travel. Most grow in terrestrial environments, though several species live partly or solely in aquatic habitats, such as the chytrid fungus *Batrachochytrium dendrobatidis*, a parasite that has been responsible for a worldwide decline in amphibian populations. This organism spends part of its life cycle as a motile zoospore, enabling it to propel itself through water and enter its amphibian host. Other examples of aquatic fungi include those living in hydrothermal areas of the ocean.

Around 100,000 species of fungi have been formally described by taxonomists, but the global biodiversity of the fungus kingdom is not fully understood. On the basis of observations of the ratio of the number of fungal species to the number of plant species in selected environments, the fungal kingdom has been estimated to contain about 1.5 million species. In mycology, species have historically been distinguished by a variety of methods and concepts. Classification based on morphological characteristics, such as the size and shape of spores or fruiting structures, has traditionally dominated fungal taxonomy. Species may also be distinguished by their biochemical and physiological characteristics, such as their ability to metabolize certain biochemicals, or their reaction to chemical tests. The biological species concept discriminates species based on their ability to mate. The application of molecular tools, such as DNA sequencing and phylogenetic analysis, to study diversity has greatly enhanced the resolution and added robustness to estimates of genetic diversity within various taxonomic groups.

Morphology

Microscopic structures



An environmental isolate of *Penicillium*
1. hypha 2. conidiophore 3. phialide 4. conidia 5. septa

Most fungi grow as hyphae, which are cylindrical, thread-like structures 2–10 µm in diameter and up to several centimeters in length. Hyphae grow at their tips (apices); new hyphae are typically formed by emergence of new tips along existing hyphae by a process called *branching*, or occasionally growing hyphal tips bifurcate (fork) giving rise to two parallel-growing hyphae. The combination of apical growth and branching/forking leads to the development of a mycelium, an interconnected network of hyphae. Hyphae can be either septate or coenocytic: septate hyphae are divided into compartments separated by cross walls (internal cell walls, called septa, that are formed at right angles to the cell wall giving the hypha its shape), with each compartment containing one or more nuclei; coenocytic hyphae are not compartmentalized. Septa have pores that allow cytoplasm, organelles, and sometimes nuclei to pass through; an example is the dolipore septum in the fungi of the phylum Basidiomycota. Coenocytic hyphae are essentially multinucleate supercells.

Many species have developed specialized hyphal structures for nutrient uptake from living hosts; examples include haustoria in plant-parasitic species of most fungal phyla, and arbuscules of several mycorrhizal fungi, which penetrate into the host cells to consume nutrients.

Although fungi are opisthokonts—a grouping of evolutionarily related organisms broadly characterized by a single posterior flagellum—all phyla except for the chytrids have lost their posterior flagella. Fungi are unusual among the eukaryotes in having a cell wall that, in addition to glucans (e.g., β-1,3-glucan) and other typical components, also contains the biopolymer chitin.

Macroscopic structures

Armillaria solidipes

Fungal mycelia can become visible to the naked eye, for example, on various surfaces and substrates, such as damp walls and on spoiled food, where they are commonly called molds. Mycelia grown on solid agar media in laboratory petri dishes are usually referred to as colonies. These colonies can exhibit growth shapes and colors (due to spores or pigmentation) that can be used as diagnostic features in the identification of species or groups. Some individual fungal colonies can reach extraordinary dimensions and ages as in the case of a clonal colony of *Armillaria solidipes*, which extends over an area of more than 900 ha (3.5 square miles), with an estimated age of nearly 9,000 years.

The apothecium—a specialized structure important in sexual reproduction in the ascomycetes—is a cup-shaped fruiting body that holds the hymenium, a layer of tissue containing the spore-bearing cells. The fruiting bodies of the basidiomycetes

(basidiocarps) and some ascomycetes can sometimes grow very large, and many are well-known as mushrooms.

Growth and physiology

The growth of fungi as hyphae on or in solid substrates or as single cells in aquatic environments is adapted for the efficient extraction of nutrients, because these growth forms have high surface area to volume ratios. Hyphae are specifically adapted for growth on solid surfaces, and to invade substrates and tissues. They can exert large penetrative mechanical forces; for example, the plant pathogen *Magnaporthe grisea* forms a structure called an appressorium which evolved to puncture plant tissues. The pressure generated by the appressorium, directed against the plant epidermis, can exceed 8 megapascals (1,200 psi). The filamentous fungus *Paecilomyces lilacinus* uses a similar structure to penetrate the eggs of nematodes.

Mold covering a decaying peach. The frames were taken approximately 12 hours apart over a period of six days.

The mechanical pressure exerted by the appressorium is generated from physiological processes that increase intracellular turgor by producing osmolytes such as glycerol. Morphological adaptations such as these are complemented by hydrolytic enzymes secreted into the environment to digest large organic molecules—such as polysaccharides, proteins, lipids, and other organic substrates—into smaller molecules that may then be absorbed as nutrients. The vast majority of filamentous fungi grow in a polar fashion—i.e., by extension into one direction—by elongation at the tip (apex) of the hypha. Alternative forms of fungal growth include intercalary extension (i.e., by longitudinal expansion of hyphal compartments that are below the apex) as in the case of some endophytic fungi, or growth by volume expansion during the development of mushroom stipes and other large organs. Growth of fungi as multicellular structures consisting of somatic and reproductive cells—a feature independently evolved in animals and plants—has several functions, including the development of fruiting bodies for dissemination of sexual spores and biofilms for substrate colonization and intercellular communication.

Traditionally, the fungi are considered heterotrophs, organisms that rely solely on carbon fixed by other organisms for metabolism. Fungi have evolved a high degree of metabolic versatility that allows them to use a diverse range of organic substrates for growth, including simple compounds such as nitrate, ammonia, acetate, or ethanol. For some species it has been shown that the pigment melanin may play a role in extracting energy from ionizing radiation, such as gamma radiation; however, this form of "radiotrophic" growth has only been described for a few species, the effects on growth rates are small, and the underlying biophysical and biochemical processes are not known. The authors speculate that this process might bear similarity to CO_2 fixation via visible light, but instead utilizing ionizing radiation as a source of energy.

Reproduction

Polyporus squamosus

Fungal reproduction is complex, reflecting the differences in lifestyles and genetic makeup within this kingdom of organisms. It is estimated that a third of all fungi reproduce by different modes of propagation; for example, reproduction may occur in two well-differentiated stages within the life cycle of a species, the teleomorph and the anamorph. Environmental conditions trigger genetically determined developmental states that lead to the creation of specialized structures for sexual or asexual reproduction. These structures aid reproduction by efficiently dispersing spores or spore-containing propagules.

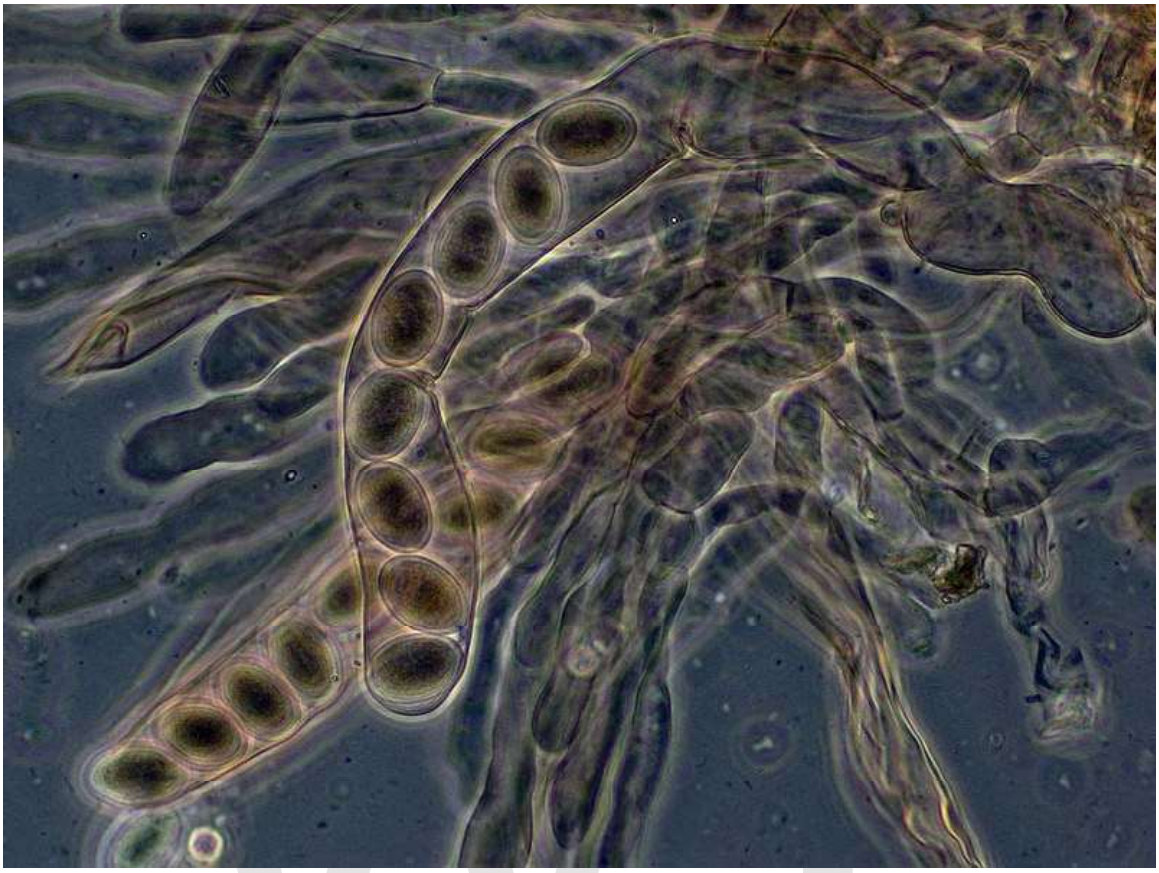
Asexual reproduction

Asexual reproduction via vegetative spores (conidia) or through mycelial fragmentation is common; it maintains clonal populations adapted to a specific niche, and allows more rapid dispersal than sexual reproduction. The "Fungi imperfecti" (fungi lacking the perfect or sexual stage) or Deuteromycota comprise all the species which lack an observable sexual cycle.

Sexual reproduction

Sexual reproduction with meiosis exists in all fungal phyla (with the exception of the Glomeromycota). It differs in many aspects from sexual reproduction in animals or plants. Differences also exist between fungal groups and can be used to discriminate species by morphological differences in sexual structures and reproductive strategies. Mating experiments between fungal isolates may identify species on the basis of biological species concepts. The major fungal groupings have initially been delineated based on the morphology of their sexual structures and spores; for example, the spore-containing structures, asci and basidia, can be used in the identification of ascomycetes and basidiomycetes, respectively. Some species may allow mating only between individuals of opposite mating type, while others can mate and sexually reproduce with any other individual or itself. Species of the former mating system are called heterothallic, and of the latter homothallic.

Most fungi have both an haploid and diploid stage in their life cycles. In sexually reproducing fungi, compatible individuals may combine by fusing their hyphae together into an interconnected network; this process, anastomosis, is required for the initiation of the sexual cycle. Ascomycetes and basidiomycetes go through a dikaryotic stage, in which the nuclei inherited from the two parents do not combine immediately after cell fusion, but remain separate in the hyphal cells.



The 8-spored asci of *Morchella elata*, viewed with phase contrast microscopy

In ascomycetes, dikaryotic hyphae of the hymenium (the spore-bearing tissue layer) form a characteristic *hook* at the hyphal septum. During cell division, formation of the hook ensures proper distribution of the newly divided nuclei into the apical and basal hyphal compartments. An ascus (plural *asci*) is then formed, in which karyogamy (nuclear fusion) occurs. Asci are embedded in an ascocarp, or fruiting body. Karyogamy in the asci is followed immediately by meiosis and the production of ascospores. After dispersal, the ascospores may germinate and form a new haploid mycelium.

Sexual reproduction in basidiomycetes is similar to that of the ascomycetes. Compatible haploid hyphae fuse to produce a dikaryotic mycelium. However, the dikaryotic phase is more extensive in the basidiomycetes, often also present in the vegetatively growing mycelium. A specialized anatomical structure, called a clamp connection, is formed at each hyphal septum. As with the structurally similar hook in the ascomycetes, the clamp connection in the basidiomycetes is required for controlled transfer of nuclei during cell division, to maintain the dikaryotic stage with two genetically different nuclei in each hyphal compartment. A basidiocarp is formed in which club-like structures known as basidia generate haploid basidiospores after karyogamy and meiosis. The most commonly known basidiocarps are mushrooms, but they may also take other forms.

In glomeromycetes (formerly zygomycetes), haploid hyphae of two individuals fuse, forming a gametangium, a specialized cell structure that becomes a fertile gamete-producing cell. The gametangium develops into a zygospore, a thick-walled spore formed by the union of gametes. When the zygospore germinates, it undergoes meiosis, generating new haploid hyphae, which may then form asexual sporangiospores. These sporangiospores allow the fungus to rapidly disperse and germinate into new genetically identical haploid fungal mycelia.

Spore dispersal

Both asexual and sexual spores or sporangiospores are often actively dispersed by forcible ejection from their reproductive structures. This ejection ensures exit of the spores from the reproductive structures as well as travelling through the air over long distances.

The bird's nest fungus *Cyathus stercoreus*

Specialized mechanical and physiological mechanisms, as well as spore surface structures (such as hydrophobins), enable efficient spore ejection. For example, the structure of the spore-bearing cells in some ascomycete species is such that the buildup of substances affecting cell volume and fluid balance enables the explosive discharge of spores into the air. The forcible discharge of single spores termed *ballistospores* involves

formation of a small drop of water (Buller's drop), which upon contact with the spore leads to its projectile release with an initial acceleration of more than 10,000 g; the net result is that the spore is ejected 0.01–0.02 cm, sufficient distance for it to fall through the gills or pores into the air below. Other fungi, like the puffballs, rely on alternative mechanisms for spore release, such as external mechanical forces. The bird's nest fungi use the force of falling water drops to liberate the spores from cup-shaped fruiting bodies. Another strategy is seen in the stinkhorns, a group of fungi with lively colors and putrid odor that attract insects to disperse their spores.

Other sexual processes

Besides regular sexual reproduction with meiosis, certain fungi, such as those in the genera *Penicillium* and *Aspergillus*, may exchange genetic material via parasexual processes, initiated by anastomosis between hyphae and plasmogamy of fungal cells. The frequency and relative importance of parasexual events is unclear and may be lower than other sexual processes. It is known to play a role in intraspecific hybridization and is likely required for hybridization between species, which has been associated with major events in fungal evolution.

Evolution

In contrast to plants and animals, the early fossil record of the fungi is meager. Factors that likely contribute to the under-representation of fungal species among fossils include the nature of fungal fruiting bodies, which are soft, fleshy, and easily degradable tissues and the microscopic dimensions of most fungal structures, which therefore are not readily evident. Fungal fossils are difficult to distinguish from those of other microbes, and are most easily identified when they resemble extant fungi. Often recovered from a permineralized plant or animal host, these samples are typically studied by making thin-section preparations that can be examined with light microscopy or transmission electron microscopy. Compression fossils are studied by dissolving the surrounding matrix with acid and then using light or scanning electron microscopy to examine surface details.

The earliest fossils possessing features typical of fungi date to the Proterozoic eon, some 1,430 million years ago (Ma); these multicellular benthic organisms had filamentous structures with septa, and were capable of anastomosis. More recent studies (2009) estimate the arrival of fungal organisms at about 760–1060 Ma on the basis of comparisons of the rate of evolution in closely related groups. For much of the Paleozoic Era (542–251 Ma), the fungi appear to have been aquatic and consisted of organisms similar to the extant Chytrids in having flagellum-bearing spores. The evolutionary adaptation from an aquatic to a terrestrial lifestyle necessitated a diversification of ecological strategies for obtaining nutrients, including parasitism, saprobism, and the development of mutualistic relationships such as mycorrhiza and lichenization. Recent (2009) studies suggest that the ancestral ecological state of the Ascomycota was saprobism, and that independent lichenization events have occurred multiple times.

The fungi probably colonized the land during the Cambrian (542–488.3 Ma), long before land plants. Fossilized hyphae and spores recovered from the Ordovician of Wisconsin (460 Ma) resemble modern-day Glomerales, and existed at a time when the land flora likely consisted of only non-vascular bryophyte-like plants. Prototaxites, which was probably a fungus or lichen, would have been the tallest organism of the late Silurian. Fungal fossils do not become common and uncontroversial until the early Devonian (416–359.2 Ma), when they are abundant in the Rhynie chert, mostly as Zygomycota and Chytridiomycota. At about this same time, approximately 400 Ma, the Ascomycota and Basidiomycota diverged, and all modern classes of fungi were present by the Late Carboniferous (Pennsylvanian, 318.1–299 Ma).

Lichen-like fossils have been found in the Doushantuo Formation in southern China dating back to 635–551 Ma. Lichens were a component of the early terrestrial ecosystems, and the estimated age of the oldest terrestrial lichen fossil is 400 Ma; this date corresponds to the age of the oldest known sporocarp fossil, a *Paleopyrenomycites* species found in the Rhynie Chert. The oldest fossil with microscopic features resembling modern-day basidiomycetes is *Palaeoancistrus*, found permineralized with a fern from the Pennsylvanian. Rare in the fossil record are the homobasidiomycetes (a taxon roughly equivalent to the mushroom-producing species of the agaricomycetes). Two amber-preserved specimens provide evidence that the earliest known mushroom-forming fungi (the extinct species *Archaeomarasmius legletti*) appeared during the mid-Cretaceous, 90 Ma.

Some time after the Permian-Triassic extinction event (251.4 Ma), a fungal spike (originally thought to be an extraordinary abundance of fungal spores in sediments) formed, suggesting that fungi were the dominant life form at this time, representing nearly 100% of the available fossil record for this period. However, the relative proportion of fungal spores relative to spores formed by algal species is difficult to assess, the spike did not appear worldwide, and in many places it did not fall on the Permian-Triassic boundary.

Taxonomy

Although commonly included in botany curricula and textbooks, fungi are more closely related to animals than to plants and are placed with the animals in the monophyletic group of opisthokonts. Analyses using molecular phylogenetics support a monophyletic origin of the Fungi. The taxonomy of the Fungi is in a state of constant flux, especially due to recent research based on DNA comparisons. These current phylogenetic analyses often overturn classifications based on older and sometimes less discriminative methods based on morphological features and biological species concepts obtained from experimental matings.

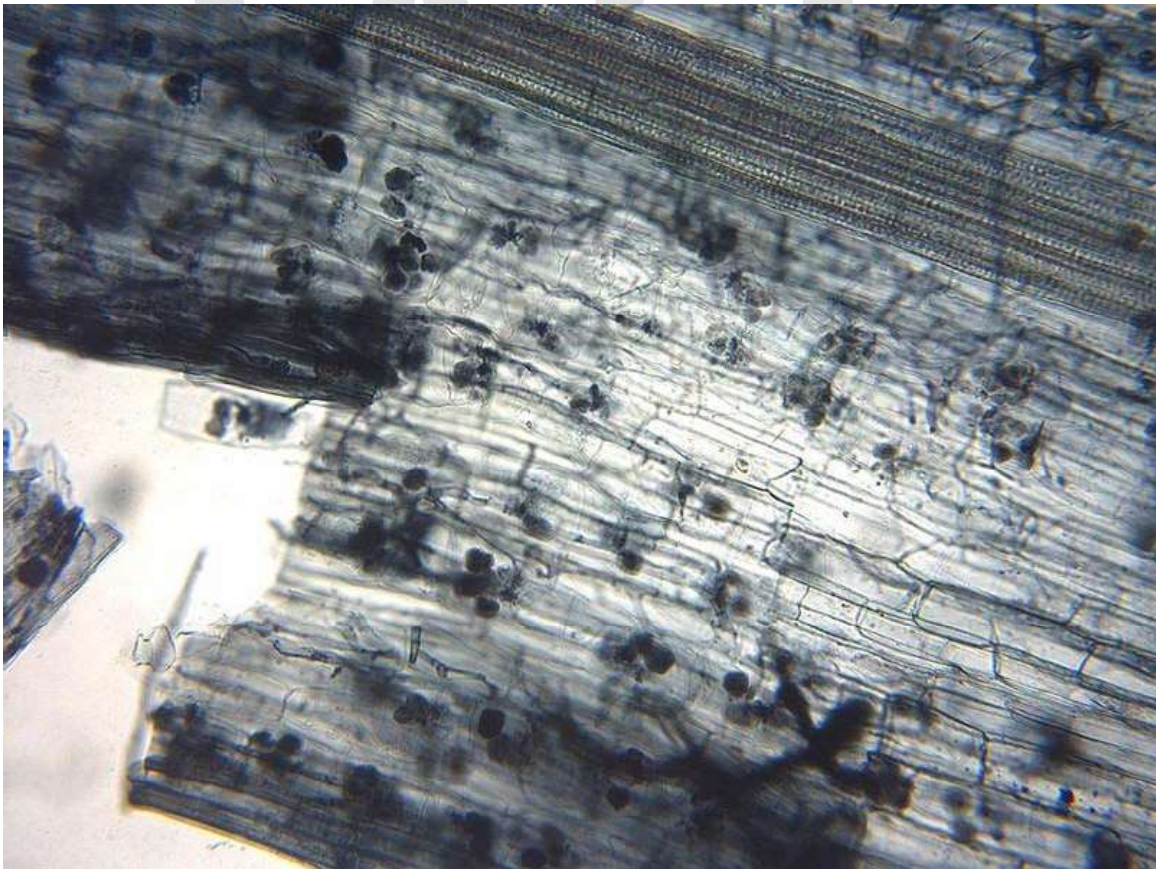
There is no unique generally accepted system at the higher taxonomic levels and there are frequent name changes at every level, from species upwards. Efforts among researchers are now underway to establish and encourage usage of a unified and more consistent nomenclature. Fungal species can also have multiple scientific names depending on their

life cycle and mode (sexual or asexual) of reproduction. Web sites such as Index Fungorum and ITIS list current names of fungal species (with cross-references to older synonyms).

The 2007 classification of Kingdom Fungi is the result of a large-scale collaborative research effort involving dozens of mycologists and other scientists working on fungal taxonomy. It recognizes seven phyla, two of which—the Ascomycota and the Basidiomycota—are contained within a branch representing subkingdom Dikarya. The below cladogram depicts the major fungal taxa and their relationship to opisthokont and unikont organisms. The lengths of the branches in this tree are not proportional to evolutionary distances.

Taxonomic groups

The major phyla (sometimes called divisions) of fungi have been classified mainly on the basis of characteristics of their sexual reproductive structures. Currently, seven phyla are proposed: Microsporidia, Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Ascomycota, and Basidiomycota.



Arbuscular mycorrhiza seen under microscope. Flax root cortical cells containing paired arbuscules.

Phylogenetic analysis has demonstrated that the Microsporidia, unicellular parasites of animals and protists, are fairly recent and highly derived endobiotic fungi (living within the tissue of another species). One 2006 study concludes that the Microsporidia are a sister group to the true fungi, that is, they are each other's closest evolutionary relative. Hibbett and colleagues suggest that this analysis does not clash with their classification of the Fungi, and although the Microsporidia are elevated to phylum status, it is acknowledged that further analysis is required to clarify evolutionary relationships within this group.

The Chytridiomycota are commonly known as chytrids. These fungi are distributed worldwide. Chytrids produce zoospores that are capable of active movement through aqueous phases with a single flagellum, leading early taxonomists to classify them as protists. Molecular phylogenies, inferred from rRNA sequences in ribosomes, suggest that the Chytrids are a basal group divergent from the other fungal phyla, consisting of four major clades with suggestive evidence for paraphyly or possibly polyphyly.

The Blastocladiomycota were previously considered a taxonomic clade within the Chytridiomycota. Recent molecular data and ultrastructural characteristics, however, place the Blastocladiomycota as a sister clade to the Zygomycota, Glomeromycota, and Dikarya (Ascomycota and Basidiomycota). The blastocladiomycetes are saprotrophs, feeding on decomposing organic matter, and they are parasites of all eukaryotic groups. Unlike their close relatives, the chytrids, which mostly exhibit zygotic meiosis, the blastocladiomycetes undergo sporic meiosis.

The Neocallimastigomycota were earlier placed in the phylum Chytridomycota. Members of this small phylum are anaerobic organisms, living in the digestive system of larger herbivorous mammals and possibly in other terrestrial and aquatic environments. They lack mitochondria but contain hydrogenosomes of mitochondrial origin. As the related chrytrids, neocallimastigomycetes form zoospores that are posteriorly uniflagellate or polyflagellate.

Members of the Glomeromycota form arbuscular mycorrhizae, a form of symbiosis where fungal hyphae invade plant root cells and both species benefit from the resulting increased supply of nutrients. All known Glomeromycota species reproduce asexually. The symbiotic association between the Glomeromycota and plants is ancient, with evidence dating to 400 million years ago. Formerly part of the Zygomycota (commonly known as 'sugar' and 'pin' molds), the Glomeromycota were elevated to phylum status in 2001 and now replace the older phylum Zygomycota. Fungi that were placed in the Zygomycota are now being reassigned to the Glomeromycota, or the subphyla incertae sedis Mucoromycotina, Kickxellomycotina, the Zoopagomycotina and the Entomophthoromycotina. Some well-known examples of fungi formerly in the Zygomycota include black bread mold (*Rhizopus stolonifer*), and *Pilobolus* species, capable of ejecting spores several meters through the air. Medically relevant genera include *Mucor*, *Rhizomucor*, and *Rhizopus*.

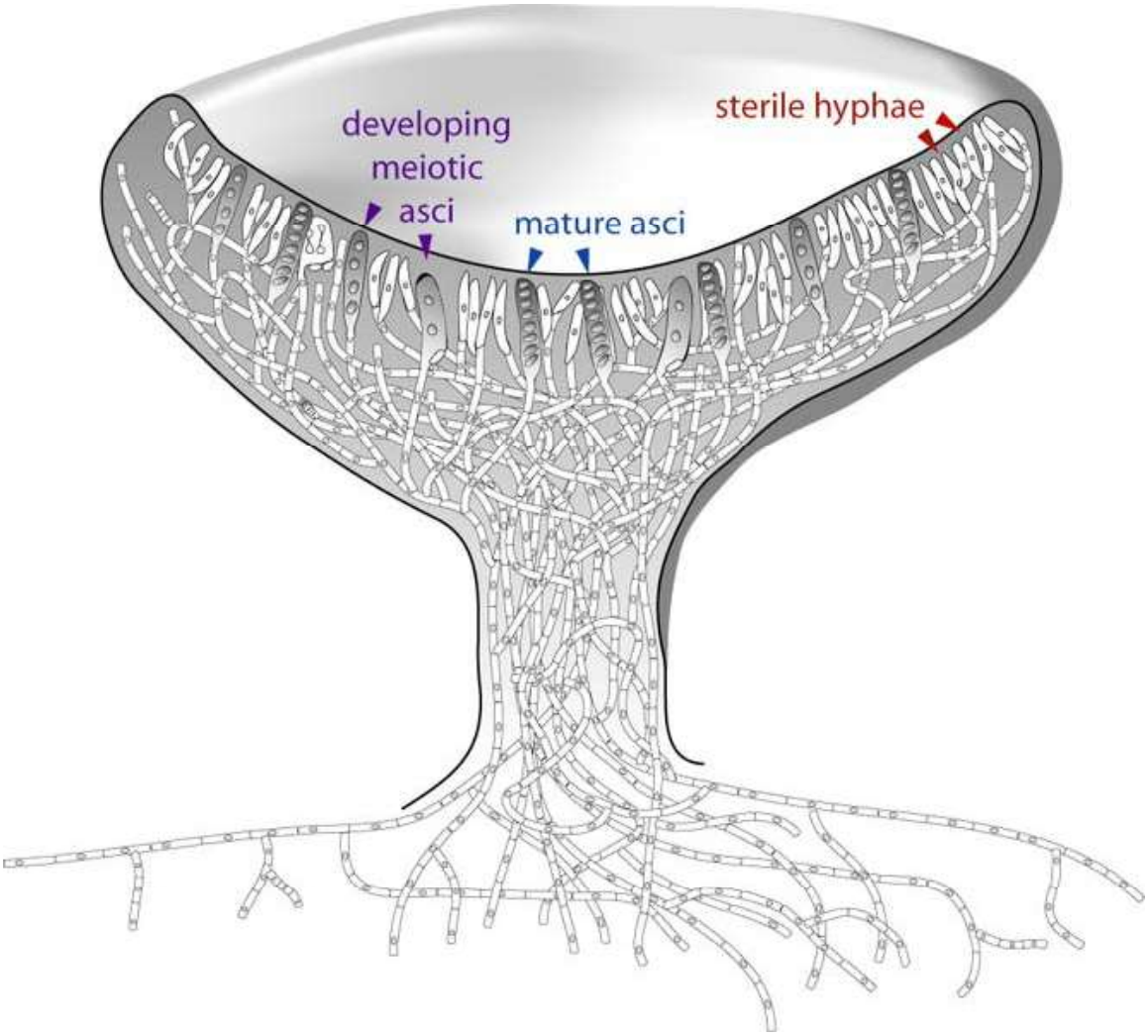


Diagram of an apothecium (the typical cup-like reproductive structure of Ascomycetes) showing sterile tissues as well as developing and mature asci.

The Ascomycota, commonly known as sac fungi or ascomycetes, constitute the largest taxonomic group within the Eumycota. These fungi form meiotic spores called ascospores, which are enclosed in a special sac-like structure called an ascus. This phylum includes morels, a few mushrooms and truffles, single-celled yeasts (e.g., of the genera *Saccharomyces*, *Kluyveromyces*, *Pichia*, and *Candida*), and many filamentous fungi living as saprotrophs, parasites, and mutualistic symbionts. Prominent and important genera of filamentous ascomycetes include *Aspergillus*, *Penicillium*, *Fusarium*, and *Claviceps*. Many ascomycete species have only been observed undergoing asexual reproduction (called anamorphic species), but analysis of molecular data has often been able to identify their closest teleomorphs in the Ascomycota. Because the products of meiosis are retained within the sac-like ascus, ascomycetes have been used for elucidating principles of genetics and heredity (e.g. *Neurospora crassa*).

Members of the Basidiomycota, commonly known as the club fungi or basidiomycetes, produce meiospores called basidiospores on club-like stalks called basidia. Most common mushrooms belong to this group, as well as rust and smut fungi, which are major pathogens of grains. Other important basidiomycetes include the maize pathogen *Ustilago maydis*, human commensal species of the genus *Malassezia*, and the opportunistic human pathogen, *Cryptococcus neoformans*.

Fungus-like organisms

Because of similarities in morphology and lifestyle, the slime molds (myxomycetes) and water molds (oomycetes) were formerly classified in the kingdom Fungi. Unlike true fungi the cell walls of these organisms contain cellulose and lack chitin. Myxomycetes are unikonts like fungi, but are grouped in the Amoebozoa. Oomycetes are diploid bikonts, grouped in the Chromalveolate kingdom. Neither water molds nor slime molds are closely related to the true fungi, and, therefore, taxonomists no longer group them in the kingdom Fungi. Nonetheless, studies of the oomycetes and myxomycetes are still often included in mycology textbooks and primary research literature.

The nucleariids, currently grouped in the Choanozoa, may be a sister group to the eumycete clade, and as such could be included in an expanded fungal kingdom.

Ecology

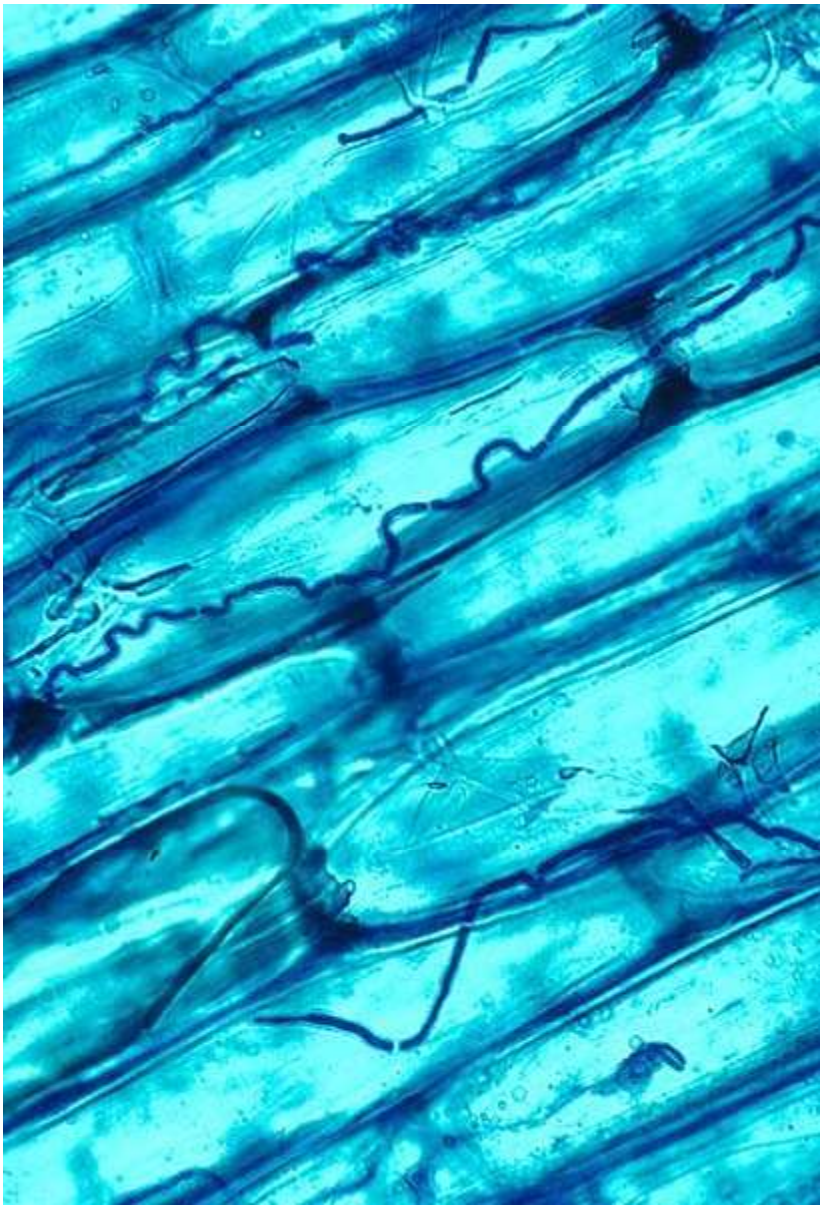
Although often inconspicuous, fungi occur in every environment on Earth and play very important roles in most ecosystems. Along with bacteria, fungi are the major decomposers in most terrestrial (and some aquatic) ecosystems, and therefore play a critical role in biogeochemical cycles and in many food webs. As decomposers, they play an essential role in nutrient cycling, especially as saprotrophs and symbionts, degrading organic matter to inorganic molecules, which can then re-enter anabolic metabolic pathways in plants or other organisms.

Symbiosis

Many fungi have important symbiotic relationships with organisms from most if not all Kingdoms. These interactions can be mutualistic or antagonistic in nature, or in the case of commensal fungi are of no apparent benefit or detriment to the host.

With plants

Mycorrhizal symbiosis between plants and fungi is one of the most well-known plant–fungus associations and is of significant importance for plant growth and persistence in many ecosystems; over 90% of all plant species engage in mycorrhizal relationships with fungi and are dependent upon this relationship for survival.



The dark filaments are hyphae of the endophytic fungus *Neotyphodium coenophialum* in the intercellular spaces of tall fescue leaf sheath tissue

The mycorrhizal symbiosis is ancient, dating to at least 400 million years ago. It often increases the plant's uptake of inorganic compounds, such as nitrate and phosphate from soils having low concentrations of these key plant nutrients. The fungal partners may also mediate plant-to-plant transfer of carbohydrates and other nutrients. Such mycorrhizal communities are called "common mycorrhizal networks". A special case of mycorrhiza is myco-heterotrophy, whereby the plant parasitizes the fungus, obtaining all of its nutrients from its fungal symbiont. Some fungal species inhabit the tissues inside roots, stems, and leaves, in which case they are called endophytes. Similar to mycorrhiza, endophytic colonization by fungi may benefit both symbionts; for example, endophytes of grasses

impart to their host increased resistance to herbivores and other environmental stresses and receive food and shelter from the plant in return.

With algae and cyanobacteria

The lichen *Lobaria pulmonaria*, a symbiosis of fungal, algal, and cyanobacterial species

Lichens are formed by a symbiotic relationship between algae or cyanobacteria (referred to in lichen terminology as "photobionts") and fungi (mostly various species of ascomycetes and a few basidiomycetes), in which individual photobiont cells are embedded in a tissue formed by the fungus. Lichens occur in every ecosystem on all continents, play a key role in soil formation and the initiation of biological succession, and are the dominating life forms in extreme environments, including polar, alpine, and semiarid desert regions. They are able to grow on inhospitable surfaces, including bare soil, rocks, tree bark, wood, shells, barnacles and leaves. As in mycorrhizas, the photobiont provides sugars and other carbohydrates via photosynthesis, while the fungus provides minerals and water. The functions of both symbiotic organisms are so closely intertwined that they function almost as a single organism; in most cases the resulting organism differs greatly from the individual components. Lichenization is a common mode of nutrition; around 20% of fungi—between 17,500 and 20,000 described species—are lichenized. Characteristics common to most lichens include obtaining organic carbon by photosynthesis, slow growth, small size, long life, long-lasting (seasonal) vegetative reproductive structures, mineral nutrition obtained largely from

airborne sources, and greater tolerance of desiccation than most other photosynthetic organisms in the same habitat.

With insects

Many insects also engage in mutualistic relationships with fungi. Several groups of ants cultivate fungi in the order Agaricales as their primary food source, while ambrosia beetles cultivate various species of fungi in the bark of trees that they infest. Similarly, females of several wood wasp species (genus *Sirex*) inject their eggs together with spores of the wood-rotting fungus *Amylostereum areolatum* into the sapwood of pine trees; the growth of the fungus provides ideal nutritional conditions for the development of the wasp larvae. Termites on the African savannah are also known to cultivate fungi, and yeasts of the genera *Candida* and *Lachancea* inhabit the gut of a wide range of insects, including neuropterans, beetles, and cockroaches; it is not known whether these fungi benefit their hosts.

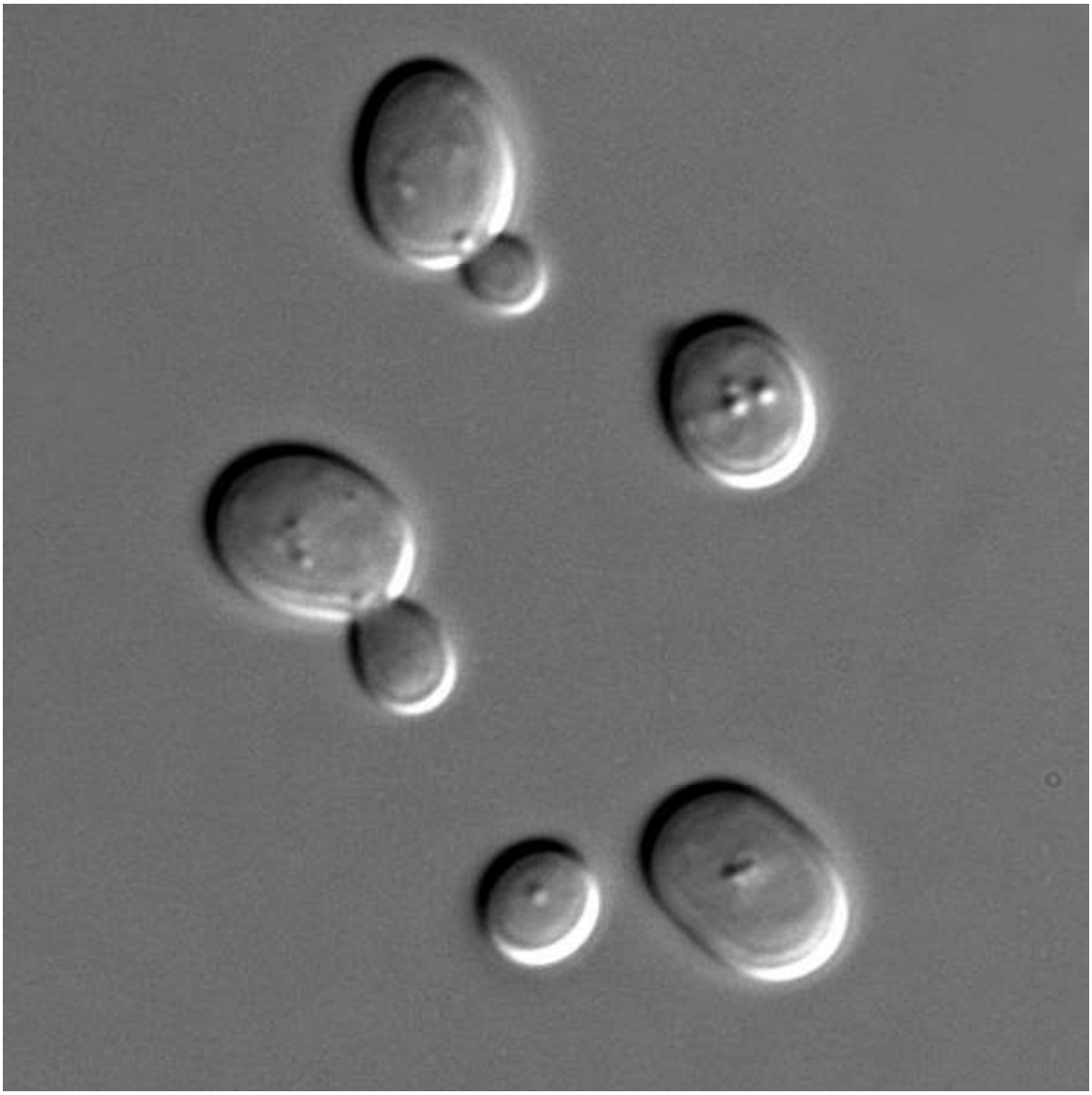
As pathogens and parasites

The plant pathogen *Aecidium magellanicum* causes calafate rust, seen here on a *Berberis* shrub in Chile.

Many fungi are parasites on plants, animals (including humans), and other fungi. Serious pathogens of many cultivated plants causing extensive damage and losses to agriculture and forestry include the rice blast fungus *Magnaporthe oryzae*, tree pathogens such as *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* causing Dutch elm disease, and *Cryphonectria parasitica* responsible for chestnut blight, and plant pathogens in the genera *Fusarium*, *Ustilago*, *Alternaria*, and *Cochliobolus*. Some carnivorous fungi, like *Paecilomyces lilacinus*, are predators of nematodes, which they capture using an array of specialized structures such as constricting rings or adhesive nets.

Some fungi can cause serious diseases in humans, several of which may be fatal if untreated. These include aspergilloses, candidoses, coccidioidomycosis, cryptococcosis, histoplasmosis, mycetomas, and paracoccidioidomycosis. Furthermore, persons with immuno-deficiencies are particularly susceptible to disease by genera such as *Aspergillus*, *Candida*, *Cryptoccocus*, *Histoplasma*, and *Pneumocystis*. Other fungi can attack eyes, nails, hair, and especially skin, the so-called dermatophytic and keratinophilic fungi, and cause local infections such as ringworm and athlete's foot. Fungal spores are also a cause of allergies, and fungi from different taxonomic groups can evoke allergic reactions.

Human use



Saccharomyces cerevisiae cells shown with DIC microscopy.

The human use of fungi for food preparation or preservation and other purposes is extensive and has a long history. Mushroom farming and mushroom gathering are large industries in many countries. The study of the historical uses and sociological impact of fungi is known as ethnomycology. Because of the capacity of this group to produce an enormous range of natural products with antimicrobial or other biological activities, many species have long been used or are being developed for industrial production of antibiotics, vitamins, and anti-cancer and cholesterol-lowering drugs. More recently, methods have been developed for genetic engineering of fungi, enabling metabolic engineering of fungal species. For example, genetic modification of yeast species—which are easy to grow at fast rates in large fermentation vessels—has opened up ways of

pharmaceutical production that are potentially more efficient than production by the original source organisms.

Drugs

Many species produce metabolites that are major sources of pharmacologically active drugs. Particularly important are the antibiotics, including the penicillins, a structurally related group of β-lactam antibiotics that are synthesized from small peptides. Although naturally occurring penicillins such as penicillin G (produced by *Penicillium chrysogenum*) have a relatively narrow spectrum of biological activity, a wide range of other penicillins can be produced by chemical modification of the natural penicillins. Modern penicillins are semisynthetic compounds, obtained initially from fermentation cultures, but then structurally altered for specific desirable properties. Other antibiotics produced by fungi include: ciclosporin, commonly used as an immunosuppressant during transplant surgery; and fusidic acid, used to help control infection from methicillin-resistant *Staphylococcus aureus* bacteria. Widespread use of these antibiotics for the treatment of bacterial diseases, such as tuberculosis, syphilis, leprosy, and many others began in the early 20th century and continues to play a major part in anti-bacterial chemotherapy. In nature, antibiotics of fungal or bacterial origin appear to play a dual role: at high concentrations they act as chemical defense against competition with other microorganisms in species-rich environments, such as the rhizosphere, and at low concentrations as quorum-sensing molecules for intra- or interspecies signaling.

Other drugs produced by fungi include griseofulvin isolated from *Penicillium griseofulvum*, used to treat fungal infections, and statins (HMG-CoA reductase inhibitors), used to inhibit cholesterol synthesis. Examples of statins found in fungi include mevastatin from *Penicillium citrinum* and lovastatin from *Aspergillus terreus* and the oyster mushroom.

Cultured foods

Baker's yeast or *Saccharomyces cerevisiae*, a single-celled fungus, is used to make bread and other wheat-based products, such as pizza dough and dumplings. Yeast species of the genus *Saccharomyces* are also used to produce alcoholic beverages through fermentation. Shoyu koji mold (*Aspergillus oryzae*) is an essential ingredient in brewing Shoyu (soy sauce) and sake, and the preparation of miso, while *Rhizopus* species are used for making tempeh. Several of these fungi are domesticated species that were bred or selected according to their capacity to ferment food without producing harmful mycotoxins, which are produced by very closely related *Aspergilli*. Quorn, a meat substitute, is made from *Fusarium venenatum*.

Medicinal use

The medicinal fungi *Ganoderma lucidum* (left) and *Cordyceps sinensis* (right).

Certain mushrooms enjoy usage as therapeutics in folk medicines, such as Traditional Chinese medicine. Notable medicinal mushrooms with a well-documented history of use include *Agaricus subrufescens*,[1] *Ganoderma lucidum*, and *Cordyceps sinensis*. Research has identified compounds produced by these and other fungi that have inhibitory biological effects against viruses and cancer cells. Specific metabolites, such as polysaccharide-K, ergotamine, and β-lactam antibiotics, are routinely used in clinical medicine. The shiitake mushroom is a source of lentinan, a clinical drug approved for use in cancer treatments in several countries, including Japan. In Europe and Japan, polysaccharide-K (brand name Krestin), a chemical derived from *Trametes versicolor*, is an approved adjuvant for cancer therapy.

Edible and poisonous species

Amanita phalloides accounts for the majority of fatal mushroom poisonings worldwide.

Edible mushrooms are well-known examples of fungi. Many are commercially raised, but others must be harvested from the wild. *Agaricus bisporus*, sold as button mushrooms when small or Portobello mushrooms when larger, is a commonly eaten species, used in salads, soups, and many other dishes. Many Asian fungi are commercially grown and have increased in popularity in the West. They are often available fresh in grocery stores and markets, including straw mushrooms (*Volvariella volvacea*), oyster mushrooms (*Pleurotus ostreatus*), shiitakes (*Lentinula edodes*), and enokitake (*Flammulina* spp.).

There are many more mushroom species that are harvested from the wild for personal consumption or commercial sale. Milk mushrooms, morels, chanterelles, truffles, black trumpets, and *porcini* mushrooms (*Boletus edulis*) (also known as king boletes) demand a high price on the market. They are often used in gourmet dishes.

Certain types of cheeses require inoculation of milk curds with fungal species that impart a unique flavor and texture to the cheese. Examples include the blue color in cheeses such as Stilton or Roquefort, which are made by inoculation with *Penicillium roqueforti*. Molds used in cheese production are non-toxic and are thus safe for human consumption; however, mycotoxins (e.g., aflatoxins, roquefortine C, patulin, or others) may accumulate because of growth of other fungi during cheese ripening or storage.

Stilton cheese veined with *Penicillium roqueforti*

Many mushroom species are poisonous to humans, with toxicities ranging from slight digestive problems or allergic reactions as well as hallucinations to severe organ failures and death. Genera with mushrooms containing deadly toxins include *Conocybe*, *Galerina*, *Lepiota*, and most infamously, *Amanita*. The latter genus includes the destroying angel *(A. virosa)* and the death cap *(A. phalloides)*, the most common cause of deadly mushroom poisoning. The false morel (*Gyromitra esculenta*) is occasionally considered a delicacy when cooked, yet can be highly toxic when eaten raw. *Tricholoma*

equestre was considered edible until being implicated in serious poisonings causing rhabdomyolysis. Fly agaric mushrooms (*Amanita muscaria*) also cause occasional non-fatal poisonings, mostly as a result of ingestion for use as a recreational drug for its hallucinogenic properties. Historically, fly agaric was used by different peoples in Europe and Asia and its present usage for religious or shamanic purposes is reported from some ethnic groups such as the Koryak people of north-eastern Siberia.

As it is difficult to accurately identify a safe mushroom without proper training and knowledge, it is often advised to assume that a wild mushroom is poisonous and not to consume it.

Pest control

Grasshoppers killed by *Beauveria bassiana*

In agriculture, fungi may be useful if they actively compete for nutrients and space with pathogenic microorganisms such as bacteria or other fungi via the competitive exclusion principle, or if they are parasites of these pathogens. For example, certain species may be used to eliminate or suppress the growth of harmful plant pathogens, such as insects, mites, weeds, nematodes and other fungi that cause diseases of important crop plants. This has generated strong interest in practical applications that use these fungi in the biological control of these agricultural pests. Entomopathogenic fungi can be used as biopesticides, as they actively kill insects. Examples that have been used as biological insecticides are *Beauveria bassiana*, *Metarhizium* spp, *Hirsutella* spp, *Paecilomyces* (*Isaria*) spp, and *Lecanicillium lecanii*. Endophytic fungi of grasses of the genus *Neotyphodium*, such as *N. coenophialum*, produce alkaloids that are toxic to a range of invertebrate and vertebrate herbivores. These alkaloids protect grass plants from herbivory, but several endophyte alkaloids can poison grazing animals, such as cattle and sheep. Infecting cultivars of pasture or forage grasses with *Neotyphodium* endophytes is one approach being used in grass breeding programs; the fungal strains are selected for producing only alkaloids that increase resistance to herbivores such as insects, while being non-toxic to livestock.

Bioremediation

Certain fungi, in particular "white rot" fungi, can degrade insecticides, herbicides, pentachlorophenol, creosote, coal tars, and heavy fuels and turn them into carbon dioxide, water, and basic elements. Fungi have been shown to biomineralize uranium oxides, suggesting they may have application in the bioremediation of radioactively polluted sites.

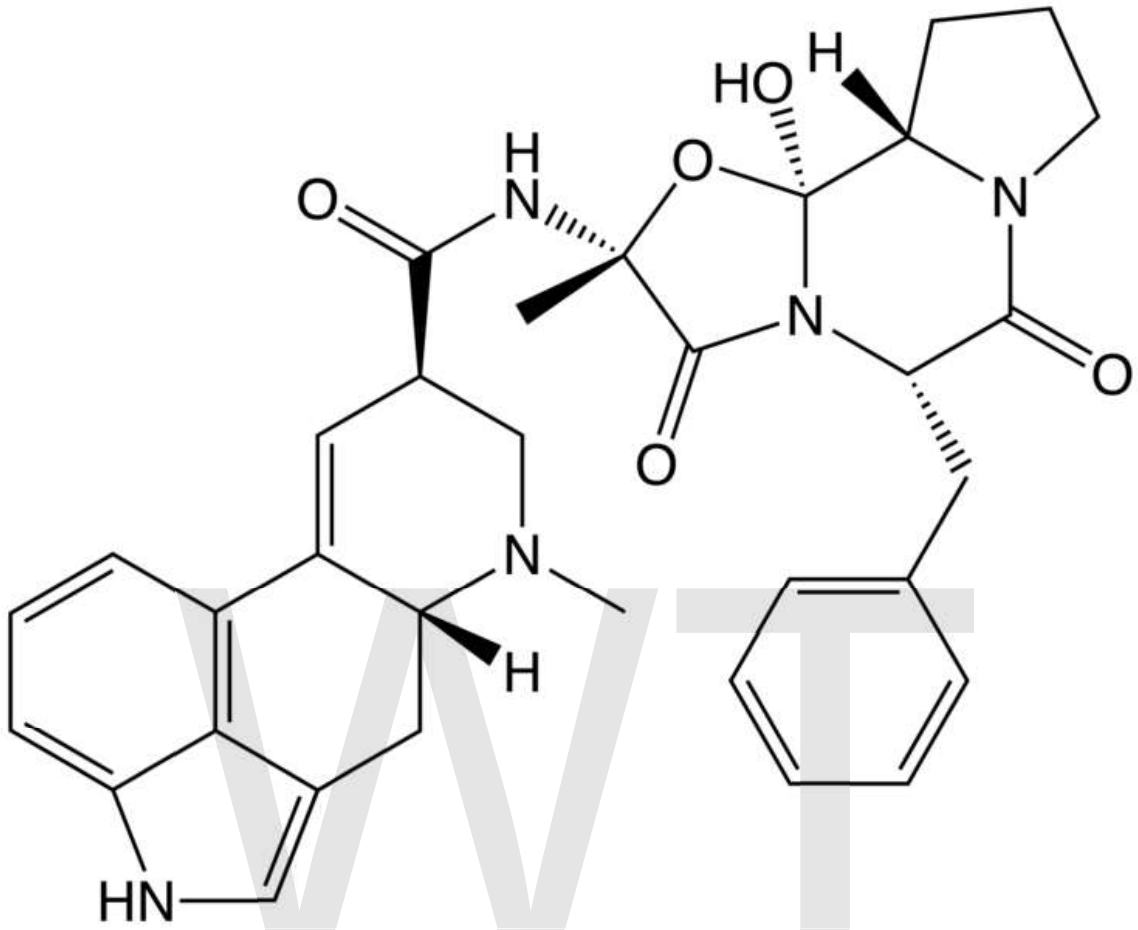
Model organisms

Several pivotal discoveries in biology were made by researchers using fungi as model organisms, that is, fungi that grow and sexually reproduce rapidly in the laboratory. For example, the one gene-one enzyme hypothesis was formulated by scientists who used the bread mold *Neurospora crassa* to test their biochemical theories. Other important model fungi are *Aspergillus nidulans* and the yeasts, *Saccaromyces cerevisiae* and *Schizosaccharomyces pombe*, each of which has a long history of use to investigate issues in eukaryotic cell biology and genetics, such as cell cycle regulation, chromatin structure, and gene regulation. Other fungal models have more recently emerged that each address specific biological questions relevant to medicine, plant pathology, and industrial uses; examples include *Candida albicans*, a dimorphic, opportunistic human pathogen, *Magnaporthe grisea*, a plant pathogen, and *Pichia pastoris*, a yeast widely used for eukaryotic protein expression.

Others

Fungi are used extensively to produce industrial chemicals like citric, gluconic, lactic, and malic acids, and industrial enzymes, such as lipases used in biological detergents, cellulases used in making cellulosic ethanol and stonewashed jeans, and amylases, invertases, proteases and xylanases. Several species, most notably *Psilocybin mushrooms* (colloquially known as *magic mushrooms*), are ingested for their psychedelic properties, both recreationally and religiously.

Mycotoxins



Ergotamine, a major mycotoxin produced by *Claviceps* species, which if ingested can cause gangrene, convulsions, and hallucinations

Many fungi produce biologically active compounds, several of which are toxic to animals or plants and are therefore called mycotoxins. Of particular relevance to humans are mycotoxins produced by molds causing food spoilage, and poisonous mushrooms. Particularly infamous are the lethal amatoxins in some *Amanita* mushrooms, and ergot alkaloids, which have a long history of causing serious epidemics of ergotism (St Anthony's Fire) in people consuming rye or related cereals contaminated with sclerotia of the ergot fungus, *Claviceps purpurea*. Other notable mycotoxins include the aflatoxins, which are insidious liver toxins and highly carcinogenic metabolites produced by certain *Aspergillus* species often growing in or on grains and nuts consumed by humans, ochratoxins, patulin, and trichothecenes (e.g., T-2 mycotoxin) and fumonisins, which have significant impact on human food supplies or animal livestock.

Mycotoxins are secondary metabolites (or natural products), and research has established the existence of biochemical pathways solely for the purpose of producing mycotoxins and other natural products in fungi. Mycotoxins may provide fitness benefits in terms of

physiological adaptation, competition with other microbes and fungi, and protection from consumption (fungivory).

Mycology

Mycology is the branch of biology concerned with the systematic study of fungi, including their genetic and biochemical properties, their taxonomy, and their use to humans as a source of medicine, food, and psychotropic substances consumed for religious purposes, as well as their dangers, such as poisoning or infection. The field of phytopathology, the study of plant diseases, is closely related because many plant pathogens are fungi.

Use of fungi by humans dates back to prehistory; Ötzi the Iceman, a well-preserved mummy of a 5,300 year old Neolithic man found frozen in the Austrian Alps, carried two species of polypore mushrooms that may have been used as tinder (*Fomes fomentarius*), or for medicinal purposes (*Piptoporus betulinus*). Ancient peoples have used fungi as food sources–often unknowingly–for millennia, in the preparation of leavened bread and fermented juices. Some of the oldest written records contain references to the destruction of crops that were probably caused by pathogenic fungi.

History

Mycology is a relatively new science that became systematic after the development of the microscope in the 16th century. Although fungal spores were first observed by Giambattista della Porta in 1588, the seminal work in the development of mycology is considered to be the publication of Pier Antonio Micheli's 1729 work *Nova plantarum genera*. Micheli not only observed spores, but showed that under the proper conditions, they could be induced into growing into the same species of fungi from which they originated. Extending the use of the binomial system of nomenclature introduced by Carl Linnaeus in his *Species plantarum* (1753), the Dutch Christian Hendrik Persoon (1761–1836) established the first classification of mushrooms with such skill so as to be considered a founder of modern mycology. Later, Elias Magnus Fries (1794–1878) further elaborated the classification of fungi, using spore color and various microscopic characteristics, methods still used by taxonomists today. Other notable early contributors to mycology in the 17th–19th and early 20th centuries include Miles Joseph Berkeley, August Carl Joseph Corda, Anton de Bary, the brothers Louis René and Charles Tulasne, Arthur H. R. Buller, Curtis G. Lloyd, and Pier Andrea Saccardo. The 20th century has seen a modernization of mycology that has come from advances in biochemistry, genetics, molecular biology, and biotechnology. The use of DNA sequencing technologies and phylogenetic analysis has provided new insights into fungal relationships and biodiversity, and has challenged traditional morphology-based groupings in fungal taxonomy.

Chapter 12

Ascomycota

Ascomycota

Sarcoscypha coccinea

Scientific classification	
Domain:	Eukarya
Kingdom:	Fungi
Subkingdom:	Dikarya
Phylum:	**Ascomycota** (Berk. 1857) Caval.-Sm. 1998

Subphyla/Classes

Pezizomycotina
Arthoniomycetes
Dothideomycetes
Eurotiomycetes
Geoglossomycetes
Laboulbeniomycetes
Lecanoromycetes
Leotiomycetes

Lichinomycetes
Orbiliomycetes
Pezizomycetes
Sordariomycetes
"Unplaced orders"
Lahmiales
Medeolariales
Triblidiales
Saccharomycotina
Saccharomycetes
Taphrinomycotina
Neolectomycetes
Pneumocystidomycetes
Schizosaccharomycetes
Taphrinomycetes

The **Ascomycota** are a Division/Phylum of the kingdom Fungi, and subkingdom Dikarya. Its members are commonly known as the **Sac fungi**. They are the largest phylum of Fungi, with over 64,000 species. The defining feature of this fungal group is the "ascus" (from Greek: ἀσκός (*askos*), meaning "sac" or "wineskin"), a microscopic sexual structure in which nonmotile spores, called ascospores, are formed. However, some species of the Ascomycota are asexual, meaning that they do not have a sexual cycle and thus do not form asci or ascospores. Previously placed in the Deuteromycota along with asexual species from other fungal taxa, asexual (or anamorphic) ascomycetes are now identified and classified based on morphological or physiological similarities to ascus-bearing taxa, and by phylogenetic analyses of DNA sequences.

The ascomycetes are a monophyletic group, i.e., all of its members trace back to one common ancestor. This group is of particular relevance to humans as sources for medicinally important compounds, such as antibiotics and for making bread, alcoholic beverages, and cheese, but also as pathogens of humans and plants. Familiar examples of sac fungi include morels, truffles, brewer's yeast and baker's yeast, Dead Man's Fingers, and cup fungi. The fungal symbionts in the majority of lichens (loosely termed "ascolichens") such as *Cladonia* belong to the Ascomycota. There are many plant-pathogenic ascomycetes, including apple scab, rice blast, the ergot fungi, black knot, and the powdery mildews. Several species of ascomycetes are biological model organisms in laboratory research. Most famously *Neurospora crassa*, several species of yeasts, and *Aspergillus* species are used in many genetics and cell biology studies. *Penicillium* species on cheeses and those producing antibiotics for treating bacterial infectious diseases are examples of taxa that belong to the Ascomycota.

Ascomycetes versus Ascomycota

Before the recognition of the fungal kingdom, the sac fungi were considered to be a *Class*, not a *Phylum*. The original collective term for these taxa was "Ascomycetes", which was first coined in the 1800s for a rankless nonlichenized taxon that possessed

asci. The names Ascomycota, Ascomycetes, and others with the same root are based upon the term "ascus". "Ascomycetes" was soon used to include lichenized taxa, and became the standard term, at the class level, for all ascus-bearing species, just as the term "Basidiomycetes" became used for their basidium-bearing counterparts. Elevation of the taxonomic rank of the Ascomycetes resulted in the names Ascomycetae, Ascomycotina, and finally Ascomycota. Together, the Ascomycota and the Basidiomycota form the subkingdom Dikarya. The more familiar term, Ascomycetes, is still loosely used, e.g. at fungal forays it is often said of a fungus, such as *Peziza*, "It is an ascomycete, not a basidiomycete" in reference to their sexual reproductive mode. The terms are further abbreviated to "ascos" and "basidos" which are not officially sanctioned technical names.

Modern classification of Ascomycota

There are three subphyla that are described and accepted:

- The *Pezizomycotina* is the largest subphylum and contains all ascomycetes that produce ascocarps (fruiting bodies), except for one genus, *Neolecta*, in the Taphrinomycotina. It is roughly equivalent to the previous taxon, *Euascomycetes*. The Pezizomycotina includes most macroscopic "ascos" such as truffles, ergot, ascolichens, cup fungi (discomycetes), pyrenomycetes, lorchels, and caterpillar fungus. It also contains microscopic fungi such as powdery mildews, dermatophytic fungi, and Laboulbeniales.
- The *Saccharomycotina* comprises most of the "true" yeasts, such as baker's yeast and *Candida* which are single-celled (unicellular) fungi, which reproduce vegetatively by budding. Most of these species were previously classified in a taxon called *Hemiascomycetes*.
- The *Taphrinomycotina* includes a disparate and basal group within the Ascomycota that was recognized following molecular (DNA) analyses. The taxon was originally named *Archiascomycetes* (or *Archaeascomycetes*). It includes both hyphal fungi (*Neolecta*, *Taphrina*), fission yeasts (*Schizosaccharomyces*), and the mammalian lung parasite, *Pneumocystis*.

Ribosomal RNA gene sequencing of soil suggests that there may be a fourth subphylum of Ascomycota (termed Soil Clone Group I or SCGI), that has not been described in cultures or based on fruiting bodies. SCGI organisms are only known from DNA sequences found in soils worldwide and are placed between the Taphriomycotina and the Saccharomycotina.

Outdated taxon names

Several outdated taxon names—based on morphological features—are still occasionally used for species of the Ascomycota. These include the following sexual (teleomorphic) groups, defined by the structures of their sexual fruiting bodies: the Discomycetes, which included all species forming apothecia; the Pyrenomycetes, which included all sac fungi that formed perithecia or pseudothecia, or any structure resembling these morphological structures; and the Plectomycetes, which included those species that form cleistothecia.

Hemiascomycetes included the yeasts and yeast-like fungi that have now been placed into the Saccharomycotina or Taphrinomycotina, while the Euascomycetes included the remaining species of the Ascomycota which are now in the Pezizomycotina, and the Neolecta which are in the Taphrinomycotina.

Some ascomycetes do not reproduce sexually or are not known to produce asci and are therefore anamorphic species. Those anamorphs that produce conidia (mitospores) were previously described as Mitosporic Ascomycota. Some taxonomists placed this group into a separate artificial phylum, the Deuteromycota (or "Fungi Imperfecti"). Where recent molecular analyses have identified close relationships with ascus-bearing taxa, anamorphic species have been grouped into the Ascomycota, despite the absence of the defining ascus. Sexual and asexual isolates of the same species commonly carry different binomial species names, as, for example, *Aspergillus nidulans* and *Emericella nidulans*, for asexual and sexual isolates, respectively, of the same species.

Species of the Deuteromycota were classified as Coelomycetes if they produced their conidia in minute flask- or saucer-shaped conidiomata, known technically as *pycnidia* and *acervuli*. The Hyphomycetes were those species where the conidiophores (*i.e.*, the hyphal structures that carry conidia-forming cells at the end) are free or loosely organized. They are mostly isolated but sometimes also appear as bundles of cells aligned in parallel (described as *synnematal*) or as cushion-shaped masses (described as *sporodochial*).

Morphology

Most species grow as filamentous, microscopic structures called hyphae. Many interconnected hyphae form a mycelium, which—when visible to the naked eye (macroscopic)—is commonly called mold (or, in botanical terminology, thallus). During sexual reproduction, many Ascomycota typically produce large numbers of asci. The asci is often contained in a multicellular, occasionally readily visible fruiting structure, the ascocarp (also called an *ascoma*). Ascocarps come in a very large variety of shapes: cup-shaped, club-shaped, potato-like, spongy, seed-like, oozing and pimple-like, coral-like, nit-like, golf-ball-shaped, perforated tennis ball-like, cushion-shaped, plated and feathered in miniature (Laboulbeniales), microscopic classic Greek shield-shaped, stalked or sessile. They can appear solitary or clustered. Their texture can likewise be very variable, including fleshy, like charcoal (carbonaceous), leathery, rubbery, gelatinous, slimy, powdery, or cob-web-like. Ascocarps come in multiple colors such as red, orange, yellow, brown, black, or, more rarely, green or blue. Some ascomyceous fungi, such as *Saccharomyces cerevisiae*, grow as single-celled yeasts, which—during sexual reproduction—develop into an ascus, and do not form fruiting bodies.

The "candlesnuff fungus", *Xylaria hypoxylon*

In lichenized species, the thallus of the fungus defines the shape of the symbiotic colony. Some dimorphic species, such as *Candida albicans*, can switch between growth as single cells and as filamentous, multicellular hyphae. Other species are pleomorphic, exhibiting asexual (anamorphic) as well as a sexual (teleomorphic) growth forms.

Except for lichens, the non-reproductive (vegetative) mycelium of most ascomycetes is usually inconspicuous because it is commonly embedded in the substrate, such as soil, or grows on or inside a living host, and only the ascoma may be seen when fruiting. Pigmentation, such as melanin in hyphal walls, along with prolific growth on surfaces can result in visible mold colonies; examples include *Cladosporium* species, which form black spots on bathroom caulking and other moist areas. Many ascomycetes cause food spoilage, and, therefore, the pellicles or moldy layers that develop on jams, juices, and other foods are the mycelia of these species or occasionally Mucoromycotina and almost never Basidiomycota. Sooty molds that develop on plants, especially in the tropics are the thalli of many species.

The ascocarp of a morel contains numerous apothecia.

Large masses of yeast cells, asci or ascus-like cells, or conidia can also form macroscopic structures. For example. *Pneumocystis* species can colonize lung cavities (visible in x-rays), causing a form of pneumonia. Asci of *Ascosphaera* fill honey bee larvae and pupae causing mummification with a chalk-like appearance, hence the name "chalkbrood". Yeasts for small colonies in vitro and in vivo and excessive growth of *Candida* species in the mouth or vagina causes "thrush", a form of candidiasis.

The cell walls of the ascomycetes almost always contain chitin and β-glucans, and divisions within the hyphae, called "septa", are the internal boundaries of individual cells (or compartments). The cell wall and septa give stability and rigidity to the hyphae and may prevent loss of cytoplasm in case of local damage to cell wall and cell membrane.

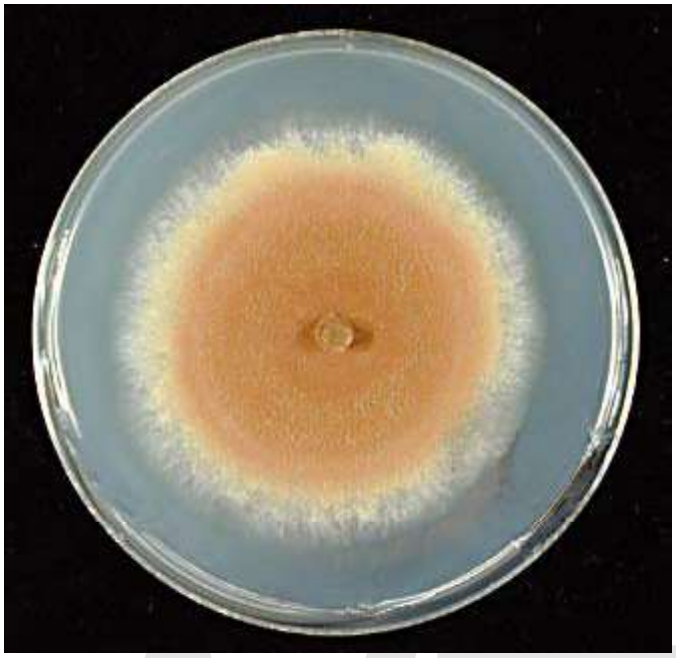
The septa commonly have a small opening in the center, which functions as a cytoplasmic connection between adjacent cells, also sometimes allowing cell-to-cell movement of nuclei within a hypha. Vegetative hyphae of most ascomycetes contain only one nucleus per cell (*uninucleate* hyphae), but multinucleate cells—especially in the apical regions of growing hyphae—can also be present.

Metabolism

In common with other fungal phyla, the Ascomycota are heterotrophic organisms that require organic molecules as energy sources. These are obtained by feeding on a variety of organic substrates including dead matter, foodstuffs, or as symbionts in or on other living organisms. To obtain these nutrients from their surroundings, ascomycetous fungi secrete powerful digestive enzymes which break down organic substances into smaller molecules, which are then taken up into the cell. Many species live on dead plant material such as leaves, twigs, or logs. Several species colonize plants, animals, or other fungi as parasites or mutualistic symbionts and derive all their metabolic energy in form of nutrients from the tissues of their hosts.

Owing to their long evolutionary history, the Ascomycota have evolved the capacity to break down almost every organic substance. Unlike most organisms, they are able to use their own enzymes to digest plant biopolymers such as cellulose or lignin. Collagen, an abundant structural protein in animals, and keratin—a protein that forms hair and nails—, can also serve as food sources. Unusual examples include *Aureobasidium pullulans*, which feeds on wall paint, and the kerosene fungus *Amorphotheca resinae*, which feeds on aircraft fuel (causing occasional problems for the airline industry), and may sometimes block fuel pipes. Other species can resist high osmotic stress and grow, for example, on salted fish, and a few ascomycetes are aquatic.

The Ascomycota is characterized by a high degree of specialization; for instance, certain species of Laboulbeniales attack only one particular leg of one particular insect species. Many Ascomycota engage in symbiotic relationships such as in lichens—symbiotic associations with green algae or cyanobacteria—in which the fungal symbiont directly obtains products of photosynthesis. In common with many basidiomycetes and Glomeromycota, some ascomycetes form symbioses with plants by colonizing the roots to form mycorrhizal associations. The Ascomycota also represents several carnivorous fungi, which have developed hyphal traps to capture small protists such as amoebae, as well as roundworms (*Nematoda*), rotifers, tardigrades, and small arthropods such as springtails (*Collembola*).



Hypomyces completus on culture medium

Distribution and living environment

The Ascomycota are represented in all land ecosystems worldwide, occurring on all continents including Antarctica. Spores and hyphal fragments are dispersed through the atmosphere and freshwater environments, as well as ocean beaches and tidal zones. The distribution of species is variable; while some are found on all continents, others, as for example the white truffle *Tuber magnatum*, only occur in isolated locations in Italy and Eastern Europe. The distribution of plant-parasitic species is often restricted by host distributions; for example, *Cyttaria* is only found on *Nothofagus* (Southern Beech) in the Southern Hemisphere.

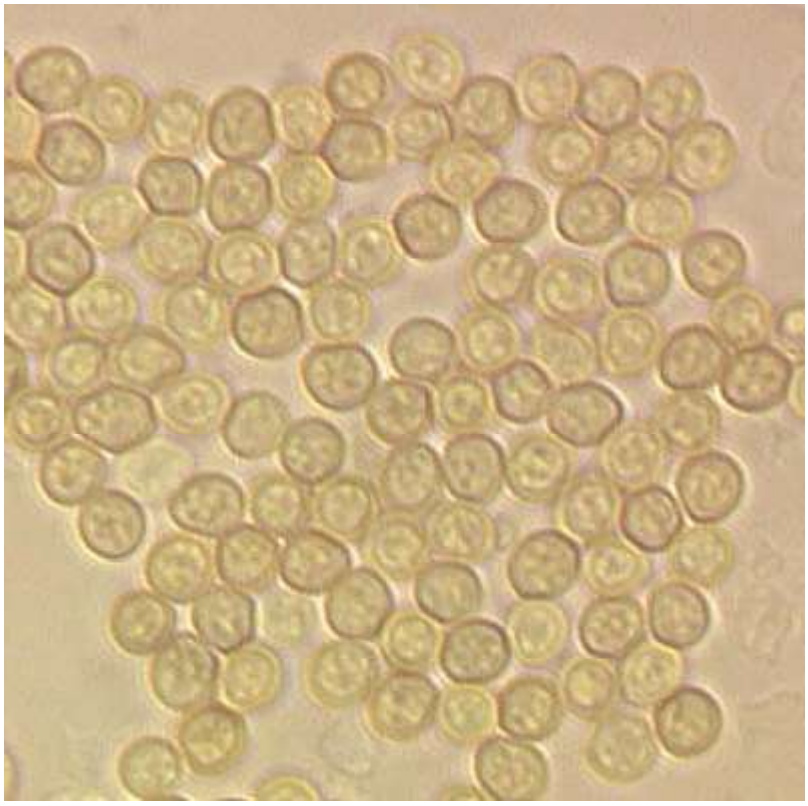
Reproduction

Asexual reproduction

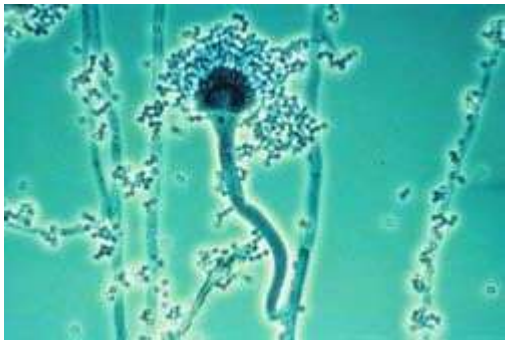
Asexual reproduction is the dominant form of propagation in the Ascomycota, and is responsible for the rapid spread of these fungi into new areas. It occurs through vegetative reproductive spores, the conidia. The conidiospores commonly contain one nucleus and are products of mitotic cell divisions and thus are sometimes call mitospores, which are genetically identical to the mycelium from which they originate. They are typically formed at the ends of specialized hyphae, the *conidiophores*. Depending on the species they may be dispersed by wind or water, or by animals.

Asexual spores

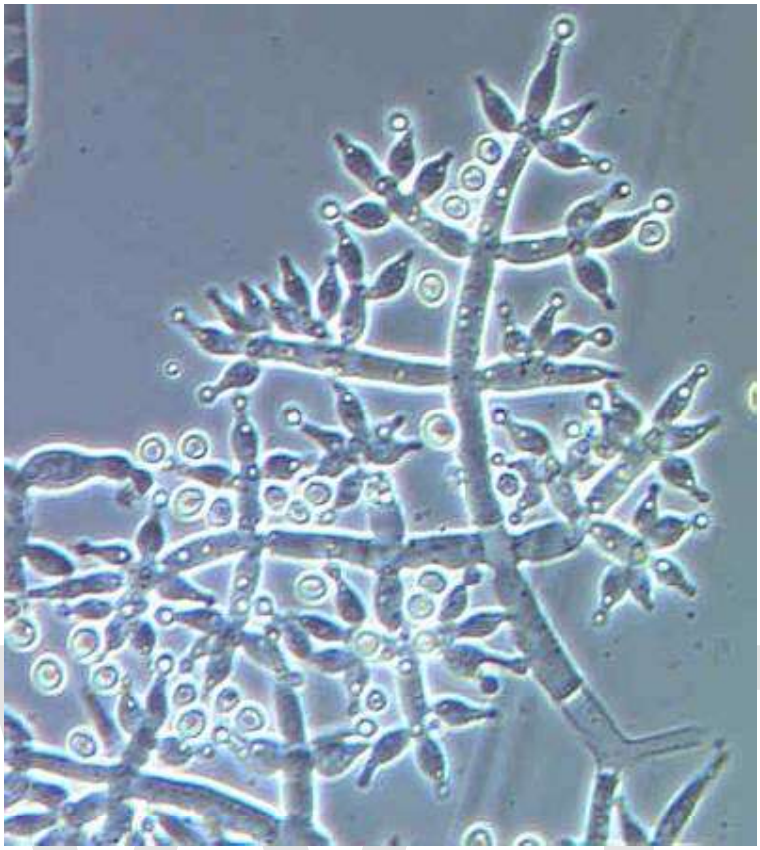
Different types of asexual spores can be identified by colour, shape, and how they are released as individual spores. Spore types can be used as taxonomic characters in the classification within the Ascomycota. The most frequent types are the single-celled spores, which are designated *amerospores*. If the spore is divided into two by a cross-wall (septum), it is called a *didymospore*.



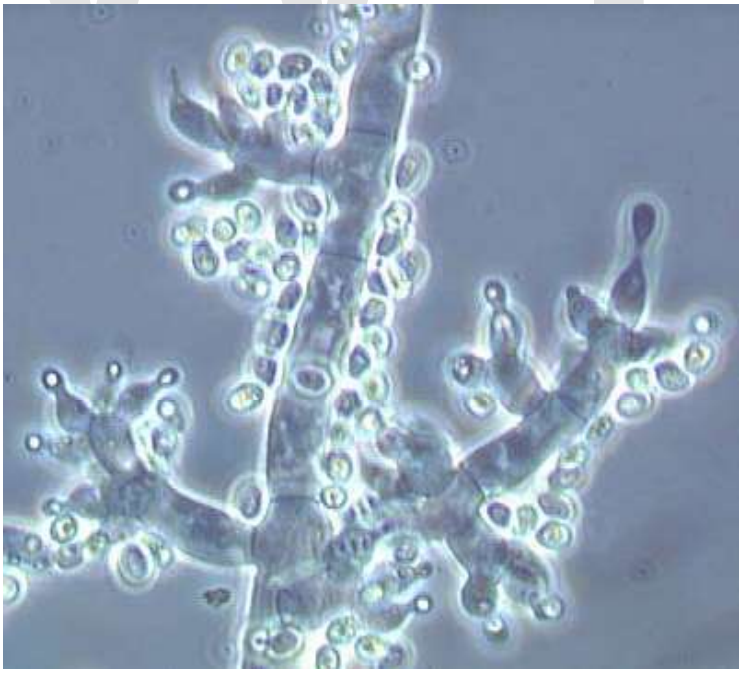
Conidiospores of *Trichoderma aggressivum*, Diameter approx. 3µm



Conidiophores of molds of the genus *Aspergillus*, conidiogenesis is blastic-phialidic



Conidiophores of *Trichoderma harzianum*, conidiogenesis is blastic-phialidic



Conidiophores of *Trichoderma fertile* with vase-shaped phialides and newly formed conidia on their ends (bright points)

When there are two or more cross-walls, the classification depends on spore shape. If the septa are *transversal*, like the rungs of a ladder, it is a *phragmospore*, and if they possess a net-like structure it is a *dictyospore*. In *staurospores* ray-like arms radiate from a central body; in others (*helicospores*) the entire spore is wound up in a spiral like a spring. Very long worm-like spores with a length-to-diameter ratio of more than 15:1, are called *scolecospores*.

Conidiogenesis and dehiscence

Important characteristics of the anamorphs of the Ascomycota are *conidiogenesis*, which includes spore formation and dehiscence (separation from the parent structure). Conidiogenesis corresponds to Embryology in animals and plants and can be divided into two fundamental forms of development: *blastic* conidiogenesis, where the spore is already evident before it separates from the conidiogenic hypha, and *thallic* conidiogenesis, during which a cross-wall forms and the newly created cell develops into a spore. The spores may or may not be generated in a large-scale specialized structure which helps to spread them.

These two basic types can be further classified as follows:

- *blastic-acropetal* (repeated budding at the tip of the conidiogenic hypha, so that a chain of spores is formed with the youngest spores at the tip),
- *blastic-synchronous* (simultaneous spore formation from a central cell, sometimes with secondary acropetal chains forming from the initial spores),
- *blastic-sympodial* (repeated sideways spore formation from behind the leading spore, so that the oldest spore is at the main tip),
- *blastic-annellidic* (each spore separates and leaves a ring-shaped scar which is inside the scar left by the previous spore),
- *blastic-phialidic* (the spores arise and are ejected from the open ends of special conidiogenic cells called phialides which remain constant in length),
- *basauxic* (where a chain of conidia, in successively younger stages of development, is emitted from the mother cell),
- *blastic-retrogressive* (spores separate by formation of crosswalls near the tip of the conidiogenic hypha, which thus becomes progressively shorter),
- *thallic-arthric* (double cell walls split the conidiogenic hypha into cells which develop into short, cylindrical spores called *arthroconidia*; sometimes every second cell dies off, leaving the arthroconidia free),
- *thallic-solitary* (a large bulging cell separates from the conidiogenic hypha, forms internal walls, and develops to a *phragmospore*).

Sometimes the conidia are produced in structures visible to the naked eye, which help to distribute the spores. These structures are called "conidiomata" (singular: conidioma), and may take the form of *pycnidia* (which are flask-shaped and arise in the fungal tissue) or *acervuli* (which are cushion-shaped and arise in host tissue).

Dehiscence happens in two ways. In *schizolytic* dehiscence, a double-dividing wall with a central lamella (layer) forms between the cells; the central layer then breaks down thereby releasing the spores. In*rhexolytic* dehiscence, the cell wall which joins the spores on the outside degenerates and releases the conidia.

Heterokaryosis and parasexuality

Several Ascomycota species are not known to have a sexual cycle. Such asexual species may be able to undergo genetic recombination between individuals by processes involving *heterokaryosis* and *parasexual* events.

Parasexuality refers to the process of heterokaryosis, caused by merging of two hyphae belonging to different individuals, by a process called *anastomosis*, followed by a series of events resulting in genetically different cell nuclei in the mycelium. The merging of nuclei is not followed by meiotic events, such as gamete formation and results in an increased number of chromosomes per nuclei. *Mitotic crossover* may enable recombination, i.e., an exchange of genetic material between homologous chromosomes. The chromosome number may then be restored to its haploid state by nuclear division, with each daughter nuclei being genetically different from the original parent nuclei. Alternatively, nuclei may lose some chromosomes, resulting in aneuploid cells.

Sexual reproduction

Ascus of *Hypocrea virens* with eight two-celled Ascospores

Sexual reproduction in the Ascomycota leads to the formation of the *ascus*, the structure that defines this fungal group and distinguishes it from other fungal phyla. The ascus is a tube-shaped vessel, a *meiosporangium*, which contains the sexual spores produced by meiosis and which are called *ascospores*.

Apart from a few exceptions, such as *Candida albicans*, most ascomycetes are haploid, i.e., they contain one set of chromosomes per nuclei. During sexual reproduction there is a diploid phase which commonly is very short, and meiosis restores the haploid state.

Formation of sexual spores

The sexual part of the life cycle commences when two hyphal structures mate. In the case of *homothallic* species, mating is enabled between hyphae of the same fungal clone, whereas in *heterothallic* species, the two hyphae must originate from fungal clones that differ genetically, i.e., those that are of a different mating type. Mating types are typical of the fungi and correspond roughly to the sexes in plants and animals; however one species may have more than two mating types, resulting in sometimes complex vegetative incompatibility systems.

Gametangia are sexual structures formed from hyphae, and are the generative cells. A very fine hypha, called trichogyne emerges from one gametangium, the *ascogonium*, and merges with a gametangium (the *antheridium*) of the other fungal isolate. The nuclei in the antheridium then migrate into the ascogonium, and plasmogamy—the mixing of the cytoplasm—occurs. Unlike in animals and plants, plasmogamy is not immediately followed by the merging of the nuclei (called *karyogamy*). Instead, the nuclei from the two hyphae form pairs, initiating the *dikaryophase* of the sexual cycle, during which time the pairs of nuclei synchronously divide. Fusion of the paired nuclei leads to mixing of the genetic material and recombination and is followed by meiosis. A similar sexual cycle is present in the red algae (Rhodophyta).

Unitunicate-inoperculate Asci of *Hypomyces chrysospermus*

From the fertilized ascogonium, *dinucleate* hyphae emerge in which each cell contains two nuclei. These hyphae are called *ascogenous* or fertile hyphae. They are supported by the vegetative mycelium containing uni– (or mono–) nucleate hyphae, which are sterile. The mycelium containing both sterile and fertile hyphae may grows into fruiting body, the *ascocarp*, which may contain millions of fertile hyphae.

The sexual structures are formed in the fruiting layer of the ascocarp, the hymenium. At one end of ascogenous hyphae, characteristic U-shaped hooks develop, which curve back opposite to the growth direction of the hyphae. The two nuclei contained in the apical part of each hypha divide in such a way that the threads of their mitotic spindles run parallel, creating two pairs of genetically different nuclei. One daughter nucleus migrates close to the hook, while the other daughter nucleus locates to the basal part of the hypha. The formation of two parallel cross-walls then divides the hypha into three sections: one at the hook with one nucleus, one at the basal of the original hypha that contains one nucleus, and one that separates the U-shaped part which contains the other two nuclei.

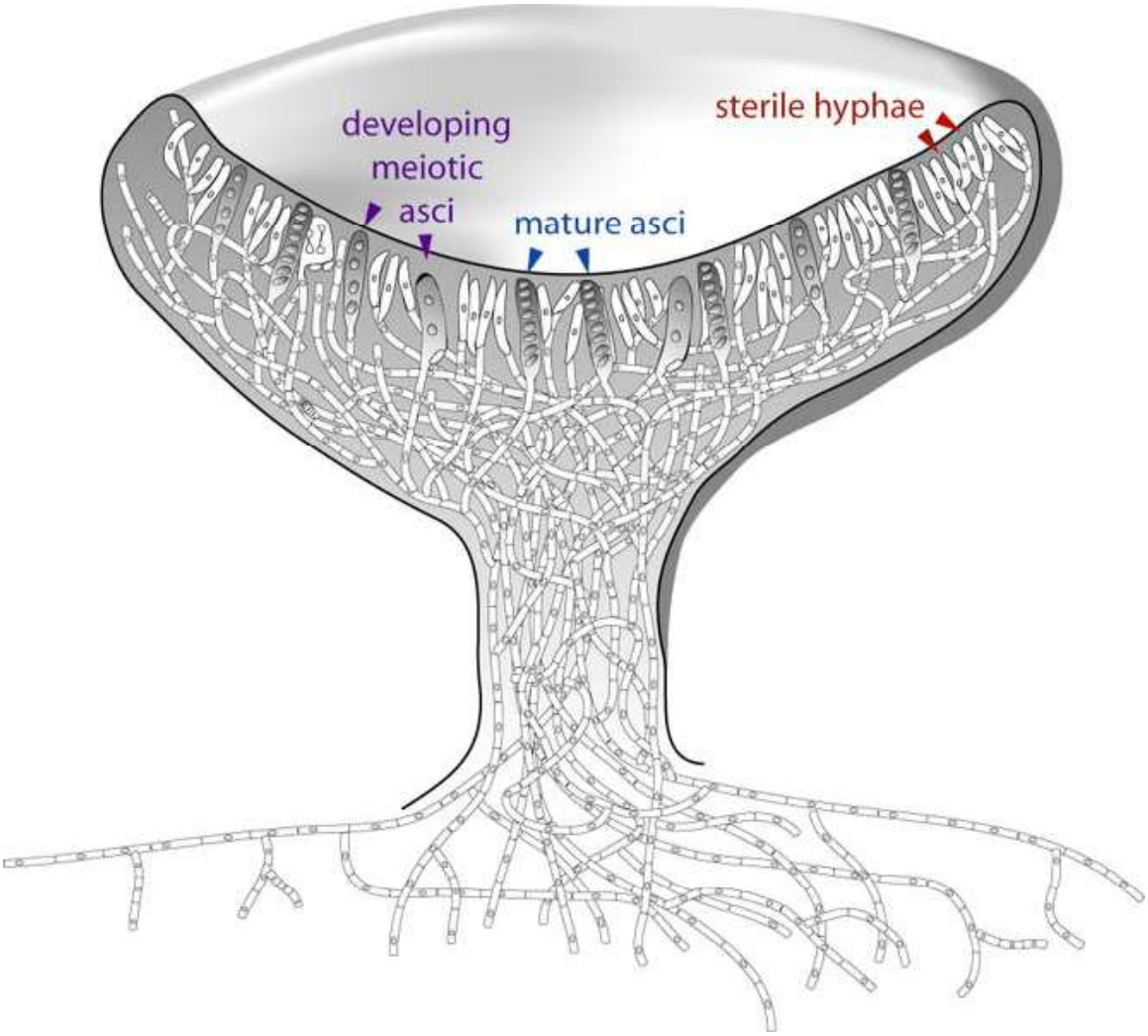


Diagram of an apothecium (the typical cup-like reproductive structure of Ascomycetes) showing sterile tissues as well as developing and mature asci.

Fusion of the nuclei (karyogamy) takes place in the U-shaped cells in the hymenium, and results in the formation of a diploid zygote. The zygote grows into the ascus, an elongated tube-shaped or cylinder-shaped capsule. Meiosis then gives rise to four haploid nuclei, usually followed by a further mitotic division that results in eight nuclei in each ascus. The nuclei along with some cytoplasma become enclosed within membranes and a cell wall to give rise to ascospores that are aligned inside the ascus like peas in a pod.

Upon opening of the ascus, ascospores may be dispersed by the wind, while in some cases the spores are forcibly ejected form the ascus; certain species have evolved spore cannons, which can eject ascospores up to 30 cm. away. When the spores reach a suitable substrate, they germinate, form new hyphae, which restarts the fungal life cycle.

The form of the ascus is important for classification and is divided into four basic types: unitunicate-operculate, unitunicate-inoperculate, bitunicate, or prototunicate.

Ecology

The Ascomycota fulfil a central role in most land-based ecosystems. They are important decomposers which break down organic materials, such as dead leaves and animals, and help the detritivores (animals which feed on decomposing material) to obtain their nutrients. Ascomycetes along with other fungi can break down large molecules such as cellulose or lignin, and thus have important roles in nutrient cycling such as the carbon cycle.

The fruiting bodies of the Ascomycota provide food for many animals ranging from insects and slugs and snails (*Gastropoda*) to rodents and larger mammals such as deer and wild boars.

Many ascomycetes also form symbiotic relationships with other organisms, including plants and animals.

Lichens

Probably since early in their evolutionary history, the Ascomycota have formed symbiotic associations with green algae (*Chlorophyta*), and other types of algae and cyanobacteria. These mutualistic associations are commonly known as lichens, and can grow and persist in terrestrial regions of the earth that are inhospitable to other organisms and characterized by extremes in temperature and humidity, including the Arctic, the Antarctic, deserts, and mountaintops. While the photoautotrophic algal partner generates metabolic energy through photosynthesis, the fungus offers a stable, supportive matrix and protects cells from radiation and dehydration. Around 42% of the Ascomycota (about 18,000 species) form lichens, and almost all the fungal partners of lichens belong to the Ascomycota.

Mycorrhizal fungi and endophytes

Members of the Ascomycota form two important types of relationship with plants: as mycorrhizal fungi and as endophytes. Mycorrhiza are symbiotic associations of fungi with the root systems of the plants, which can be of vital importance for growth and persistence for the plant. The fine mycelial network of the fungus enables the increased uptake of mineral salts that occur at low levels in the soil. In return, the plant provides the fungus with metabolic energy in the form of photosynthetic products.

Endophytic fungi live inside plants, and those that form mutualistic or commensal associations with their host, do not damage their hosts. The exact nature of the relationship between endophytic fungus and host depends on the species involved, and in some cases fungal colonization of plants can bestow a higher resistance against insects, roundworms (nematodes), and bacteria; in the case of grass endophytes the fungal symbiont produces poisonous alkaloids, which can affect the health of plant-eating (herbivorous) mammals and deter or kill insect herbivores.

Symbiotic relationships with animals

Several ascomycetes of the genus *Xylaria* colonize the nests of leafcutter ants and other fungus-growing ants of the tribe Attini, and the fungal gardens of termites (Isoptera). Since they do not generate fruiting bodies until the insects have left the nests, it is suspected that, as confirmed in several cases of Basidiomycota species, they may be cultivated.

Bark beetles (family Scolytidae) are important symbiotic partners of ascomycetes. The female beetles transport fungal spores to new hosts in characteristic tucks in their skin, the *mycetangia*. The beetle tunnels into the wood and into large chambers in which they lay their eggs. Spores released from the mycetangia germinate into hyphae, which can break down the wood. The beetle larvae then feed on the fungal mycelium, and, on reaching maturity, carry new spores with them to renew the cycle of infection. A well-known example of this is Dutch elm disease, caused by *Ophiostoma ulmi*, which is carried by the European elm bark beetle, *Scolytus multistriatus*.

Importance for humans

Tree attacked by the Bluestain fungus, *Ophiostoma minus*

Ascomycetes make many contributions to the good of humanity, and also have many ill effects.

Harmful interactions

One of their most harmful roles is as the agent of many plant diseases. For instance:

- Dutch Elm Disease, caused by the closely related species *Ophiostoma ulmi* and *Ophiostoma novo-ulmi*, has led to the death of many elms in Europe and North America.

Claviceps purpurea on rye (*Secale cereale*)

- The originally Asian *Cryphonectria parasitica* is responsible for attacking Sweet Chestnuts (*Castanea sativa*), and virtually eliminated the once-widespread American Chestnut (*Castanea dentata*),
- A disease of Maize (*Zea mays*), which is especially prevalent in North America, is brought about by *Cochliobolus heterostrophus*.
- *Taphrina deformans* causes leaf curl of peach.
- *Uncinula necator* is responsible for the disease Powdery mildew, which attacks grapevines.
- Species of *Monilia* cause brown rot of stone fruit such as peaches (*Prunus persica*) and sour cherries (*Prunus ceranus*).

- Members of the Ascomycota such as *Stachybotrys chartarum* are responsible for fading of woollen textiles, which is a common problem especially in the tropics.
- Blue-green, red and brown moulds attack and spoil foodstuffs - for instance *Penicillium italicum* rots oranges.
- Cereals infected with *Fusarium graminearum* contain mycotoxins like deoxynivalenol (DON), which can lead to skin and mucous membrane lesions when eaten by pigs.
- Ergot (*Claviceps purpurea*) is a direct menace to humans when it attacks wheat or rye and produces highly poisonous and carcinogenic alkaloids, causing ergotism if consumed. Symptoms include hallucinations, stomach cramp, and a burning sensation in the limbs ("Saint Anthony's Fire").
- *Aspergillus flavus*, which grows on peanuts and other hosts, generates aflatoxin, which damages the liver and is highly carcinogenic.
- *Candida albicans*, a yeast which attacks the mucous membranes, can cause an infection of the mouth or vagina called thrush or candidiasis, and is also blamed for "yeast allergies".
- Fungi like *Epidermophyton* cause skin infections but are not very dangerous for people with healthy immune systems. However if the immune system is damaged they can be life-threatening; for instance, *Pneumocystis jiroveci* is responsible for severe lung infections which occur in AIDS patients.

Positive effects

On the other hand, ascus fungi have brought some important benefits to humanity.

- The most famous case may be that of the mould *Penicillium chrysogenum* (formerly *Penicillium notatum*), which, probably to attack competing bacteria, produces an antibiotic which, under the name of Penicillin, triggered a revolution in the treatment of bacterial infectious diseases in the 20th century.
- The medical importance of *Tolypocladium niveum* as an immunosuppressor can hardly be exaggerated. It excretes Ciclosporin, which, as well as being given during organ transplants to prevent rejection, is also prescribed for auto-immune diseases such as multiple sclerosis, although there is some doubt over the long-term side-effects of the treatment.

Stilton cheese veined with *Penicillium roqueforti*

- Some ascomycete fungi can be altered relatively easily through genetic engineering procedures. They can then produce useful proteins such as insulin, human growth hormone, or TPa, which is employed to dissolve blood clots.
- Several species are common model organisms in biology, including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Neurospora crassa*. The genomes of a number of ascomycete fungi have been fully sequenced.
- Baker's Yeast (*Saccharomyces cerevisiae*) is used to make bread, beer and wine, during which process sugars such as glucose or sucrose are fermented to make ethanol and carbon dioxide. Bakers use the fungus for the carbon dioxide production, causing the bread to rise, with the ethanol boiling off during cooking. Most vintners use it for the ethanol production, with the carbon dioxide being released into the atmosphere during fermentation. Brewers and traditional producers of sparkling wine use both, with a primary fermentation for the alcohol and a secondary one to produce the carbon dioxide bubbles that provide the drinks with "sparkling" texture in the case of wine and the desirable foam in the case of beer.
- Enzymes of *Penicillium camemberti* play a role in the manufacture of the cheeses Camembert and Brie, while those of *Penicillium roqueforti* do the same for Gorgonzola, Roquefort and Stilton.

- In Asia *Aspergillus oryzae* is added to a pulp of soaked soya beans to make soy sauce.
- Finally, some members of the Ascomycota are eaten with relish; morelss (*Morchella*) and truffles (*Tuber*) are some of the most sought-after fungus delicacies.

Chapter 13

Basidiomycota

Basidiomycota

Basidiomycetes from Ernst Haeckel's 1904 *Kunstformen der Natur*

Scientific classification

Domain:	Eukarya
Kingdom:	Fungi
Subkingdom:	Dikarya
Phylum:	**Basidiomycota** R.T. Moore, 1980

Subphyla/Classes

Agaricomycotina

Pucciniomycotina
Ustilaginomycotina
Incertae sedis (no phylum)

Wallemiomycetes

Basidiomycota is one of two large phyla that, together with the Ascomycota, comprise the subkingdom Dikarya (often referred to as the **"higher fungi"**) within the Kingdom Fungi. More specifically the Basidiomycota include mushrooms, puffballs, stinkhorns, bracket fungi, other polypores, jelly fungi, boletes, chanterelles, earth stars, smuts, bunts, rusts, mirror yeasts, and the human pathogenic yeast *Cryptococcus*. Basically, Basidiomycota are filamentous fungi composed of hyphae (except for those forming yeasts), and reproducing sexually via the formation of specialized club-shaped end cells called basidia that normally bear external meiospores (usually four). These specialized spores are called basidiospores. However, some Basidiomycota reproduce asexually, and may or may not also reproduce sexually. Asexually reproducing Basidiomycota (discussed below) can be recognized as members of this phylum by gross similarity to others, by the formation of a distinctive anatomical feature, cell wall components, and definitively by phylogenetic molecular analysis of DNA sequence data.

Classification

The most recent classification adopted by a coalition of 67 mycologists recognizes three subphyla (Pucciniomycotina, Ustilaginomycotina, Agaricomycotina) and two other class level taxa (Wallemiomycetes, Entorrhizomycetes) outside of these, among the Basidiomycota. As now classified, the subphyla join and also cut across various obsolete taxonomic groups previously commonly used to describe various Basidiomycota. According to a 2008 estimate, Basidiomycota comprises three subphyla (including six unassigned classes) 16 classes, 52 orders, 177 families, 1,589 genera, and 31,515 species.

The Basidiomycota had traditionally been divided into two obsolete classes, the Homobasidiomycetes (including true mushrooms); and the Heterobasidiomycetes (the jelly, rust and smut fungi). Previously the entire Basidiomycota were called **Basidiomycetes**, an invalid class level name coined in 1959 as a counterpart to the **Ascomycetes**, when neither of these taxa were recognized as phyla. The terms basidiomycetes and ascomycetes are frequently used loosely to refer to Basidiomycota and Ascomycota. They are often abbreviated to "basidios" and "ascos" as mycological slang.

Agaricomycotina

The Agaricomycotina include what had previously been called the Hymenomycetes (an obsolete morphological based class of Basidiomycota that formed hymenial layers on their fruitbodies), the Gasteromycetes (another obsolete class that included species mostly lacking hymenia and mostly forming spores in enclosed fruitbodies), as well as

most of the jelly fungi. The three classes in the Agaricomycotina are the Agaricomycetes, the Dacrymycetes, and the Tremellomycetes.

Pucciniomycotina

The Pucciniomycotina includes the rust fungi, the insect parasitic/symbiotic genus *Septobasidium*, a former group of smut fungi (in the Microbotryomycetes, which includes mirror yeasts), and a mixture of odd, infrequently seen or seldom recognized fungi, often parasitic on plants. The eight classes in the Pucciniomycotina are Agaricostilbomycetes, Atractiellomycetes, Classiculomycetes, Cryptomycocolacomycetes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes, and Pucciniomycetes.

Ustilaginomycotina

The Ustilaginomycotina are most (but not all) of the former smut fungi and along with the Exobasidiales. The classes of the Ustilaginomycotina are the Exobasidiomycetes, the Entorrhizomycetes, and the Ustilaginomycetes.

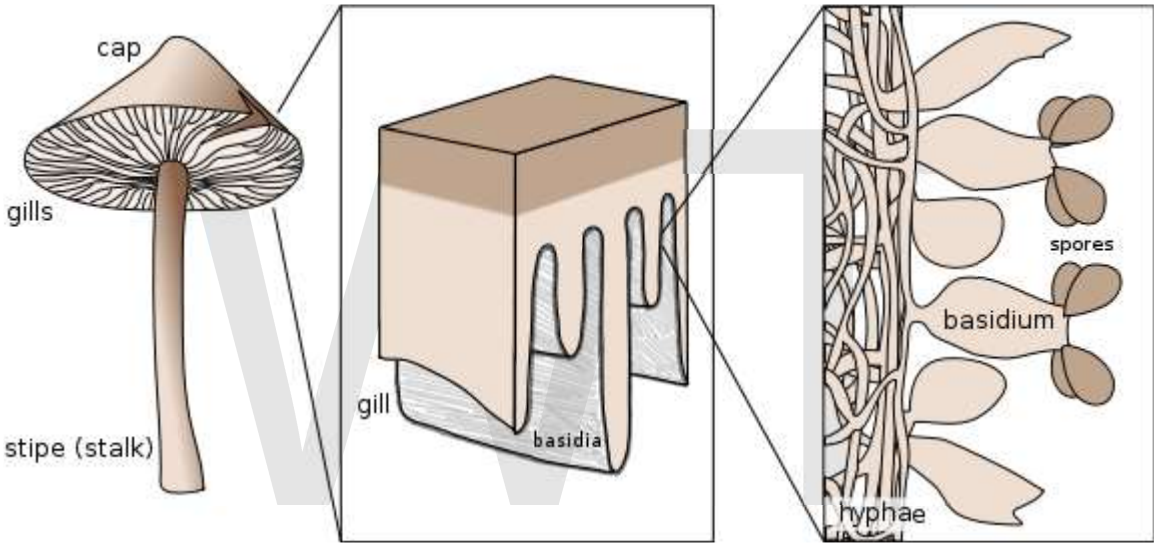
The class Wallemiomycetes is not yet placed in a subphylum.

Typical life-cycle

Unlike higher animals and plants which have readily recognizable male and female counterparts, Basidiomycota (except for the Rust (Pucciniales)) tend to have mutually indistinguishable, compatible haploids which are usually mycelia being composed of filamentous hyphae. Typically haploid Basidiomycota mycelia fuse via plasmogamy and then the compatible nuclei migrate into each other's mycelia and pair up with the resident nuclei. Karyogamy is delayed, so that the compatible nuclei remain in pairs, called a **dikaryon**. The hyphae are then said to be **dikaryotic**. Conversely, the haploid mycelia are called **monokaryons**. Often, the dikaryotic mycelium is more vigorous than the individual **monokaryotic** mycelia, and proceeds to take over the substrate in which they are growing. The dikaryons can be long-lived, lasting years, decades, or centuries. *The monokaryons are neither male nor female*. They have either a **bipolar** (**unifactorial**) or a **tetrapolar** (**bifactorial**) mating system. This results in the fact that following meiosis, the resulting haploid basidiospores and resultant monokaryons, have nuclei that are compatible with 50% (if bipolar) or 25% (if tetrapolar) of their sister basidiospores (and their resultant monokaryons) because the mating genes must differ for them to be compatible. However, there are many variations of these genes in the population, and therefore, over 90% of monokaryons are compatible with each other. It is as if there were multiple sexes.

The maintenance of the dikaryotic status in dikaryons in many Basidiomycota is facilitated by the formation of clamp connections that physically appear to help coordinate and re-establish pairs of compatible nuclei following synchronous mitotic nuclear divisions. Variations are frequent and multiple. In a typical Basidiomycota lifecycle the long lasting dikaryons periodically (seasonally or occasionally) produce

basidia, the specialized usually club-shaped end cells, in which a pair of compatible nuclei fuse (karyogamy) to form a diploid cell. Meiosis follows shortly with the production of 4 haploid nuclei that migrate into 4 external, usually apical basidiospores. Variations occur, however. Typically the basidiospores are ballistic, hence they are sometimes also called ballistospores. In most species, the basidiospores disperse and each can start a new haploid mycelium, continuing the lifecycle. Basidia are microscopic but they are often produced on or in multicelled large fructifications called basidiocarps or basidiomes, or fruitbodies), variously called mushrooms, puffballs, etc. Ballistic basidiospores are formed on **sterigmata** which are tapered spine-like projections on basidia, and are typically curved, like the horns of a bull. In some Basidiomycota the spores are not ballistic, and the sterigmata may be straight, reduced to stubbs, or absent. The basidiospores of these non-ballistosporic basidia may either bud off, or be released via dissolution or disintegration of the basidia.



Schematic of a typical basidiocarp, the dipoid reproductive structure of a basidiomycete, showing fruiting body, hymenium and basidia.

In summary, meiosis takes place in a diploid basidium. Each one of the four haploid nuclei migrates into its own basidiospore. The basidiospores are ballistically discharged and start new haploid mycelia called monokaryons. There are no males or females, rather there are compatible thalli with multiple compatibility factors. Plasmogamy between compatible individuals leads to delayed karyogamy leading to establishment of a dikaryon. The dikaryon is long lasting but ultimately gives rise to either fruitbodies with basidia or directly to basidia without fruitbodies. The paired dikaryon in the basidium fuse (i.e. karyogamy takes place). The diploid basidium begins the cycle again.

Variations in life-cycles

Many variations occur. Some are self compatible and spontaneously form dikaryons without a separate compatible thallus being involved. These fungi are said to be *homothallic*, versus the normal *heterothallic* species with mating types. Others are

secondarily homothallic, in that two compatible nuclei following meiosis migrate into each basidiospore, which is then dispersed as a pre-existing dikaryon. Often such species form only two spores per basidium, but that too varies. Following meiosis, mitotic divisions can occur in the basidium. Multiple numbers of basidiospores can result, including odd numbers via degeneration of nuclei, or pairing up of nuclei, or lack of migration of nuclei. For example, the chanterelle genus *Craterellus* often has 6-spored basidia, while some corticioid *Sistotrema* species can have 2-, 4-, 6-, or 8-spored basidia, and the cultivated button mushroom, *Agaricus bisporus*. can have 1-, 2-, 3- or 4-spored basidia under some circumstances. Occasionally monokaryons of some taxa can form morphologically fully formed basidiomes and anatomically correct basidia and ballistic basidiospores in the absence of dikaryon formation, diploid nuclei, and meiosis. A rare few number of taxa have extended diploid life-cycles, but can be common species. Examples exist in the mushroom genera *Armillaria* and *Xerula*, both in the Physalacriaceae. Occasionally basidiospores are not formed and parts of the "basidia" act as the dispersal agents, e.g. the peculiar mycoparasitic jelly fungus, *Tetragoniomyces* or the entire "basidium" acts as a "spore", e.g. in some false puffballs (*Scleroderma*). In the human pathogenic genus *Cryptococcus*, 4 nuclei following meiosis remain in the basidium but continually divide mitotically, each nucleus migrating into synchronously forming nonballistic basidiospores that are then pushed upwards by another set forming below them, resulting in 4 parallel chains of dry "basidiospores".

Other variations occur, some as standard life-cycles (that themselves have variations within variations) within specific orders.

Rusts

Rusts (Pucciniales, previously known as Uredinales) at their greatest complexity produce five different types of spores on two different hosts in two unrelated host families. Such rusts are heteroecious (requiring 2 hosts) and macrocyclic (producing all 5 spores types). Wheat stem rust is an example. By convention the stages and spore states are numbered by Roman numerals. Typically, basidiospores infect host one, the mycelium forms pycnidia, called spermagonia, which are miniature, flask-shaped, hollow, submicroscopic bodies embedded in host tissue (such as a leaf). This stage, numbered "0", produces single-celled, minute spores that ooze out in a sweet liquid and that act as nonmotile spermatia, and also protruding receptive hyphae. Insects and probably other vectors such as rain carry the spermatia from spermagonia to spermagonia, cross inoculating the mating types. Neither thallus is male or female. Once crossed, the dikaryons are established and a second spore stage is formed, numbered "I" and called aecia, which form dikaryotic aeciospores in dry chains in inverted cup-shaped bodies embedded in host tissue. These aeciospores then infect the second host genus and cannot infect the host on which they are formed (in macrocyclic rusts). On the second host a repeating spore stage is formed, numbered "II", the urediospores in dry pustules called uredinia. Urediospores are dikaryotic and can infect the same host that produced them. They repeatedly infect this host over the growing season. At the end of the season, a fourth spore type, the teliospore, is formed. It is thicker-walled and serves to overwinter or to survive other harsh conditions. It does not continue the infection process, rather it

remains dormant for a period and then germinates to form basidia (stage "IV"), sometimes called a promycelium. In the Pucciniales, the basidia are cylindrical and become 3-septate after meiosis, with each of the 4 cells bearing one basidiospore each. The basidospores disperse and start the infection process on host 1 again. Autoecious rusts complete their life-cycles on one host instead of two, and **microcyclic** rusts cut out one or more stages.

Smuts

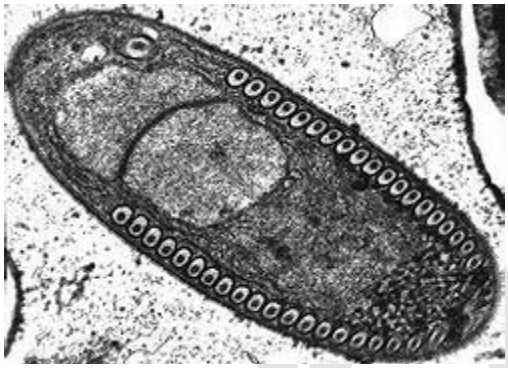
The characteristic part of the life-cycle of smuts is the thick-walled, often darkly pigmented, ornate, teliospore that serves to survive harsh conditions such as overwintering and also serves to help disperse the fungus as dry diaspores. The teliospores are initially dikaryotic but become diploid via karyogamy. Meiosis takes place at the time of germination. A promycelim is formed that consists to a short hypha (equated to a basidium). In some smuts such as *Ustilago maydis* the nuclei migrate into the promycelium that becomes septate, and haploid yeast-like conidia/basidiospores sometimes called sporidia, bud off laterally from each cell. In various smuts, the yeast phase may proliferate, or they may fuse, or they may infect plant tissue and become hyphal. In other smuts, such as *Tilletia caries*, the elongated haploid basidiospores form apically, often in compatible pairs that fuse centrally resulting in "H"-shaped diaspores which are by then dikaryotic. Dikaryotic conidia may then form. Eventually the host is infected by infectious hyphae. Teliospores form in host tissue. Many variations on these general themes occur.

Smuts with both a yeast phase and an infectious hyphal state are examples of dimorphic Basidiomycota. In plant parasitic taxa, the saprotrophic phase is normally the yeast while the infectious stage is hyphal. However, there are examples of animal and human parasites where the species are dimorphic but it is the yeast-like state that is infectious. The genus *Filobasidiella* forms basidia on hyphae but the main infectious stage is more commonly known by the anamorphic yeast name *Cryptococcus*, e.g. *Cryptococcus neoformans* and *Cryptococcus gattii*.

The dimorphic Basidiomycota with yeast stages and the pleiomorphic rusts are examples of fungi with anamorphs, which are the asexual stages. Some Basidiomycota are only known as anamorphs. Many are yeasts, collectively called basidiomycetous yeasts to differentiate them from ascomycetous yeasts in the Ascomycota. Aside from yeast anamorphs, and uredinia, aecia and pycnidia, some Basidiomycota form other distinctive anamorphs as parts of their life-cycles. Examples are *Collybia tuberosa* with its apple-seed-shaped and coloured sclerotium, *Dendrocollybia racemosa* with its sclerotium and its *Tilachlidiopsis racemosa* conidia, *Armillaria* with their rhizomorphs, *Hohenbuehelia* with their *Nematoctonus* nematode infectious, state and the coffee leaf parasite, *Mycena citricolor* and its *Decapitatus flavidus* propagules called gemmae.

Chapter 14

Microsporidia

Microsporidia

Sporoblast of *Fibrillanosema crangonycis*
Scientific classification
Kingdom: Fungi
Division: **Microsporidia**
Classes and orders
Dihaplophasea Dissociodihaplophasida Meiodihaplophasida Haplophasea Chyridiopsida Glugeida

The **microsporidia** constitute a phylum of spore-forming unicellular parasites. They were once thought to be protists but are now known to be fungi. Loosely 1500 of the probably more than one million species are named now. Microsporidia are restricted to animal hosts, and all major groups of animals host microsporidia. Most infect insects, but they are also responsible for common diseases of crustaceans and fish. The distinguished species of microsporidia usually infect one specific host or a related group of hosts. Several species, most of which are opportunistic, also infect humans.

Approximately 10 percent of the species are parasites of vertebrates, including in humans.

After infection they influence their hosts in various ways and all organs and tissues are invaded. Some species are lethal, and a few are used in biological control of insect pests. Parasitic castration, gigantism, change of host sex are effects of microsporidian parasitism. In the most advanced cases of parasitism the microsporidium rules the host cell completely and controls its metabolism and reproduction, forming a xenoma. .

Replication takes place within the host's cells, which are infected by means of unicellular spores. These vary from 1-40 μm, making them some of the smallest eukaryotes. They also have the smallest eukaryotic genomes.

Microsporidium was once the vernacular name for a member of the class Microsporea.

Xenoma on flatfish caused by *Glugea stephani*

Anatomy

Microsporidia lack mitochondria and possess, instead, mitosomes. They also lack motile structures such as flagella.
Microsporidia produce highly resistant spores to survive outside the host for up to several years. Spore morphology is useful in distinguishing between different species. Spores of

most species are oval or pyriform, but rod-shaped or spherical spores are not unusual. A few genera produce spores of unique shape for the genus.

The spore is protected by a wall, consisting of three layers:

- an outer electron-dense *exospore*
- a median, wide and seemingly structureless *endospore*, containing chitin
- a thin internal *plasma membrane*

In most cases there are two closely associated nuclei, forming a *diplokaryon*, but sometimes there is only one.
The anterior half of the spore contains a harpoon-like apparatus with a long thread-like *polar filament*, which is coiled up in the posterior half of the spore. The anterior part of the polar filament is surrounded by a *polaroplast*, a lamella of membranes. Behind the polar filament there is a posterior *vacuole*.

Infection

In the gut of the host the spore germinates. It builds up osmotic pressure until its rigid wall ruptures at its thinnest point at the apex. The posterior vacuole swells, forcing the polar filament to rapidly eject the infectious content into the cytoplasm of the potential host. Simultaneously the material of the filament is rearranged to form a tube which functions as a hypodermic needle and penetrates the gut epithelium.

Once inside the host cell, a sporoplasm grows, dividing or forming a multinucleate plasmodium, before producing new spores. The life cycle varies considerably. Some have a simple asexual life cycle, while others have a complex life cycle involving multiple hosts and both asexual and sexual reproduction. Different types of spores may be produced at different stages, probably with different functions including autoinfection (transmission within a single host).

Medical implications

The microsporidia often cause chronic, debilitating diseases rather than lethal infections. Effects on the host include reduced longevity, fertility, weight, and general vigor. Vertical transmission of microsporidia is frequently reported. In the case of insect hosts, vertical transmission often occurs as transovarial transmission, where the microsporidian parasites pass from the ovaries of the female host into eggs and eventually multiply in the infected larvae. *Amblyospora salinaria* n. sp. which infects the mosquito *Culex salinarius* Coquillett, and *Amblyospora californica* which infects the mosquito *Culex tarsalis* Coquillett, provide typical examples of transovarial transmission of microsporidia.

Microsporidia, specifically the mosquito-infecting *Vavraia culicis*, are being explored as a possible 'evolution-proof' malaria-control method. Microsporidian infection of *Anopheles gambiae* (the principal vector of *Plasmodium falciparum* malaria) reduces malarial infection within the mosquito, and shortens the mosquito lifespan. As the

majority of malaria-infected mosquitoes naturally die before the malaria parasite is mature enough to transmit, any increase in mosquito mortality through microsporidian-infection may reduce malaria in humans.

Classification

For some time microsporidia were considered as very primitive eukaryotes, especially because of the lack of mitochondria, and placed along with the other protozoa *diplomonads*, *parabasalia* and *archamoebae* in the protist-group **"Archezoa"**. More recent research has falsified this theory of early origin (for all of these). Yet microsporidia are proposed to be highly developed and specialized organisms, which just dispensed functions that are needed no longer, because they are supplied by the host. Furthermore, spore-forming organisms in general do have a complex system of reproduction, both sexual and asexual, which look far from primitive.

Nowadays microsporidia are placed within the Fungi or as a sister-group of the Fungi with a common ancestor.

Forming of clades is largely based on habitat and host. Three classes of Microsporidia are proposed by Vossbrinck and Debrunner-Vossbrinck, based on the habitat: Aquasporidia, Marinosporidia and Terresporidia.

One classification could be:

1. Subclass: *Dihaplophasea*

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  - Order: *Meiodihaplophasida*
    - Superfamily *Thelohanioidea*
      - Family *Thelohaniidae*
      - Family *Duboscqiidae*
      - Family *Janacekiidae*
      - Family *Pereziidae*
      - Family *Striatosporidae*
      - Family *Cylindrosporidae*
    - Superfamily *Burenelloidea*
      - Family *Burenellidae*
    - Superfamily *Amblyosporoidae*
      - Family *Amblyosporidae*
  - Order *Dissociodihaplophasida*
    - Superfamily *Nosematoidea*
      - Family *Nosematidae*
      - Family *Ichthyosporidiidae*
      - Family *Caudosporidae*
      - Family *Pseudopleistophoridae*
      - Family *Mrazekiidae*

- Superfamily *Culicosporoidea*
  - Family *Culicosporidae*
  - Family *Culicosporellidae*
  - Family *Golbergiidae*
  - Family *Spragueidae*
- Superfamily *Ovavesiculoidea*
  - Family *Ovavesiculidae*
  - Family *Tetramicridae*

2. Subclass *Haplophasea*

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  - Order *Glugeida*
    -
    - Family *Glugeidae*
    - Family *Pleistophoridae*
    - Family *Encephalitozoonidae*
    - Family *Abelsporidae*
    - Family *Tuzetiidae*
    - Family *Microfilidae*
    - Family *Unikaryonidae*
  - Order *Chyridiopsida*
    -
    - Family *Chytridiopsida*
    - Family *Buxtehudiidae*
    - Family *Enterocytozoonidae*
    - Family *Burkeidae*

Chapter 15

Glomeromycota and Chytridiomycota

Glomeromycota

Glomeromycota (informally **glomeromycetes**) is one of seven currently recognized phyla within the kingdom Fungi, with approximately 200 described species. Members of the Glomeromycota form arbuscular mycorrhizas (AMs) with the roots or thalli (e.g. in bryophytes) of land plants. *Geosiphon pyriformis* forms an endocytobiotic association with *Nostoc* cyanobacteria. AM formation has not yet been shown for all species. The majority of evidence shows that the Glomeromycota are obligate biotrophs, dependent on symbiosis with land plants (*Nostoc* in the case of *Geosiphon*) for carbon and energy, but there is recent circumstantial evidence that some species may be able to lead an independent existence. The arbuscular mycorrhizal species are terrestrial and widely distributed in soils worldwide where they form symbioses with the roots of the majority of plant species (>80%). They can also be found in wetlands, including salt-marshes, and associated with epiphytic plants.

Reproduction

The Glomeromycota have generally coenocytic (occasionally sparsely septate) mycelia and reproduce asexually through blastic development of the hyphal tip to produce spores (Glomerospores) with diameters of 80-500µm. In some, complex spores form within a terminal saccule.

Phylogeny

Initial studies of the Glomeromycota were based on the morphology of soil-borne sporocarps (spore clusters) found in or near colonized plant roots. Distinguishing features such as wall morphologies, size, shape, color, hyphal attachment and reaction to staining compounds allowed a phylogeny to be constructed. Superficial similarities led to the initial placement of genus *Glomus* in the unrelated family Endogonaceae. Following broader reviews that cleared up the sporocarp confusion, the Glomeromycota were first proposed in the genera *Acaulospora* and *Gigaspora* before being accorded their own

order with the three families Glomaceae (now Glomeraceae), Acaulosporaceae and Gigasporaceae.

With the advent of molecular techniques this classification has undergone major revision. An analysis of small subunit (SSU) rRNA sequences indicated that they share a common ancestor with the Dikarya.

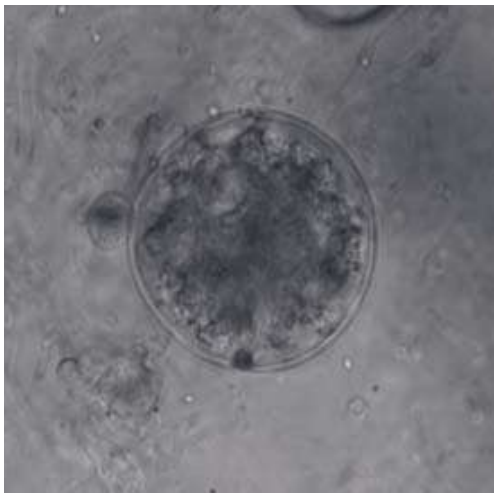
Several species which produce glomoid spores (i.e. spores similar to *Glomus*) in fact belong to other deeply divergent lineages and were placed in the orders, Paraglomerales and Archaeosporales. This new classification includes the Geosiphonaceae, which presently contains one fungus (*Geosiphon pyriformis*) that forms endosymbiotic associations with the cyanobacterium *Nostoc punctiforme* and produces spores typical to this phylum, in the Archaeosporales.

Work in this field is incomplete, and members of *Glomus* may be better suited to different genera or families.

Molecular biology

The biochemical and genetic characterization of the Glomeromycota has been hindered by their biotrophic nature, which impedes laboratory culturing. This obstacle was eventually surpassed with the use of root cultures. The first mycorrhizal gene to be sequenced was the small-subunit ribosomal RNA (SSU rRNA). This gene is highly conserved and commonly used in phylogenetic studies so was isolated from spores of each taxonomic group before amplification through the polymerase chain reaction (PCR). A molecular clock approach, based on the substitution rates of SSU sequences, was used to estimate the time of divergence of the fungi. The molecular analysis found that they are between 462 and 353 million years old. The data enforces the long-held theory that they were instrumental in the colonization of land by plants.

Chytridiomycota



Chytridiomycota is a division of the Fungi kingdom. The name is derived from the Greek *chytridion*, meaning "little pot", describing the structure containing unreleased spores. In older classifications, chytrids (except the recently established order Spizellomycetales) were placed in the Class Phycomycetes under the subdivision Myxomycophyta of the Kingdom Fungi. Also, in an older and more restricted sense (not used here), the term "chytrids" referred just to those fungi in the order Chytridiales. The chytrids have also been included among the Protista, but are now regularly classed as fungi.

The chytrids are the most primitive of the fungi and are mostly saprobic (degrading chitin and keratin). The thalli are coenocytic and usually form no true mycelium (having rhizoids instead). Some species are unicellular. As with other fungi, the cell wall in chytrids is composed of chitin.

Many chytrids are aquatic (mostly found in fresh water). There are approximately 1,000 chytrid species, in 127 genera, distributed among 5 orders.

Reproduction

Both zoospores and gametes of the chytrids are mobile by their flagella, one whiplash per individual.

An example of a Chytrid species is the water mold - Allomyces sap, it is a saprotroph found in water or wet soil. The species has an interesting life cycle. The thallus (body) is attached by rhizoids, and has an erect trunk on which reproductive organs are formed at the end of branches. The life cycle has the ability to change from haploid and diploid generations. The haploid thallus forms male and female gametangia from which flagellated gametes are released and merge to form a Zygote. Gametes and female gametangia attract the opposite sex by producing pheromones. The germinated zygote

produces a diploid thallus with two sorts of sporangia; thin-walled zoosporangia which release diploid zoospores resulting in a diploid thalli and thick-walled sporangia which after meiosis release haploid zoospores which form haploid thalli.

As a parasite

The chytrid Batrachochytrium dendrobatidis (itself commonly known as "Chytrid") is responsible for a recently discovered disease of amphibians, chytridiomycosis. Discovered in 1998 in Australia and Panama this disease is known to kill amphibians in large numbers, and has been suggested as a principal cause for the worldwide amphibian decline. In one example an outbreak of the fungus was found responsible for killing much of the Kihansi Spray Toad population in its native habitat of Tanzania. The actual process leading to mortality is, however, unknown. A popular theory is the fungus hardens the skin of amphibians which hinders respiration.

Chytrids may also infect plant species; in particular, maize-attacking and alfalfa-attacking species have been described. *Synchytrium endobioticum* is an important potato pathogen.

Fossil record

The earliest fossils of chytrids are from the Scottish Rhynie chert, a Devonian-age locality with anatomical preservation of plants and fungi. Among the microfossils are chytrids preserved as parasites on rhyniophytes. These fossils closely resemble the genus *Allomyces*.