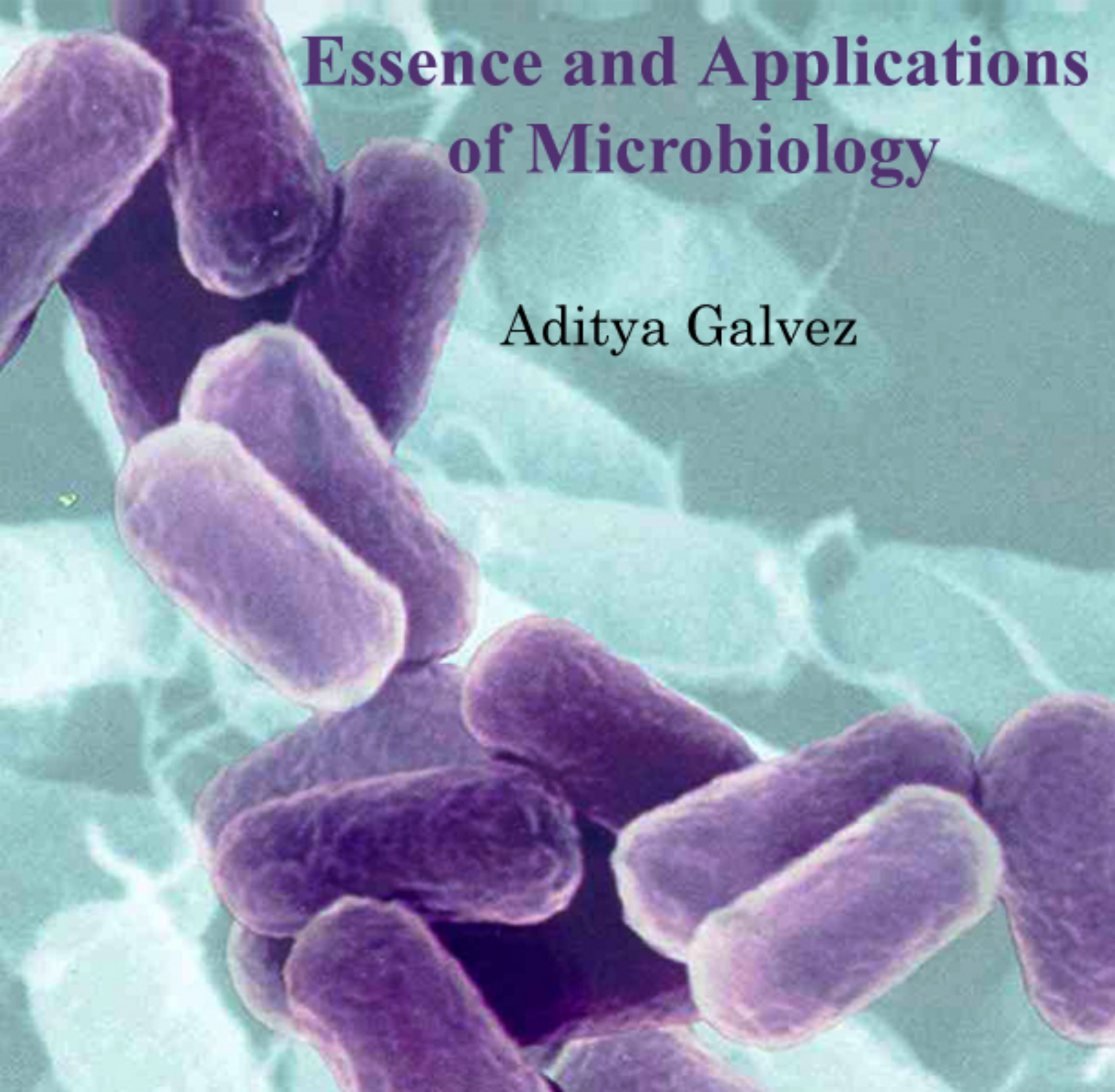
Essence and Applications of Microbiology

Aditya Galvez



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Chapter 1

Food Microbiology

Food microbiology is the study of the microorganisms that inhabit, create, or contaminate food. Of major importance is the study of microorganisms causing food spoilage. "Good" bacteria, however, such as probiotics, are becoming increasingly important in food science. In addition, microorganisms are essential for the production of foods such as cheese, yogurt, other fermented foods, bread, beer and wine.

Food safety

Food safety is a major focus of food microbiology. Pathogenic bacteria, viruses and toxins produced by microorganisms are all possible contaminants of food. However, microorganisms and their products can also be used to combat these pathogenic microbes. Probiotic bacteria, including those that produce bacteriocins, can kill and inhibit pathogens. Alternatively, purified bacteriocins such as nisin can be added directly to food products. Finally, bacteriophages, viruses that only infect bacteria, can be used to kill bacterial pathogens. Thorough preparation of food, including proper cooking, eliminates most bacteria and viruses. However, toxins produced by contaminants may not be heat-labile, and some are not eliminated by cooking.

Fermentation

Fermentation is one way microorganisms can change a food. Yeast, especially *Saccharomyces cerevisiae*, is used to leaven bread, brew beer and make wine. Certain bacteria, including lactic acid bacteria, are used to make yogurt, cheese, hot sauce, pickles, fermented sausages and dishes such as kimchi. A common effect of these fermentations is that the food product is less hospitable to other microorganisms, including pathogens and spoilage-causing microorganisms, thus extending the food's shelf-life.

Food fermentations are ancient technologies that harness microorganisms and their enzymes to improve the human diet. Fermented foods keep better, have enhanced flavours, textures and aromas, and may also possess certain health benefits, including superior digestibility. For vegetarians, fermented foods serve as palatable, protein-rich meat substitutes.

Some cheese varieties also require molds to ripen and develop their characteristic flavors.

Asian cuisines rely on a large repertoire of fermented foods. In particular, *Aspergillus oryzae* and *A. sojae*, sometimes called koji molds, are employed in many ways. Their hydrolytic enzymes suit them for growth on starch and other carbohydrate-rich substrates. In the koji process, fungal enzymes perform the same function as the malting enzymes used in the beer fermentations of western cultures. The koji molds release amylases that break down rice starch, which in turn can be fermented to make rice wine. Fermented rice beverages have numerous local variations and names, depending on country and region. Rice wine is called *shaoshing* in parts of China, *sake* in Japan, *takj* or *yakju* in Korea, as well as by many other names across Asia. The koji molds are also effective in a variety of legume fermentations, of which miso and soy sauce are best known. Miso is a mixture of soybeans and cereals usually used to flavour soups. Soy sauce is a flavourful, salty liquid sauce made from soybeans that have been fermented by koji molds, yeasts, as well as several halophilic bacteria. Other names for soy sauce include *jiangyou* (China), *makjang* and *kanjang* (Korea), *toyo* (Philippines) and *siiu* (Thailand).

Probiotics

Probiotics are living organisms that, when consumed, have beneficial health benefits outside their inherent nutritional effects. There is a growing body of evidence for the role of probiotics in gastrointestinal infections, irritable bowel syndrome and inflammatory bowel disease.

Lactobacillus species are used for the production of yogurt, cheese, sauerkraut, pickles, beer, wine, cider, kimchi, chocolate and other fermented foods, as well as animal feeds such as silage. In recent years, much interest has been shown in the use of lactobacilli as probiotic organisms and their potential for disease prevention in humans and animals.

Bifidobacteria are considered as important probiotics, and are used in the food industry to relieve and treat many intestinal disorders. Bifidobacteria exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules.

Microbial biopolymers

A variety of biopolymers, such as polysaccharides, polyesters and polyamides, are naturally produced by microorganisms. Several microbially-produced polymers are used in the food industry.

Xanthan

Plant-pathogenic bacteria of the genus *Xanthomonas* are able to produce the acidic exopolysaccharide xanthan gum. Because of its physical properties, it is widely used as a viscosifer, thickener, emulsifier or stabilizer in the food industry. Xanthan consists of pentasaccharide repeat units composed of D-glucosyl, D-mannosyl, and D-glucuronyl acid residues in a molar ratio of 2:2:1 and variable proportions of O-acetyl and pyruvyl residues.

Alginate

Alginate is the main representative of a family of polysaccharides that neither show branching nor repeating blocks or unit patterns and this property distinguishes it from to other polymers like xanthan or dextran. Alginates can be used as thickening agents. Although listed here under the category 'Microbial polysaccharides', commercial alginates are currently only produced by extraction from brown seaweeds such as *Laminaria hyperborea* or *L. japonica*.

Cellulose

Cellulose is a simple polysaccharide, in that it consists only of one type of sugar (glucose), and the units are linearly arranged and linked together by β-1,4 linkages only. The mechanism of biosynthesis is, however, rather complex, partly because in native celluloses, the chains are organized as highly ordered water-insoluble fibers. Currently, the key genes involved in cellulose biosynthesis and regulation are known in a number of bacteria, but many details of the biochemistry of its biosynthesis are still not clear. In spite of the enormous abundance of cellulose in plants, bacterial celluloses are being investigated for industrial exploitations.

Poly-γ-glutamic acid

Poly-γ-glutamic acid (γ-PGA) produced by various strains of *Bacillus* has potential applications as a thickener in the food industry.

Levan

Levan, a homopolysaccharide composed of D-fructofuranosyl residues joined by 2,6 with multiple branches by 2,1 linkages, has great potential as a functional biopolymer in foods,

feeds, cosmetics, and the pharmaceutical and chemical industries. Levan can be used as food or a feed additive with prebiotic and hypocholesterolemic effects.

Exopolysaccharides

Microorganisms synthesize a wide spectrum of multifunctional polysaccharides, including intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides or exopolysaccharides (EPSs). EPSs generally consist of monosaccharides and some noncarbohydrate substituents (such as acetate, pyruvate, succinate, and phosphate). Owing to the wide diversity in composition, they have found multifarious applications in various food and pharmaceutical industries.

Foodborne pathogens

Foodborne pathogens are the leading causes of illness and death in less developed countries, killing approximately 1.8 million people annually. In developed countries, foodborne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of dollars in medical care and lost productivity. New foodborne pathogens and foodborne diseases are likely to emerge, driven by factors such as pathogen evolution, changes in agricultural and food manufacturing practices, and changes to the human host status. There are growing concerns that terrorists could use pathogens to contaminate food and water supplies in attempts to incapacitate thousands of people and disrupt economic growth.

Enteric viruses

Food and waterborne viruses contribute to a substantial number of illnesses throughout the world. Among those most commonly known are hepatitis A virus, rotavirus, astrovirus, enteric adenovirus, hepatitis E virus, and the human caliciviruses consisting of the noroviruses and the Sapporo viruses. This diverse group is transmitted by the fecal-oral route, often by ingestion of contaminated food and water.

Protozoan parasites

Protozoan parasites associated with food and water can cause illness in humans. Although parasites are more commonly found in developing countries, developed countries have also experienced several foodborne outbreaks. Contaminants may be inadvertently introduced to the foods by inadequate handling practices, either on the farm or during processing of foods. Protozoan parasites can be found worldwide, either infecting wild animals or in water and contaminating crops grown for human consumption. The disease can be much more severe and prolonged in immunocompromised individuals.

Mycotoxins

Molds produce mycotoxins, which are secondary metabolites that can cause acute or chronic diseases in humans when ingested from contaminated foods. Potential diseases include cancers and tumors in different organs (heart, liver, kidney, nerves), gastrointestinal disturbances, alteration of the immune system, and reproductive problems. Species of *Aspergillus*, *Fusarium*, *Penicillium*, and *Claviceps* grow in agricultural commodities or foods and produce the mycotoxins such as aflatoxins, deoxynivalenol, ochratoxin A, fumonisins, ergot alkaloids, T-2 toxin, and zearalenone and other minor mycotoxins such as cyclopiazonic acid and patulin. Mycotoxins occur mainly in cereal grains (barley, maize, rye, wheat), coffee, dairy products, fruits, nuts and spices. Control of mycotoxins in foods has focused on minimizing mycotoxin production in the field, during storage or destruction once produced. Monitoring foods for mycotoxins is important to manage strategies such as regulations and guidelines, which are used by 77 countries, and for developing exposure assessments essential for accurate risk characterization.

Aflatoxins are still recognized as the most important mycotoxins. They are synthesized by only a few *Aspergillus* species, of which *A. flavus* and *A. parasiticus* are the most problematic. The expression of aflatoxin-related diseases is influenced by factors such as age, nutrition, sex, species and the possibility of concurrent exposure to other toxins. The main target organ in mammals is the liver, so aflatoxicosis is primarily a hepatic disease. Conditions increasing the likelihood of aflatoxicosis in humans include limited availability of food, environmental conditions that favor mold growth on foodstuffs, and lack of regulatory systems for aflatoxin monitoring and control.

Yersinia enterocolitica

Yersinia enterocolitica includes pathogens and environmental strains that are ubiquitous in terrestrial and fresh water ecosystems. Evidence from large outbreaks of yersiniosis and from epidemiological studies of sporadic cases has shown that *Y. enterocolitica* is a foodborne pathogen. Pork is often implicated as the source of infection. The pig is the only animal consumed by man that regularly harbors pathogenic *Y. enterocolitica*. An important property of the bacterium is its ability to multiply at temperatures near 0°C, and therefore in many chilled foods. The pathogenic serovars (mainly O:3, O:5,27, O:8 and O:9) show different geographical distribution. However, the appearance of strains of serovars O:3 and O:9 in Europe, Japan in the 1970s, and in North America by the end of the 1980s, is an example of a global pandemic. There is a possible risk of reactive arthritis following infection with *Y. enterocolitica*.

Vibrio

Vibrio species are prevalent in estuarine and marine environments, and seven species can cause foodborne infections associated with seafood. *Vibrio cholerae* O1 and O139 serovtypes produce cholera toxin and are agents of cholera. However, fecal-oral route infections in the terrestrial environment are responsible for epidemic cholera. *V. cholerae*

non-O1/O139 strains may cause gastroenteritis through production of known toxins or unknown mechanism. *Vibrio parahaemolytitcus* strains capable of producing thermostable direct hemolysin (TDH) and/or TDH-related hemolysin are most important causes of gastroenteritis associated with seafood consumption. *Vibrio vulnificus* is responsible for seafoodborne primary septicemia, and its infectivity depends primarily on the risk factors of the host. *V. vulnificus* infection has the highest case fatality rate (50%) of any foodborne pathogen. Four other species (*V. mimicus*, *V. hollisae*, *V. fluvialis*, and *V. furnissii*) can cause gastroenteritis. Some strains of these species produce known toxins, but the pathogenic mechanism is largely not understood. The ecology of and detection and control methods for all seafoodborne *Vibrio* pathogens are essentially similar.

Staphylococcus aureus

Staphylococcus aureus is a common cause of bacterial foodborne disease worldwide. Symptoms include vomiting and diarrhea that occur shortly after ingestion of *S. aureus* toxin-contaminated food. The symptoms arise from ingestion of preformed enterotoxin, which accounts for the short incubation time. Staphylococcal enterotoxins are superantigens and, as such, have adverse effects on the immune system. The enterotoxin genes are accessory genetic elements in *S. aureus*, meaning not all strains of this organism are enterotoxin-producing. The enterotoxin genes are found on prophages, plasmids, and pathogenicity islands in different strains of *S. aureus*. Expression of the enterotoxin genes is often under the control of global virulence gene regulatory systems.

Campylobacter

Campylobacter spp., primarily *C. jejuni* subsp. *jejuni* is one of the major causes of bacterial gastroenteritis in the U.S. and worldwide. *Campylobacter* infection is primarily a foodborne illness, usually without complications; however, serious sequelae, such as Guillain-Barre Syndrome, occur in a small subset of infected patients. Detection of *C. jejuni* in clinical samples is readily accomplished by culture and nonculture methods.

Listeria monocytogenes

Listeria monocytogenes is Gram-positive foodborne bacterial pathogen and the causative agent of human listeriosis. *Listeria* infections are acquired primarily through the consumption of contaminated foods, including soft cheese, raw milk, deli salads, and ready-to-eat foods such as luncheon meats and frankfurters. Although *L. monocytogenes* infection is usually limited to individuals that are immunocompromised, the high mortality rate associated with human listeriosis makes it the leading cause of death among foodborne bacterial pathogens. As a result, tremendous effort has been made to develop methods for the isolation, detection and control of *L. monocytogenes* in foods.

Salmonella

Salmonella serotypes continue to be a prominent threat to food safety worldwide. Infections are commonly acquired by animal to human transmission though consumption of undercooked food products derived from livestock or domestic fowl. The second half of the 20th century saw the emergence of *Salmonella* serotypes that became associated with new food sources (i.e. chicken eggs) and the emergence of *Salmonella* serotypes with resistance against multiple antibiotics.

Shigella

Shigella species are members of the family Enterobacteriaceae and are Gram negative, nonmotile rods. Four subgroups exist based on O-antigen structure and biochemical properties: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C) and *S. sonnei* (subgroup D). Symptoms include mild to severe diarrhea with or without blood, fever, tenesmus and abdominal pain. Further complications of the disease may be seizures, toxic megacolon, reactive arthritis and hemolytic uremic syndrome. Transmission of the pathogen is by the fecal-oral route, commonly through food and water. The infectious dose ranges from 10-100 organisms. *Shigella* spp. have a sophisticated pathogenic mechanism to invade colonic epithelial cells of the host, man and higher primates, and the ability to multiply intracellularly and spread from cell to adjacent cell via actin polymerization. *Shigella* spp. are one of the leading causes of bacterial foodborne illnesses and can spread quickly within a population.

Escherichia coli

More information is available concerning *Escherichia coli* than any other organism, thus making *E. coli* the most thoroughly studied species in the microbial world. For many years, *E. coli* was considered a commensal of human and animal intestinal tracts with low virulence potential. It is now known that many strains of *E. coli* act as pathogens, inducing serious gastrointestinal diseases and even death in humans. There are six major categories of *E. coli* strains that cause enteric diseases in humans, including the (1) enterohemorrhagic *E. coli*, which cause hemorrhagic colitis and hemolytic uremic syndrome, (2) enterotoxigenic *E. coli*, which induce traveler's diarrhea, (3) enteropathogenic *E. coli*, which cause a persistent diarrhea in children living in developing countries, (4) enteroaggregative *E. coli*, which provokes diarrhea in children, (5) enteroinvasive *E. coli* that are biochemically and genetically related to *Shigella* species and can induce diarrhea, and (6) diffusely adherent *E. coli*, which cause diarrhea and are distinguished by a characteristic type of adherence to mammalian cells.

Clostridium botulinum and *Clostridium perfringens*

Clostridium botulinum produces extremely potent neurotoxins that result in the severe neuroparalytic disease, botulism. The enterotoxin produced by *C. perfringens* during sporulation of vegetative cells in the host intestine results in debilitating acute diarrhea and abdominal pain. Sales of refrigerated, processed foods of extended durability

including sous-vide foods, chilled ready-to-eat meals, and cook-chill foods have increased over recent years. Anaerobic spore-formers have been identified as the primary microbiological concerns in these foods. Heightened awareness over intentional food source tampering with botulinum neurotoxin has arisen with respect to genes encoding the toxins that are capable of transfer to nontoxigenic clostridia.

Bacillus cereus

The *Bacillus cereus* group comprises six members: *B. anthracis*, *B. cereus*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis* and *B. weihenstephanensis*. These species are closely related and should be placed within one species, except for *B. anthracis* that possesses specific large virulence plasmids. *B. cereus* is a normal soil inhabitant, and is frequently isolated from a variety of foods, including vegetables, dairy products and meat. It causes a vomiting or diarrhea illness that is becoming increasingly important in the industrialized world. Some patients may experience both types of illness simultaneously. The diarrheal type of illness is most prevalent in the western hemisphere, whereas the emetic type is most prevalent in Japan. Desserts, meat dishes, and dairy products are the foods most frequently associated with diarrheal illness, whereas rice and pasta are the most common vehicles of emetic illness. The emetic toxin (cereulide) has been isolated and characterized; it is a small ring peptide synthesised nonribosomally by a peptide synthetase. Three types of *B. cereus* enterotoxins involved in foodborne outbreaks have been identified. Two of these enterotoxins are three-component proteins and are related, while the last is a one-component protein (CytK). Deaths have been recorded both by strains that produce the emetic toxin and by a strain producing only CytK. Some strains of the *B. cereus* group are able to grow at refrigeration temperatures. These variants raise concern about the safety of cooked, refrigerated foods with an extended shelf life. *B. cereus* spores adhere to many surfaces and survive normal washing and disinfection (except for hypochlorite and UVC) procedures. *B. cereus* food borne illness is likely under-reported because of its relatively mild symptoms, which are of short duration.

Food authenticity

It is important to be able to detect microorganisms in food, in particular pathogenic microorganisms or genetically modified microorganisms. Real-time PCR is an accepted analytical tool within the food industry. Its principal role has been one of assisting the legislative authorities, major manufacturers and retailers to confirm the authenticity of foods. The most obvious role is the detection of genetically modified organisms, but real-time PCR makes a significant contribution to other areas of the food industry, including food safety.

Chapter 2

Algae

Algae

Laurencia, a marine genus of Red Algae from Hawaii.

Scientific classification

Domain: Eukaryota

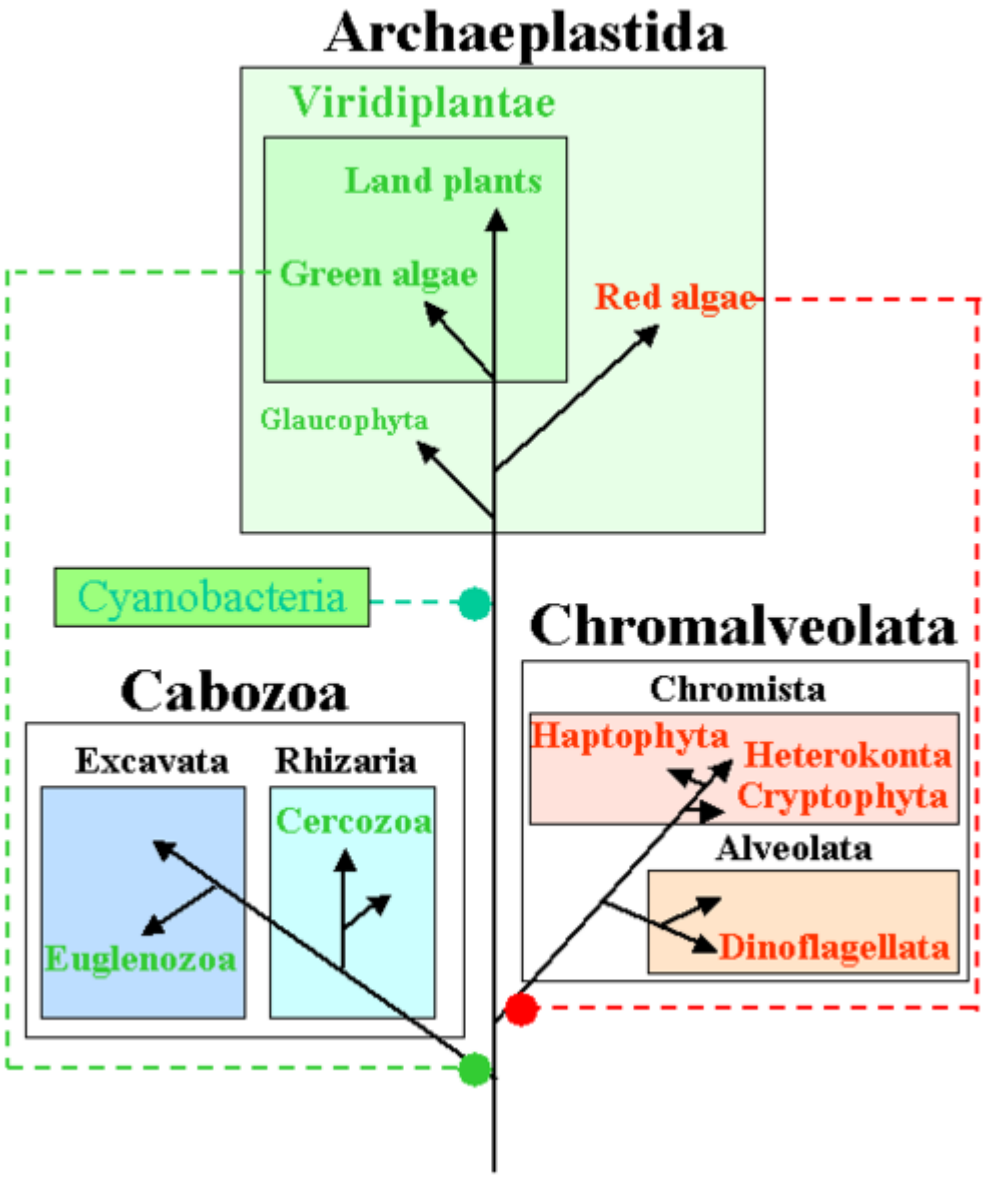
Included groups

- Archaeplastida
  - Chlorophyta (Green algae)
  - Rhodophyta (Red algae)
  - Glaucophyta
- Rhizaria, Excavata
  - Chlorarachniophytes
  - Euglenids
- Chromista, Alveolata
  - Heterokonts
    - Bacillariophyceae (Diatoms)
    - Axodine

        - Bolidomonas
        - Eustigmatophyceae
        - Phaeophyceae (Brown algae)
        - Chrysophyceae (Golden algae)
        - Raphidophyceae
        - Synurophyceae
        - Xanthophyceae (Yellow-green algae)
    - Cryptophyta
    - Dinoflagellates
    - Haptophyta

Excluded groups

- Cyanobacteria
- Plantae



The lineage of algae according to Thomas Cavalier-Smith. The exact number and placement of endosymbiotic events is not yet clear, so this diagram can be taken only as a general guide It represents the most parsimonious way of explaining the three types of endosymbiotic origins of plastids. These types include the endosymbiotic events of cyanobacteria, red algae and green algae, leading to the hypothesis of the supergroups Archaeplastida, Chromalveolata and Cabozoa respectively. However, the monophyly of Cabozoa has been refuted and the monophylies of Archaeplastida and Chromalveolata are currently strongly challenged. Endosymbiotic events are noted by dotted lines.

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms, such as the giant kelps that grow to 65 meters in length. The US Algal Collection is represented by almost 300,000 accessioned and inventoried herbarium specimens. The largest and most complex marine forms are called

seaweeds. They are photosynthetic like plants, and "simple" because their tissues are not organized into the many distinct organs found in land plants.

Though the prokaryotic cyanobacteria (commonly referred to as blue-green algae) were traditionally included as "algae" in older textbooks, many modern sources regard this as outdated as they are now considered to be bacteria. The term *algae* is now restricted to eukaryotic organisms. All true algae therefore have a nucleus enclosed within a membrane and plastids bound in one or more membranes. Algae constitute a paraphyletic and polyphyletic group, as they do not include all the descendants of the last universal ancestor nor do they all descend from a common algal ancestor, although their plastids seem to have a single origin. Diatoms are also examples of algae.

Algae are found in the fossil record dating back to approximately 3 billion years in the Precambrian. They exhibit a wide range of reproductive strategies, from simple, asexual cell division to complex forms of sexual reproduction.

Algae lack the various structures that characterize land plants, such as phyllids (leaves) and rhizoids in nonvascular plants, or leaves, roots, and other organs that are found in tracheophytes (vascular plants). Many are photoautotrophic, although some groups contain members that are mixotrophic, deriving energy both from photosynthesis and uptake of organic carbon either by osmotrophy, myzotrophy, or phagotrophy. Some unicellular species rely entirely on external energy sources and have limited or no photosynthetic apparatus.

Nearly all algae have photosynthetic machinery ultimately derived from the Cyanobacteria, and so produce oxygen as a by-product of photosynthesis, unlike other photosynthetic bacteria such as purple and green sulfur bacteria. Fossilized filamentous algae from the Vindhya basin have been dated back to 1.6 to 1.7 billion years ago.

Etymology and study

HISTORIA
FVCORVM
AVCTORE
SAMVEL GOTTLIEB GMELIN,
MED. DOCT. ACADEM. IMPER. PETROPOL. BOTA-
NICES PROFESSORE ET MEMBRO ORDINARIO

PETROPOLI
EX TYPOGRAPHIA ACADEMIAE SCIENTIARVM
cIↃ IↃcc LXVIII.

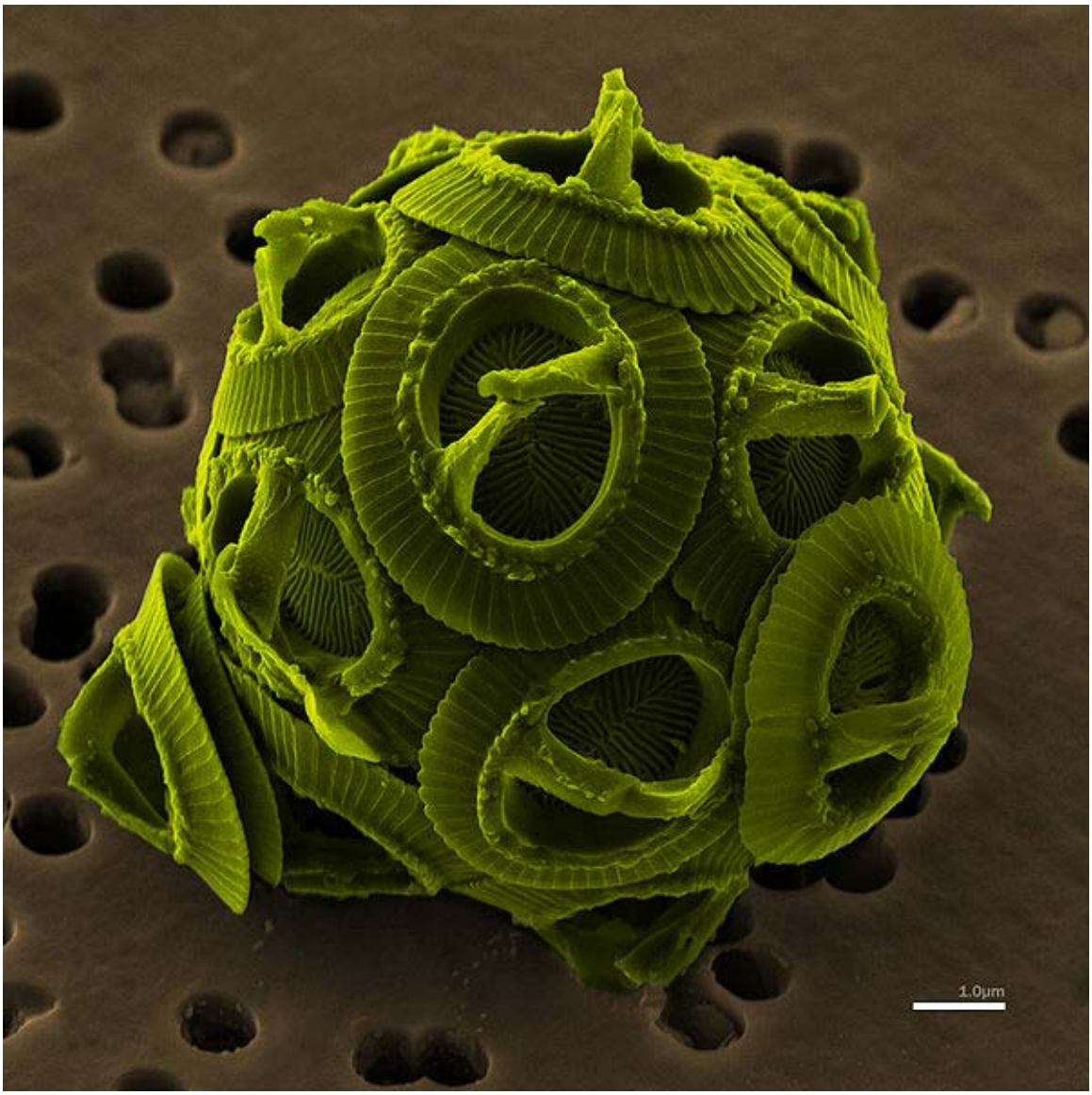
Title page of Samuel Gottlieb Gmelin, *Historia Fucorum*, dated 1768.

The singular *alga* is the Latin word for a particular seaweed and retains that meaning in English. The etymology is obscure. Although some speculate that it is related to Latin *algēre*, "be cold", there is no known reason to associate seaweed with temperature. A more likely source is *alliga*, "binding, entwining." Since Algae has become a biological classification, alga can also mean one classification under Algae, parallel to a fungus being a species of fungi, a plant being a species of plant, and so on.

The ancient Greek word for seaweed was φῦκος (fūkos or phykos), which could mean either the seaweed, probably Red Algae, or a red dye derived from it. The Latinization, *fūcus*, meant primarily the cosmetic rouge. The etymology is uncertain, but a strong candidate has long been some word related to the Biblical פוּךְ (pūk), "paint" (if not that word itself), a cosmetic eye-shadow used by the ancient Egyptians and other inhabitants of the eastern Mediterranean. It could be any color: black, red, green, blue.

Accordingly the modern study of marine and freshwater algae is called either phycology or algology. The name Fucus appears in a number of taxa.The singular form is alga.

Classification



False-colour Scanning electron micrograph of the unicellular coccolithophore, *Gephyrocapsa oceanica*.

While *Cyanobacteria* have been traditionally included among the Algae, recent works usually exclude them due to large differences such as the lack of membrane-bound organelles, the presence of a single circular chromosome, the presence of peptidoglycan in the cell walls, and ribosomes different in size and content from those of the Eukaryotes. Rather than in chloroplasts, they conduct photosynthesis on specialized infolded cytoplasmic membranes called thylakoid membranes. Therefore, they differ significantly from the Algae despite occupying similar ecological niches.

By modern definitions Algae are Eukaryotes and conduct photosynthesis within membrane-bound organelles called chloroplasts. Chloroplasts contain circular DNA and are similar in structure to Cyanobacteria, presumably representing reduced cyanobacterial endosymbionts. The exact nature of the chloroplasts is different among the different lines of Algae, reflecting different endosymbiotic events. The table below describes the composition of the three major groups of Algae. Their lineage relationships are shown in the figure in the upper right. Many of these groups contain some members that are no longer photosynthetic. Some retain plastids, but not chloroplasts, while others have lost plastids entirely. The singular form is alga.

Supergroup affiliation	Members	Endosymbiont	Summary
Primoplantae/ Archaeplastida	• Chlorophyta • Rhodophyta • Glaucophyta	Cyanobacteria	These Algae have *primary* chloroplasts, i.e. the chloroplasts are surrounded by *two membranes* and probably developed through a single endosymbiotic event. The chloroplasts of Red Algae have chlorophylls *a* and *c* (often), and phycobilins, while those of Green Algae have chloroplasts with chlorophyll *a* and *b*. Higher plants are pigmented similarly to Green Algae and probably developed from them, and thus Chlorophyta is a sister taxon to the plants; sometimes they are grouped as Viridiplantae.
Excavata and Rhizaria	• Chlorarachniophytes • Euglenids	Green Algae	These groups have green chloroplasts containing chlorophylls *a* and *b*.

			Their chloroplasts are surrounded by *four and three membranes*, respectively, and were probably retained from ingested Green Algae. **Chlorarachniophytes**, which belong to the phylum Cercozoa, contain a small nucleomorph, which is a relict of the algae's nucleus. **Euglenids**, which belong to the phylum Euglenozoa, live primarily in freshwater and have chloroplasts with only three membranes. It has been suggested that the endosymbiotic Green Algae were acquired through myzocytosis rather than phagocytosis.
Chromista and Alveolata	• Heterokonts • Haptophyta • Cryptomonads • Dinoflagellates	Red Algae	These groups have chloroplasts containing chlorophylls *a* and *c*, and phycobilins.The shape varies from plant to plant. they may be of discoid, plate-like, reticulate, cup-shaped, spiral or ribbon shaped. They have one or more pyrenoids to preserve protein and starch. The latter chlorophyll type is not known from any prokaryotes or primary chloroplasts, but genetic similarities with the Red Algae suggest a

relationship there.

In the first three of these groups (**Chromista**), the chloroplast has four membranes, retaining a nucleomorph in Cryptomonads, and they likely share a common pigmented ancestor, although other evidence casts doubt on whether the Heterokonts, Haptophyta, and Cryptomonads are in fact more closely related to each other than to other groups.

The typical **dinoflagellate** chloroplast has three membranes, but there is considerable diversity in chloroplasts within the group, and it appears there were a number of endosymbiotic events. The Apicomplexa, a group of closely related parasites, also have plastids called apicoplasts. Apicoplasts are not photosynthetic but appear to have a common origin with Dinoflagellate chloroplasts.

W.H.Harvey (1811—1866) was the first to divide the Algae into four divisions based on their pigmentation. This is the first use of a biochemical criterion in plant systematics. Harvey's four divisions are: Red Algae (Rhodophyta), Brown Algae (Heteromontophyta), Green Algae (Chlorophyta) and Diatomaceae.

Relationship to higher plants

The first plants on earth evolved from shallow freshwater algae much like *Chara* some 400 million years ago. These probably had an isomorphic alternation of generations and were probably filamentous. Fossils of isolated land plant spores suggest land plants may have been around as long as 475 million years ago.

Morphology

The kelp forest exhibit at the Monterey Bay Aquarium. A three-dimensional, multicellular thallus.

A range of algal morphologies are exhibited, and convergence of features in unrelated groups is common. The only groups to exhibit three dimensional multicellular thalli are the reds and browns, and some chlorophytes. Apical growth is constrained to subsets of these groups: the florideophyte reds, various browns, and the charophytes. The form of charophytes is quite different to those of reds and browns, because have distinct nodes, separated by internode 'stems'; whorls of branches reminiscent of the horsetails occur at the nodes. Conceptacles are another polyphyletic trait; they appear in the coralline algae and the Hildenbrandiales, as well as the browns.

Most of the simpler algae are unicellular flagellates or amoeboids, but colonial and non-motile forms have developed independently among several of the groups. Some of the more common organizational levels, more than one of which may occur in the life cycle of a species, are

- *Colonial*: small, regular groups of motile cells
- *Capsoid*: individual non-motile cells embedded in mucilage
- *Coccoid*: individual non-motile cells with cell walls
- *Palmelloid*: non-motile cells embedded in mucilage
- *Filamentous*: a string of non-motile cells connected together, sometimes branching
- *Parenchymatous*: cells forming a thallus with partial differentiation of tissues

In three lines even higher levels of organization have been reached, with full tissue differentiation. These are the brown algae,—some of which may reach 50 m in length (kelps)—the red algae, and the green algae. The most complex forms are found among the green algae, in a lineage that eventually led to the higher land plants. The point where these non-algal plants begin and algae stop is usually taken to be the presence of reproductive organs with protective cell layers, a characteristic not found in the other alga groups.

Symbiotic algae

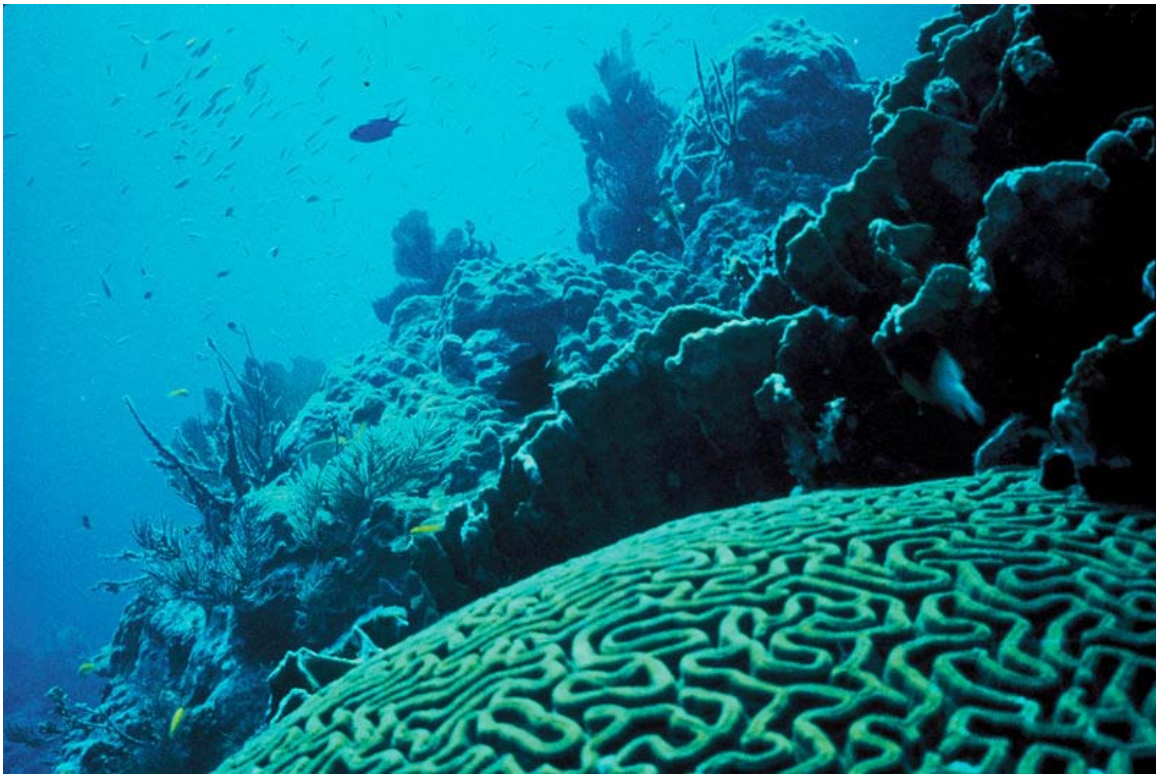
Some species of algae form symbiotic relationships with other organisms. In these symbioses, the algae supply photosynthates (organic substances) to the host organism providing protection to the algal cells. The host organism derives some or all of its energy requirements from the algae. Examples are as follows.

Lichens

Rock lichens in Ireland.

Lichens are defined by the International Association for Lichenology to be "an association of a fungus and a photosynthetic symbiont resulting in a stable vegetative body having a specific structure." The fungi, or mycobionts, are from the Ascomycota with a few from the Basidiomycota. They are not found alone in nature but when they began to associate is not known. One mycobiont associates with the same phycobiont species, rarely two, from the Green Algae, except that alternatively the mycobiont may associate with the same species of Cyanobacteria (hence "photobiont" is the more accurate term). A photobiont may be associated with many specific mycobionts or live independently; accordingly, lichens are named and classified as fungal species. The association is termed a morphogenesis because the lichen has a form and capabilities not possessed by the symbiont species alone (they can be experimentally isolated). It is possible that the photobiont triggers otherwise latent genes in the mycobiont.

Coral reefs



Floridian coral reef

Coral reefs are accumulated from the calcareous exoskeletons of marine invertebrates of the Scleractinia order; i.e., the Stony Corals. As animals they metabolize sugar and oxygen to obtain energy for their cell-building processes, including secretion of the exoskeleton, with water and carbon dioxide as byproducts. As the reef is the result of a favorable equilibrium between construction by the corals and destruction by marine erosion, the rate at which metabolism can proceed determines the growth or deterioration of the reef.

Algae of the Dinoflagellate phylum are often endosymbionts in the cells of marine invertebrates, where they accelerate host-cell metabolism by generating immediately available sugar and oxygen through photosynthesis using incident light and the carbon dioxide produced in the host. Endosymbiont algae in the Stony Corals are described by the term zooxanthellae, with the host Stony Corals called on that account hermatypic corals, which although not a taxon are not in healthy condition without their endosymbionts. Zooxanthellae belong almost entirely to the genus *Symbiodinium*. The loss of *Symbiodinium* from the host is known as coral bleaching, a condition which unless corrected leads to the deterioration and loss of the reef.

Sea sponges

Green Algae live close to the surface of some sponges, for example, breadcrumb sponge (*Halichondria panicea*). The alga is thus protected from predators; the sponge is provided with oxygen and sugars which can account for 50 to 80% of sponge growth in some species.

Life-cycle

Rhodophyta, Chlorophyta and Heterokontophyta, the three main algal Phyla, have life-cycles which show tremendous variation with considerable complexity. In general there is an asexual phase where the seaweed's cells are diploid, a sexual phase where the cells are haploid followed by fusion of the male and female gametes. Asexual reproduction is advantageous in that it permits efficient population increases, but less variation is possible. Sexual reproduction allows more variation, but is more costly. Often there is no strict alternation between the sporophyte and also because there is often an asexual phase, which could include the fragmentation of the thallus.

Numbers

Algae on coastal rocks at Shihtiping in Taiwan

The *Algal Collection of the U.S. National Herbarium* (located in the National Museum of Natural History) consists of approximately 320500 dried specimens, which, although not exhaustive (no exhaustive collection exists), gives an idea of the order of magnitude of the number of algal species (that number remains unknown). Estimates vary widely. For example, according to one standard textbook, in the British Isles the *UK Biodiversity Steering Group Report* estimated there to be 20000 algal species in the UK. Another checklist reports only about 5000 species. Regarding the difference of about 15000 species, the text concludes: "It will require many detailed field surveys before it is possible to provide a reliable estimate of the total number of species ...."

Regional and group estimates have been made as well: 5000—5500 species of Red Algae worldwide, "some 1300 in Australian Seas," 400 seaweed species for the western coastline of South Africa, 669 marine species from California (U.S.A.), 642 in the check-list of Britain and Ireland, and so on, but lacking any scientific basis or reliable sources, these numbers have no more credibility than the British ones mentioned above. Most estimates also omit the microscopic Algae, such as the phytoplankta, entirely.

Distribution

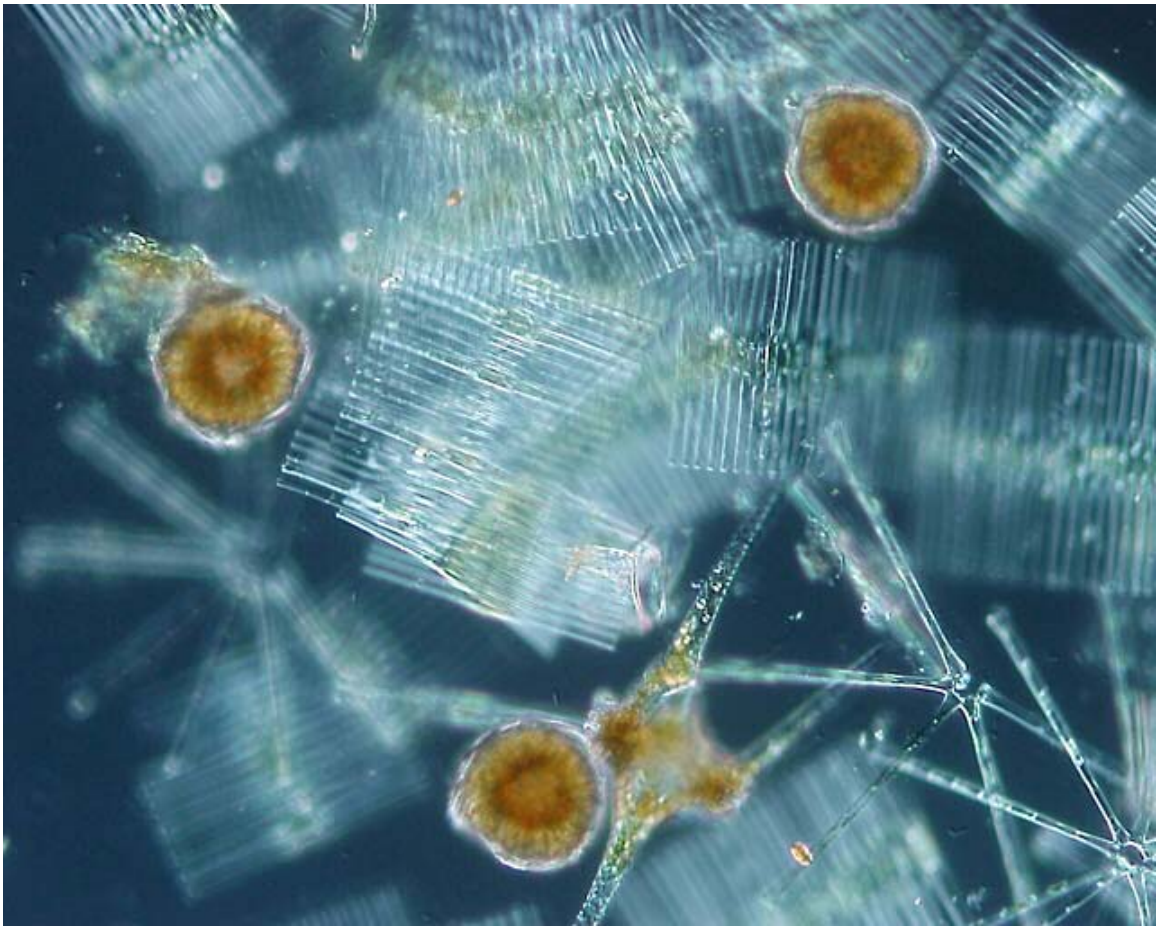
The topic of distribution of algal species has been fairly well studied since the founding of phytogeography in the mid-19th century AD. Algae spread mainly by the dispersal of spores analogously to the dispersal of Plantae by seeds and spores. Spores are everywhere in all parts of the Earth: the waters fresh and marine, the atmosphere, free-floating and in precipitation or mixed with dust, the humus and in other organisms, such as humans. Whether a spore is to grow into an organism depends on the combination of the species and the environmental conditions of where the spore lands.

The spores of fresh-water Algae are dispersed mainly by running water and wind, as well as by living carriers. The bodies of water into which they are transported are chemically selective. Marine spores are spread by currents. Ocean water is temperature selective, resulting in phytogeographic zones, regions and provinces.

To some degree the distribution of Algae is subject to floristic discontinuities caused by geographical features, such as Antarctica, long distances of ocean or general land masses. It is therefore possible to identify species occurring by locality, such as "Pacific Algae" or "North Sea Algae". When they occur out of their localities, it is usually possible to hypothesize a transport mechanism, such as the hulls of ships. For example, *Ulva reticulata* and *Ulva fasciata* travelled from the mainland to Hawaii in this manner.

Mapping is possible for select species only: "there are many valid examples of confined distribution patterns." For example, *Clathromorphum* is an arctic genus and is not mapped far south of there. On the other hand, scientists regard the overall data as insufficient due to the "difficulties of undertaking such studies."

Locations



Phytoplankton, Lake Chuzenji

Algae are prominent in bodies of water, common in terrestrial environments and are found in unusual environments, such as on snow and on ice. Seaweeds grow mostly in shallow marine waters, under 100 metres (330 ft); however some have been recorded to a depth of 360 metres (1,180 ft).

The various sorts of algae play significant roles in aquatic ecology. Microscopic forms that live suspended in the water column (phytoplankton) provide the food base for most marine food chains. In very high densities (algal blooms) these algae may discolor the water and outcompete, poison, or asphyxiate other life forms.

Algae are variously sensitive to different factors, which has made them useful as biological indicators in the Ballantine Scale and its modification.

Uses

Harvesting Algae

Agar

Agar, a gelatinous substance derived from red algae, has a number of commercial uses.

Alginates

Between 100,000 and 170,000 wet tons of *Macrocystis* are harvested annually in California for alginate extraction and abalone feed.

Energy source

To be competitive and independent from fluctuating support from (local) policy on the long run, biofuels should equal or beat the cost level of fossil fuels. Here, algae based fuels hold great promise, directly related to the potential to produce more biomass per unit area in a year than any other form of biomass. The break-even point for algae-based biofuels should be within reach in about ten to fifteen years.

Fertilizer

Seaweed is used as a fertilizer.

For centuries seaweed has been used as a fertilizer; George Owen of Henllys writing in the 16th century referring to drift weed in South Wales:

This kind of ore they often gather and lay on great heapes, where it heteth and rotteth, and will have a strong and loathsome smell; when being so rotten they cast on the land, as they do their muck, and thereof springeth good corn, especially barley ... After spring-tydes or great rigs of the sea, they fetch it in sacks on horse backes, and carie the same three, four, or five miles, and cast it on the lande, which doth very much better the ground for corn and grass.

Today Algae are used by humans in many ways; for example, as fertilizers, soil conditioners and livestock feed. Aquatic and microscopic species are cultured in clear tanks or ponds and are either harvested or used to treat effluents pumped through the ponds. Algaculture on a large scale is an important type of aquaculture in some places. Maerl is commonly used as a soil conditioner.

Nutrition

Seaweed gardens on Inisheer.

Naturally growing seaweeds are an important source of food, especially in Asia. They provide many vitamins including: A, B_1, B_2, B_6, niacin and C, and are rich in iodine, potassium, iron, magnesium and calcium. In addition commercially cultivated microalgae, including both Algae and Cyanobacteria, are marketed as nutritional supplements, such as Spirulina, Chlorella and the Vitamin-C supplement, Dunaliella, high in beta-carotene.

Algae are national foods of many nations: China consumes more than 70 species, including *fat choy*, a cyanobacterium considered a vegetable; Japan, over 20 species; Ireland, dulse; Chile, cochayuyo. Laver is used to make "laver bread" in Wales where it is known as *bara lawr*; in Korea, gim; in Japan, nori and aonori. It is also used along the west coast of North America from California to British Columbia, in Hawaii and by the Māori of New Zealand. Sea lettuce and badderlocks are a salad ingredient in Scotland, Ireland, Greenland and Iceland.

Dulse, a food.

The oils from some Algae have high levels of unsaturated fatty acids. For example, *Parietochloris incisa* is very high in arachidonic acid, where it reaches up to 47% of the triglyceride pool. Some varieties of Algae favored by vegetarianism and veganism contain the long-chain, essential omega-3 fatty acids, Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA), in addition to vitamin B_{12}. The vitamin B_{12} in algae is not biologically active. Fish oil contains the omega-3 fatty acids, but the original source is algae (microalgae in particular), which are eaten by marine life such as copepods and are passed up the food chain. Algae has emerged in recent years as a popular source of omega-3 fatty acids for vegetarians who cannot get long-chain EPA and DHA from other vegetarian sources such as flaxseed oil, which only contains the short-chain Alpha-Linolenic acid (ALA).

Pollution control

- Sewage can be treated with algae, reducing the need for greater amounts of toxic chemicals than are already used.
- Algae can be used to capture fertilizers in runoff from farms. When subsequently harvested, the enriched algae itself can be used as fertilizer.

Agricultural Research Service scientists found that 60-90% of nitrogen runoff and 70-100% of phosphorus runoff can be captured from manure effluents using an algal turf scrubber (ATS). Scientists developed the ATS, which are shallow, 100-foot raceways of nylon netting where algae colonies can form, and studied its efficacy for three years. They found that algae can readily be used to reduce the nutrient runoff from agricultural fields and increase the quality of water flowing into rivers, streams, and oceans. The enriched algae itself also can be used as a fertilizer. Researchers collected and dried the nutrient-rich algae from the ATS and studied its potential as an organic fertilizer. They found that cucumber and corn seedlings grew just as well using ATS organic fertilizer as they did with commercial fertilizers.

Pigments

The natural pigments produced by algae can be used as an alternative to chemical dyes and coloring agents.

Stabilizing substances

Carrageenan, from the red alga *Chondrus crispus*, is used as a stabiliser in milk products.

Chapter 3

Industrial Microbiology

Industrial microbiology or microbial biotechnology encompasses the use of microorganisms in the manufacture of food or industrial products. The use of microorganisms for the production of food, either human or animal, is often considered a branch of food microbiology. The microorganisms used in industrial processes may be natural isolates, laboratory selected mutants or genetically engineered organisms.

Food microbiology

Yogurt, cheese, chocolate, and silage (animal food) are all produced by industrial microbiology processes. 'Good' bacteria such as probiotics are becoming increasingly important in the food industry. Lactic Acid Bacteria and Bifidobacteria are amongst the most important groups of microorganisms used in the food industry. These bacteria are thought to have health-promoting abilities and many are used as probiotics for the prevention, alleviation and treatment of intestinal disorders in humans and animals.

Biopolymers

A huge variety of biopolymers, such as polysaccharides, polyesters, and polyamides, are produced by microorganisms. These products range from viscous solutions to plastics. The genetic manipulation of microorganisms has permitted the biotechnological production of biopolymers with tailored material properties suitable for high-value medical application such as tissue engineering and drug delivery. Industrial microbiology can be used for the biosynthesis of xanthan, alginate, cellulose, cyanophycin, poly(gamma-glutamic acid), levan, hyaluronic acid, organic acids, oligosaccharides and polysaccharides, and polyhydroxyalkanoates.

Bioremediation

Microbial biodegradation of pollutants can be used to cleanup contaminated environments. These bioremediation and biotransformation methods harness naturally occurring microbes to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals.

Waste biotreatment

Microorganisms are used to treat the vast quantities of wastes generated by modern societies. Biotreatment, the processing of wastes using living organisms, is an environmentally friendly, relatively simple and cost-effective alternative to physico-chemical clean-up options. Confined environments, such as bioreactors can be employed in biotreatment processes.

Wastewater treatment

Biological wastewater treatment is undoubtedly one of the most important biotechnological processes, which have been used for over a century to treat municipal and industrial wastewaters. Culture-independent molecular techniques have been used to study the diversity and physiology of ecologically important microorganisms in wastewater treatment processes. A number of new exciting insights into the structure, function, and dynamics of complex microbial communities in wastewater treatment processes have been gained, which have significantly expanded our understanding of process design, operation and control. Microbes play a vital role in the cycling of nitrogen in wastewater treatment processes (including anaerobic ammonia oxidation processes) and methane fermentation processes.

Health-care and medicine

Microorganisms are used to produce human or animal biologicals such as insulin, growth hormone, and antibodies. Diagnostic assays that use monoclonal antibody, DNA probe technology or real-time PCR are used as rapid tests for pathogenic organisms in the clinical laborarory.

Microorganisms may also help in the treatment of diseases such as cancer. Research shows that clostridia can selectively target cancer cells. Various strains of non-pathogenic clostridia have been shown to infiltrate and replicate within solid tumours. Clostridia therefore have the potential to deliver therapeutic proteins to tumours. *Lactobacillus* spp. and other lactic acid bacteria possess numerous potential therapeutic properties including anti-inflammatory and anti-cancer activities.

Vaccines are used to combat infectious disease, however the last decade has witnessed a revolution in the approach to vaccine design and development. Sophisticated

technologies such as genomics, proteomics, functional genomics, and synthetic chemistry can be used for the rational identification of antigens, the synthesis of complex glycans, and the generation of engineered carrier proteins.

Members of the *Streptomyces* genus are among the most prolific microorganisms producing secondary metabolites with wide uses in medicine and in agriculture. These organisms have a complex secondary metabolism producing antibiotic compounds and other metabolites with medicinal properties. Genomic studies, genomic mining and biotechnological approaches are being employed in the search for new antibiotics and other drugs in *Streptomyces*.

Archaea

Examination of microbes living in unusual environments (e.g. high temperatures, salt, low pH or temperature, high radiation) lead to discovery of microbes with new abilities that can be harnessed for industrial purposes.

Corynebacteria

Corynebacteria are a diverse group Gram-positive bacteria found in a range of different ecological niches such as soil, vegetables, sewage, skin, and cheese smear. *Corynebacterium glutamicum* is of immense industrial importance and is one of the biotechnologically most important bacterial species with an annual production of more than two million tons of amino acids, mainly L-glutamate and L-lysine. The genome sequence of *C. glutamicum* has been published.

Xanthomonas

The genus *Xanthomonas* consists of 20 plant-associated species, many of which cause important diseases of crops and other plants. Individual species comprise multiple pathovars, characterized by distinctive host specificity or mode of infection. Bacteria of the genus *Xanthomonas* are able to produce the acidic exopolysaccharide xanthan gum. Because of its physical properties, it is widely used as a viscosifer, thickener, emulsifier or stabilizer in both food and non-food industries.

Aspergillus

Species within the genus *Aspergillus* have a large chemical repertoire. Commodity products produced in *Aspergillus* cell 'factories' include citric, gluconic, itaconic and kojic acid. The use of *Aspergillus niger* in citric acid production dates back to 1917. Citric acid is one of the most widely used food ingredients. It also has found use in the pharmaceutical and cosmetic industries as an acidulant and for aiding in the dissolution of active ingredients. Other technical applications of citric acid are as a hardener in adhesive and for retarding the setting of concrete. Citric acid is a true 'bulk chemical' with

an estimated production approximating more than 1.6 billion kg each year *A. niger* also has found use in the industrial production of gluconic acid, which is used as an additive in certain metal cleaning applications, as well as for the therapy for calcium and iron deficiencies. *Aspergillus terreus* is used for itaconic acid production, a synthetic polymer. *A. oryzae* is fermented for kojic acid production which is used for skin whitening and as a precursor for synthesis of flavour enhancers.

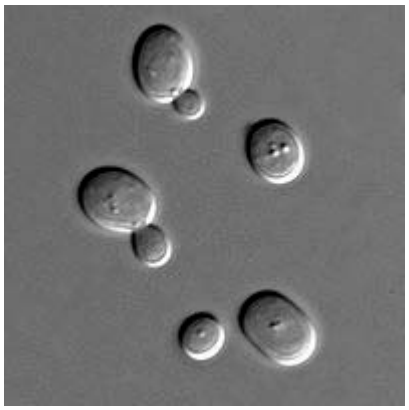
Viruses

Viruses that are pathogenic to insect pests can be exploited as biological control agents. Some viruses such as baculoviruses have been exploited for use as gene expression and delivery vectors in both insect and mammalian cells.

Chapter 4

Yeast

Yeast



Yeast of the species *Saccharomyces cerevisiae*

Scientific classification	
Domain:	Eukaryota
Kingdom:	Fungi

Phyla and Subphyla

Ascomycota

- Saccharomycotina (true yeasts)
- Taphrinomycotina
  - Schizosaccharomycetes (fission yeasts)

Basidiomycota

- Agaricomycotina
  - Tremellomycetes

- Pucciniomycotina
    - Microbotryomycetes

Yeasts are eukaryotic micro-organisms classified in the kingdom Fungi, with the 1,500 species currently described estimated to be only 1% of all yeast species. Most reproduce asexually by budding, although a few do so by mitosis. Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or false hyphae, as seen in most molds. Yeast size can vary greatly depending on the species, typically measuring 3–4 μm in diameter, although some yeasts can reach over 40 μm.

The yeast species *Saccharomyces cerevisiae* has been used in baking and in fermenting alcoholic beverages for thousands of years. It is also extremely important as a model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms. Researchers have used it to gather information about the biology of the eukaryotic cell and ultimately human biology. Other species of yeast, such as *Candida albicans*, are opportunistic pathogens and can cause infections in humans. Yeasts have recently been used to generate electricity in microbial fuel cells, and produce ethanol for the biofuel industry.

Yeasts do not form a single taxonomic or phylogenetic grouping. The term "yeast" is often taken as a synonym for *Saccharomyces cerevisiae*, but the phylogenetic diversity of yeasts is shown by their placement in two separate phyla, the Ascomycota and the Basidiomycota. The budding yeasts ("true yeasts") are classified in the order Saccharomycetales.

History

The word "yeast" comes to us from Old English *gist*, *gyst*, and from the Indo-European root *yes*-, meaning *boil*, *foam*, or *bubble*. Yeast microbes are probably one of the earliest domesticated organisms. People have used yeast for fermentation and baking throughout history. Archaeologists digging in Egyptian ruins found early grinding stones and baking chambers for yeasted bread, as well as drawings of 4,000-year-old bakeries and breweries. In 1680, the Dutch naturalist Anton van Leeuwenhoek first microscopically observed yeast, but at the time did not consider them to be living organisms, but rather globular structures. In 1857, French microbiologist Louis Pasteur proved in the paper "*Mémoire sur la fermentation alcoolique*" that alcoholic fermentation was conducted by living yeasts and not by a chemical catalyst. Pasteur showed that by bubbling oxygen into the yeast broth, cell growth could be increased, but fermentation was inhibited – an observation later called the "Pasteur effect".

By the late 18th century, two yeast strains used in brewing had been identified: *Saccharomyces cerevisiae*, so called top fermenting yeast, and *S. carlsbergensis*, bottom fermenting yeast. *S. cerevisiae* has been sold commercially by the Dutch for bread making since 1780; while around 1800, the Germans started producing *S. cerevisiae* in

the form of cream. In 1825 a method was developed to remove the liquid so the yeast could be prepared as solid blocks. The industrial production of yeast blocks was enhanced by the introduction of the filter press in 1867. In 1872, Baron Max de Springer developed a manufacturing process to create granulated yeast, a technique that was used until the first World War. In the United States, naturally occurring airborne yeasts were used almost exclusively until commercial yeast was marketed at the Centennial Exposition in 1876 in Philadelphia, where Charles L. Fleischmann exhibited the product and a process to use it, as well as serving the resultant baked bread.

Nutrition and growth

Yeasts are chemoorganotrophs, as they use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from hexose sugars, such as glucose and fructose, or disaccharides such as sucrose and maltose. Some species can metabolize pentose sugars like ribose, alcohols, and organic acids. Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes), or are anaerobic, but also have aerobic methods of energy production (facultative anaerobes). Unlike bacteria, there are no known yeast species that grow only anaerobically (obligate anaerobes). Yeasts grow best in a neutral or slightly acidic pH environment.

Yeasts vary in what temperature range they grow best. For example, *Leucosporidium frigidum* grows at -2 to 20 °C (28 to 68 °F), *Saccharomyces telluris* at 5 to 35 °C (41 to 95 °F) and *Candida slooffi* at 28 to 45 °C (82 to 113 °F). The cells can survive freezing under certain conditions, with viability decreasing over time.

Yeasts are generally grown in the laboratory on solid growth media or in liquid broths. Common media used for the cultivation of yeasts include potato dextrose agar (PDA) or potato dextrose broth, Wallerstein Laboratories nutrient (WLN) agar, yeast peptone dextrose agar (YPD), and yeast mould agar or broth (YM). Home brewers who cultivate yeast frequently use dried malt extract (DME) and agar as a solid growth medium. The antibiotic cycloheximide is sometimes added to yeast growth media to inhibit the growth of *Saccharomyces* yeasts and select for wild/indigenous yeast species. This will change the yeast process.

The appearance of a white, thready yeast, commonly known as kahm yeast, is often a byproduct of the lactofermentation (or pickling) of certain vegetables, usually the result of exposure to air. Although harmless, it can give pickled vegetables a bad flavour and so must be removed regularly during fermentation.

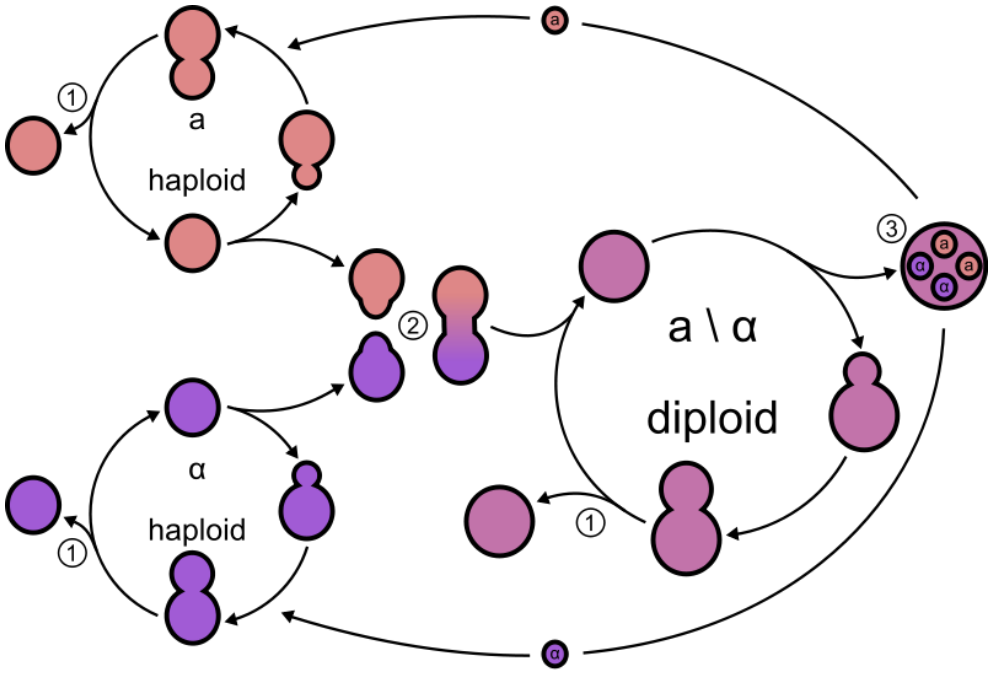
Ecology

Yeasts are very common in the environment, but are usually isolated from sugar-rich material. Examples include naturally occurring yeasts on the skins of fruits and berries (such as grapes, apples or peaches), and exudates from plants (such as plant saps or cacti). Some yeasts are found in association with soil and insects. The ecological function

and biodiversity of yeasts are relatively unknown compared to those of other microorganisms. Yeasts, including *Candida albicans*, *Rhodotorula rubra*, *Torulopsis* and *Trichosporon cutaneum*, have been found living in between people's toes as part of their skin flora. Yeasts are also present in the gut flora of mammals and some insects and even deep-sea environments host an array of yeasts.

An Indian study of seven bee species and 9 plant species found 45 species from 16 genera colonise the nectaries of flowers and honey stomachs of bees. Most were members of the *Candida* genus; the most common species in honey stomachs was *Dekkera intermedia* and in flower nectaries, *Candida blankii*. Yeast colonising nectaries of the stinking hellebore have been found to raise the temperature of the flower, which may aid in attracting pollinators by increasing the evaporation of volatile organic compounds. A black yeast has been recorded as a partner in a complex relationship between ants, their mutualistic fungus, a fungal parasite of the fungus and a bacterium that kills the parasite. The yeast have a negative effect on the bacteria that normally produce antibiotics to kill the parasite and so may affect the ants' health by allowing the parasite to spread.

Reproduction



The yeast cell's life cycle:
1. Budding
2. Conjugation
3. Spore

Yeasts have asexual and sexual reproductive cycles. The most common mode of vegetative growth in yeast is asexual reproduction by budding. Here a small bud, or daughter cell, is formed on the parent cell. The nucleus of the parent cell splits into a

daughter nucleus and migrates into the daughter cell. The bud continues to grow until it separates from the parent cell, forming a new cell. Some yeasts, including *Schizosaccharomyces pombe*, reproduce by mitosis instead of budding.

Under high stress conditions, haploid cells will generally die; under the same conditions, however, diploid cells can undergo sporulation, entering sexual reproduction (meiosis) and producing a variety of haploid spores, which can go on to mate (conjugate), reforming the diploid.

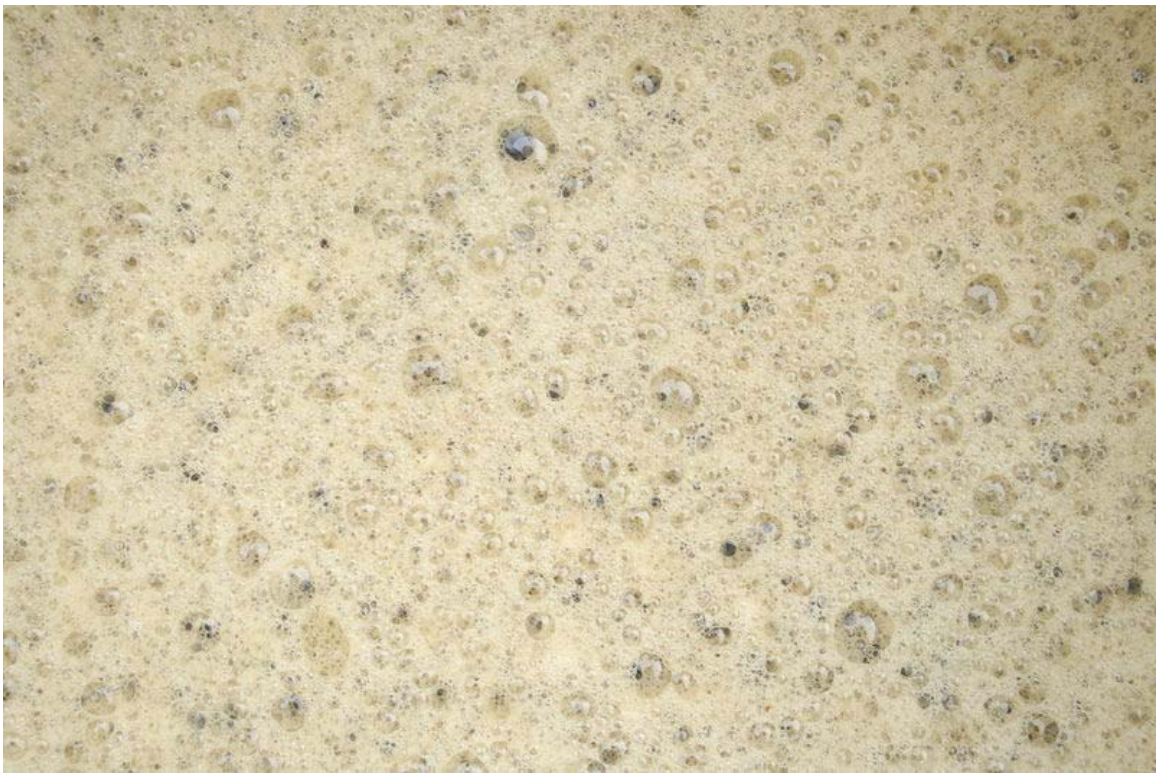
Uses

The useful physiological properties of yeast have led to their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest and largest application of this technology. Many types of yeasts are used for making many foods: baker's yeast in bread production; brewer's yeast in beer fermentation; yeast in wine fermentation and for xylitol production. So-called red rice yeast is actually a mold, *Monascus purpureus*. Yeasts include some of the most widely used model organisms for genetics and cell biology.

Alcoholic beverages

Alcoholic beverages are defined as beverages that contain ethanol (C_2H_5OH). This ethanol is almost always produced by fermentation – the metabolism of carbohydrates by certain species of yeast under anaerobic or low-oxygen conditions. Beverages such as wine, beer, or distilled spirits all use yeast at some stage of their production. A distilled beverage is a beverage containing ethanol that has been purified by distillation. Carbohydrate-containing plant material is fermented by yeast, producing a dilute solution of ethanol in the process. Spirits such as whiskey and rum are prepared by distilling these dilute solutions of ethanol. Components other than ethanol are collected in the condensate, including water, esters, and other alcohols, which account for the flavour of the beverage.

Beer



Beer being fermented by brewers yeast

Brewing yeasts may be classed as "top cropping" (or "top fermenting") and "bottom cropping" (or "bottom-fermenting"). Top cropping yeasts are so called because they form a foam at the top of the wort during fermentation. An example of a top cropping yeast is *Saccharomyces cerevisiae*, sometimes called an "ale yeast". Bottom cropping yeasts are typically used to produce lager-type beers, though they can also produce ale-type beers. These yeasts ferment more sugars, creating a dryer beer, and grow well at low temperatures. An example of bottom cropping yeast is *Saccharomyces pastorianus*, formerly known as *S. carlsbergensis*.

The most common top cropping brewer's yeast, *S. cerevisiae*, is the same species as the common baking yeast. However, baking and brewing yeasts typically belong to different strains, cultivated to favour different characteristics: baking yeast strains are more aggressive, to carbonate dough in the shortest amount of time possible; brewing yeast strains act slower, but tend to produce fewer off-flavours and tolerate higher alcohol concentrations (with some strains, up to 22%).

Brettanomyces is a genus of wild yeast important in brewing lambic, a beer produced not by the deliberate addition of brewer's yeasts, but by spontaneous fermentation by wild yeasts and bacteria. *Brettanomyces lambicus*, *B. bruxellensis* and *B. claussenii* are native to the Senne Valley region of Belgium, where lambic beer is produced.

Wine

Fresh grapes with visible bloom.

Yeast is used in winemaking, where it converts the sugars present in grape juice (must) into ethanol. Yeast is normally already present on grape skins (the white powder called "the bloom"). Fermentation can be done with this endogenous "wild yeast," but this procedure gives unpredictable results, which depend upon the exact types of yeast species present. For this reason, a pure yeast culture is usually added to the must; this yeast quickly dominates the fermentation. The wild yeasts are repressed, which ensures a reliable and predictable fermentation.

Most added wine yeasts are strains of *S. cerevisiae*, though not all strains of the species are suitable. Different *S. cerevisiae* yeast strains have differing physiological and fermentative properties, therefore the actual strain of yeast selected can have a direct impact on the finished wine. Significant research has been undertaken into the development of novel wine yeast strains that produce atypical flavour profiles or increased complexity in wines.

The growth of some yeasts, such as *Zygosaccharomyces* and *Brettanomyces*, in wine can result in wine faults and subsequent spoilage. *Brettanomyces* produces an array of metabolites when growing in wine, some of which are volatile phenolic compounds.

Together, these compounds are often referred to as "*Brettanomyces* character", and are often described as "antiseptic" or "barnyard" type aromas. *Brettanomyces* is a significant contributor to wine faults within the wine industry.

Researchers from University of British Columbia, Canada, have found a new strain of yeast that has reduced amines. The amines in red wine and Chardonnay produce off-flavors and cause headaches and hypertension in some people. About 30 percent of people are sensitive to biogenic amines, such as histamines.

Baking

Yeast, most commonly *S. cerevisiae*, is used in baking as a leavening agent, where it converts the fermentable sugars present in dough into the gas carbon dioxide. This causes the dough to expand or rise as gas forms pockets or bubbles. When the dough is baked, the yeast dies and the air pockets "set", giving the baked product a soft and spongy texture. The use of potatoes, water from potato boiling, eggs, or sugar in a bread dough accelerates the growth of yeasts. Most yeasts used in baking are of the same species common in alcoholic fermentation. Additionally, *Saccharomyces exiguus* (also known as *S. minor*), a wild yeast found on plants, fruits, and grains, is occasionally used for baking. Sugar and vinegar provide the best conditions for yeast to ferment. In bread making, the yeast initially respires aerobically, producing carbon dioxide and water. When the oxygen is depleted, anaerobic respiration begins, producing ethanol as a waste product; however, this evaporates during baking.

A block of fresh yeast

It is not known when yeast was first used to bake bread. The first records that show this use came from Ancient Egypt. Researchers speculate a mixture of flour meal and water was left longer than usual on a warm day and the yeasts that occur in natural contaminants of the flour caused it to ferment before baking. The resulting bread would have been lighter and tastier than the normal flat, hard cake.

Active dried yeast, a granulated form in which yeast is commercially sold

Today, there are several retailers of baker's yeast; one of the best-known in North America is Fleischmann’s Yeast, which was developed in 1868. During World War II, Fleischmann's developed a granulated active dry yeast, which did not require refrigeration and had a longer shelf life than fresh yeast. The company created yeast that would rise twice as fast, reducing baking time. Baker's yeast is also sold as a fresh yeast compressed into a square "cake". This form perishes quickly, and must therefore be used soon after production. A weak solution of water and sugar can be used to determine if yeast is expired. In the solution, active yeast will foam and bubble as it ferments the sugar into ethanol and carbon dioxide. Some recipes refer to this as proofing the yeast as it "proves" (tests) the viability of the yeast before the other ingredients are added. When using a sourdough starter, flour and water are added instead of sugar; this is referred to as proofing the sponge.

When yeast is used for making bread, it is mixed with flour, salt, and warm water or milk. The dough is kneaded until it is smooth, and then left to rise, sometimes until it has doubled in size. Some bread doughs are knocked back after one rising and left to rise again. A longer rising time gives a better flavour, but the yeast can fail to raise the bread in the final stages if it is left for too long initially. The dough is then shaped into loaves, left to rise until it is the correct size, and then baked. Dried yeast is usually specified for use in a bread machine, however a (wet) sourdough starter can also work.

Bioremediation

Some yeasts can find potential application in the field of bioremediation. One such yeast, *Yarrowia lipolytica*, is known to degrade palm oil mill effluent, TNT (an explosive material), and other hydrocarbons, such as alkanes, fatty acids, fats and oils. It can also tolerate high concentrations of salt and heavy metals, and is being investigated for its potential as a heavy metal biosorbent.

Industrial ethanol production

The ability of yeast to convert sugar into ethanol has been harnessed by the biotechnology industry to produce ethanol fuel. The process starts by milling a feedstock, such as sugar cane, field corn, or other cereal grains, and then adding dilute sulfuric acid, or fungal alpha amylase enzymes, to break down the starches into complex sugars. A glucoamylase is then added to break the complex sugars down into simple sugars. After this, yeasts are added to convert the simple sugars to ethanol, which is then distilled off to obtain ethanol up to 96% in concentration.

Saccharomyces yeasts have been genetically engineered to ferment xylose, one of the major fermentable sugars present in cellulosic biomasses, such as agriculture residues, paper wastes, and wood chips. Such a development means ethanol can be efficiently produced from more inexpensive feedstocks, making cellulosic ethanol fuel a more competitively priced alternative to gasoline fuels.

Nonalcoholic beverages

A Kombucha culture fermenting in a jar

Root beer and other sweet carbonated beverages can be produced using the same methods as beer, except the fermentation is stopped sooner, producing carbon dioxide, but only trace amounts of alcohol, and a significant amount of sugar is left in the drink. *Kvass*, a fermented drink made from rye, is popular in Eastern Europe; it has a recognizable, but low alcoholic content. Yeast in symbiosis with acetic acid bacteria is used in the preparation of *kombucha*, a fermented sweetened tea. Species of yeast found in the tea can vary, and may include: *Brettanomyces bruxellensis*, *Candida stellata*, *Schizosaccharomyces pombe*, *Torulaspora delbrueckii* and *Zygosaccharomyces bailii*. *Kombucha* is a popular beverage in Eastern Europe and some former Soviet republics

under the name *chajnyj grib* (Чайный гриб). *Kefir* and *kumis* are made by fermenting milk with yeast and bacteria.

Nutritional supplements

Yeast is used in nutritional supplements popular with vegans and the health conscious, where it is often referred to as "nutritional yeast". It is a deactivated yeast, usually *S. cerevisiae*. It is an excellent source of protein and vitamins, especially the B-complex vitamins, whose functions are related to metabolism, as well as other minerals and cofactors required for growth. It is also naturally low in fat and sodium. Some brands of nutritional yeast, though not all, are fortified with vitamin B_{12}, which is produced separately by bacteria. Nutritional yeast, though it has a similar appearance to brewer's yeast, is very different and has a very different taste.

Nutritional yeast has a nutty, cheesy, creamy flavor which makes it popular as an ingredient in cheese substitutes. It is often used by vegans in place of Parmesan cheese. Another popular use is as a topping for popcorn. It can also be used in mashed and fried potatoes, as well as in scrambled eggs. It comes in the form of flakes, or as a yellow powder similar in texture to cornmeal, and can be found in the bulk aisle of most natural food stores. In Australia, it is sometimes sold as "savory yeast flakes". Though "nutritional yeast" usually refers to commercial products, inadequately fed prisoners have used "home-grown" yeast to prevent vitamin deficiency.

Probiotics

Some probiotic supplements use the yeast *S. boulardii* to maintain and restore the natural flora in the gastrointestinal tract. *S. boulardii* has been shown to reduce the symptoms of acute diarrhea in children, prevent reinfection of *Clostridium difficile*, reduce bowel movements in diarrhea-predominant IBS patients, and reduce the incidence of antibiotic, traveler's, and HIV/AIDS associated diarrheas.

Aquarium hobby

Yeast is often used by aquarium hobbyists to generate carbon dioxide (CO_2) to nourish plants in planted aquariums. A homemade setup is widely used as a cheap and simple alternative to pressurized CO_2 systems. While not as effective as these, the homemade setup is considerably cheaper for less demanding hobbyists.

There are several recipes for homemade CO_2, but they are variations of the basic recipe: Baker's yeast, with sugar, baking soda and water, are added to a plastic bottle. A few drops of vegetable oil at the start reduces surface tension and speeds the release of CO_2. This will produce CO_2 for about 2 or 3 weeks; the use of a bubble counter determines production. The CO_2 is injected in the aquarium via a narrow hose and released through a diffuser that helps dissolve the gas in the water. The CO_2 is used by plants in the photosynthesis process.

Science

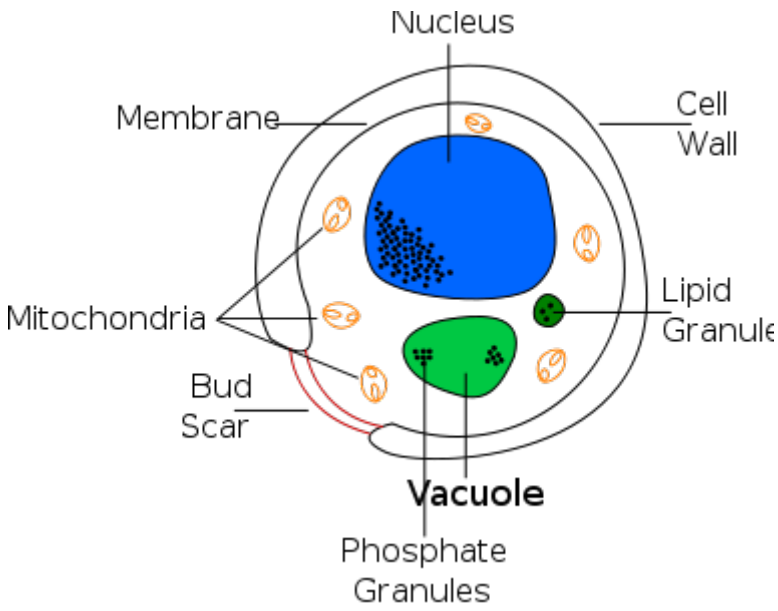


Diagram showing a yeast cell

Several yeasts, particularly *S. cerevisiae*, have been widely used in genetics and cell biology. This is largely because *S. cerevisiae* is a simple eukaryotic cell, serving as a model for all eukaryotes, including humans for the study of fundamental cellular processes such as the cell cycle, DNA replication, recombination, cell division and metabolism. Also, yeasts are easily manipulated and cultured in the laboratory, which has allowed for the development of powerful standard techniques, such as yeast two-hybrid, synthetic genetic array analysis and tetrad analysis. Many proteins important in human biology were first discovered by studying their homologues in yeast; these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes.

On 24 April 1996 *S. cerevisiae* was announced to be the first eukaryote to have its genome, consisting of 12 million base pairs, fully sequenced as part of the Genome project. At the time, it was the most complex organism to have its full genome sequenced, and took seven years and the involvement of more than 100 laboratories to accomplish. The second yeast species to have its genome sequenced was *Schizosaccharomyces pombe*, which was completed in 2002. It was the sixth eukaryotic genome sequenced and consists of 13.8 million base pairs.

Yeast extract

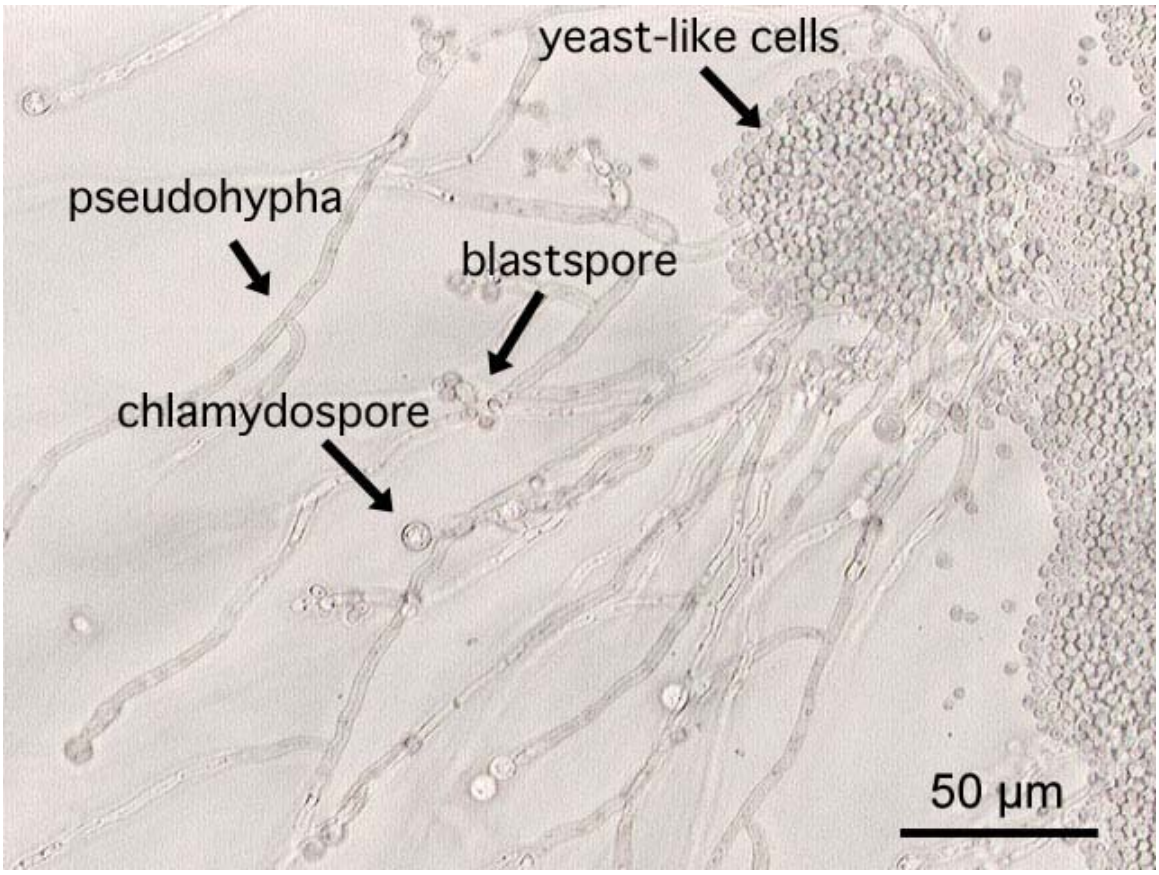
Marmite and Vegemite have a distinctive dark colour

Marmite and Vegemite, products made from yeast extract

Yeast extract is the common name for various forms of processed yeast products that are used as food additives or flavours. They are often used in the same way that monosodium glutamate (MSG) is used, and like MSG, often contain free glutamic acid. The general method for making yeast extract for food products such as Vegemite and Marmite on a commercial scale is to add salt to a suspension of yeast making the solution hypertonic, which leads to the cells shrivelling up. This triggers *autolysis*, where the yeast's digestive enzymes break their own proteins down into simpler compounds, a process of self-

destruction. The dying yeast cells are then heated to complete their breakdown, after which the husks (yeast with thick cell walls which would give poor texture) are separated. Yeast autolysates are used in Vegemite and Promite (Australia); Marmite, Bovril and Oxo (the United Kingdom, Republic of Ireland and South Africa); and Cenovis (Switzerland).

Pathogenic yeasts



A photomicrograph of *Candida albicans* showing hyphal outgrowth and other morphological characteristics.

Some species of yeast are opportunistic pathogens where they can cause infection in people with compromised immune systems.

Cryptococcus neoformans is a significant pathogen of immunocompromised people causing the disease termed cryptococcosis. This disease occurs in about 7–9% of AIDS patients in the USA, and a slightly smaller percentage (3–6%) in western Europe. The cells of the yeast are surrounded by a rigid polysaccharide capsule, which helps to prevent them from being recognised and engulfed by white blood cells in the human body.

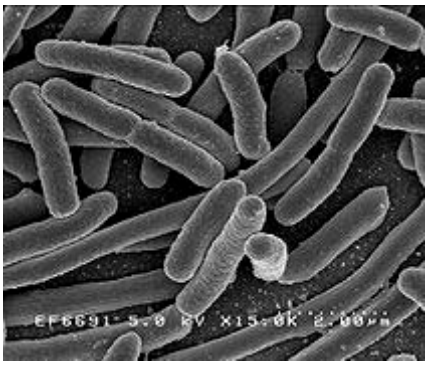
Yeasts of the *Candida* genus are another group of opportunistic pathogens which causes oral and vaginal infections in humans, known as candidiasis. *Candida* is commonly found as a commensal yeast in the mucus membranes of humans and other warm-blooded animals. However, sometimes these same strains can become pathogenic. Here the yeast cells sprout a hyphal outgrowth, which locally penetrates the mucosal membrane, causing irritation and shedding of the tissues. The pathogenic yeasts of candidiasis in probable descending order of virulence for humans are: *C. albicans*, *C. tropicalis*, *C. stellatoidea*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. viswanathii*, *C. lusitaniae* and *Rhodotorula mucilaginosa*. *Candida glabrata* is the second most common *Candida* pathogen after *C. albicans*, causing infections of the urogenital tract, and of the bloodstream (candidemia).

Food spoilage

Yeasts are able to grow in foods with a low pH, (5.0 or lower) and in the presence of sugars, organic acids and other easily metabolized carbon sources. During their growth, yeasts metabolize some food components and produce metabolic end products. This causes the physical, chemical, and sensible properties of a food to change, and the food is spoiled. The growth of yeast within food products is often seen on their surface, as in cheeses or meats, or by the fermentation of sugars in beverages, such as juices, and semi-liquid products, such as syrups and jams. The yeast of the *Zygosaccharomyces* genus have had a long history as a spoilage yeast within the food industry. This is mainly due to the fact that these species can grow in the presence of high sucrose, ethanol, acetic acid, sorbic acid, benzoic acid, and sulfur dioxide concentrations, representing some of the commonly used food preservation methods. Methylene blue is used to test for the presence of live yeast cells.

Chapter 5

Bacteria

Bacteria **Temporal range: Archean or earlier – Recent**

Scanning electron micrograph of *Escherichia coli* bacilli
Scientific classification
Domain: **Bacteria**
Phyla

- **gram positive/no outer membrane**

Actinobacteria (high-G+C)
Firmicutes (low-G+C)
Tenericutes (no wall)

- **gram negative/outer membrane present**

Aquificae
Deinococcus-Thermus

Fibrobacteres–
Chlorobi/Bacteroidetes
(FCB group)
Fusobacteria
Gemmatimonadetes
Nitrospirae
Planctomycetes–
Verrucomicrobia/Chlamydiae
(PVC group)
Proteobacteria
Spirochaetes
Synergistetes

- **unknown/ungrouped**

Acidobacteria
Chloroflexi
Chrysiogenetes
Cyanobacteria
Deferribacteres
Dictyoglomi
Thermodesulfobacteria
Thermotogae

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.

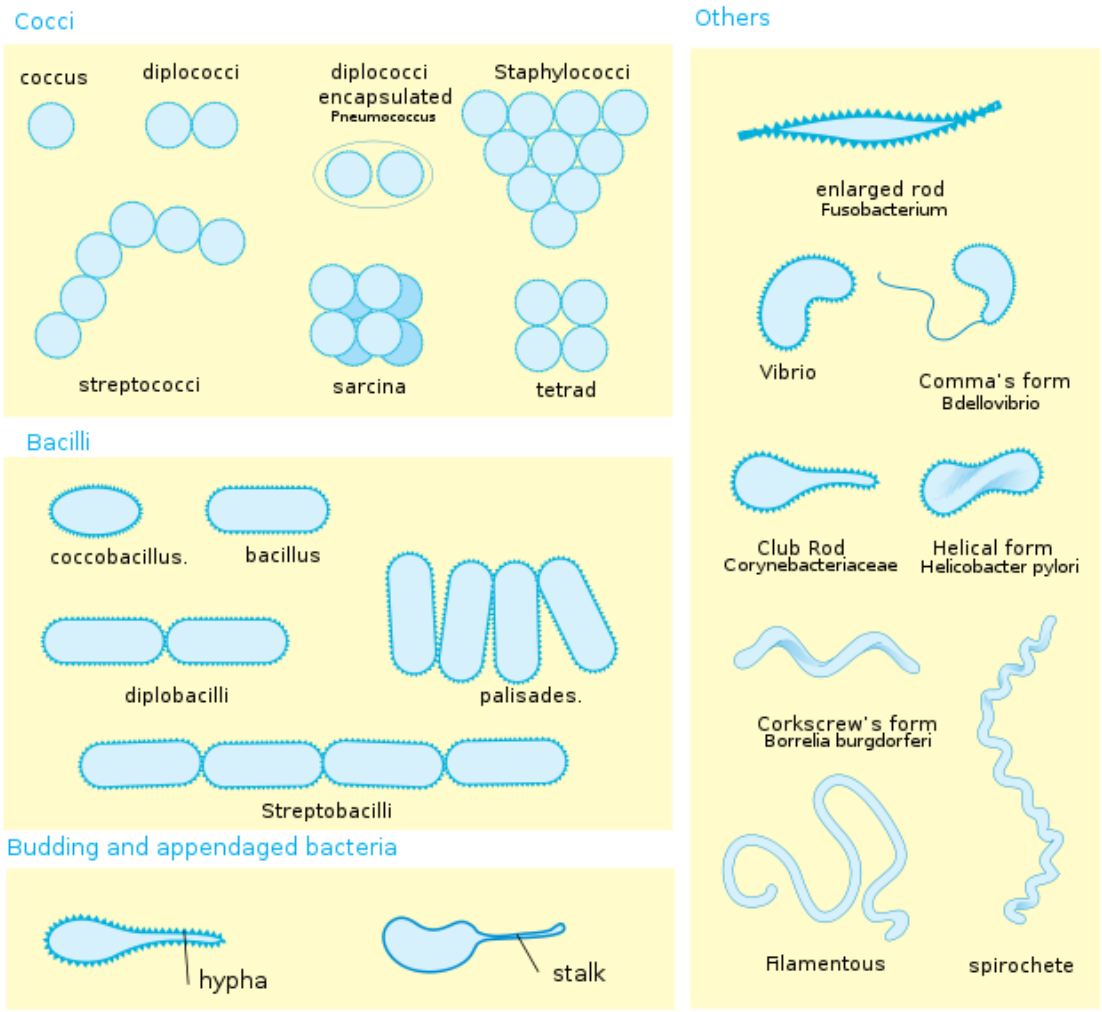
Origin and early evolution

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

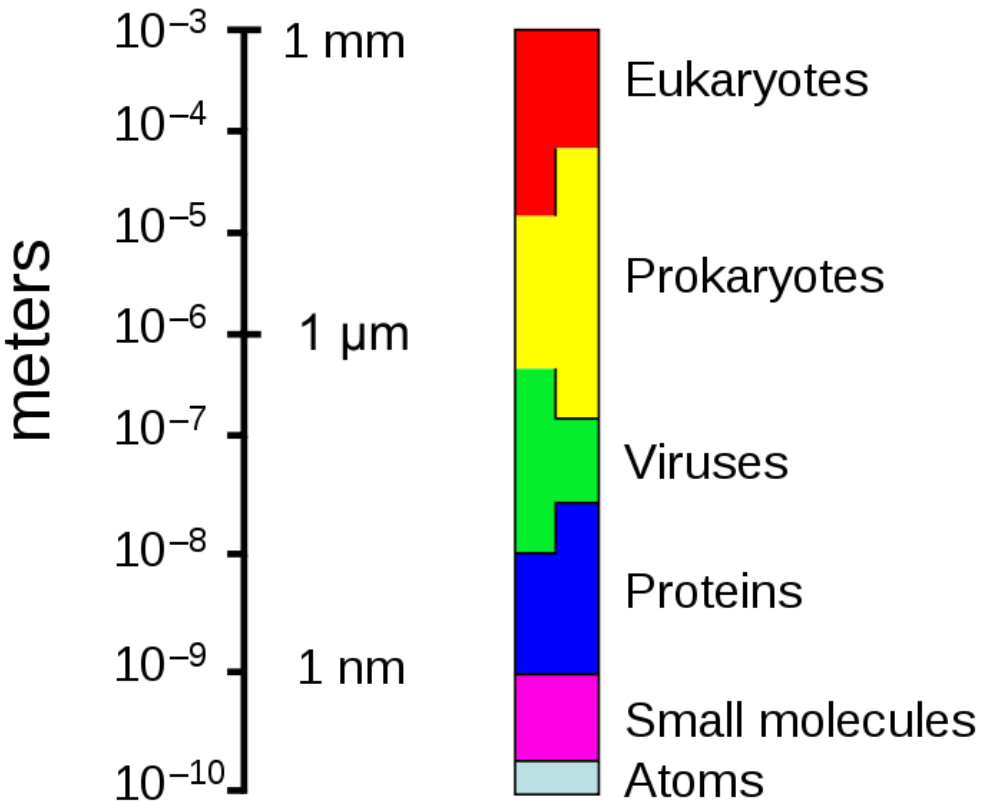


Bacteria display many cell morphologies and arrangements

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.

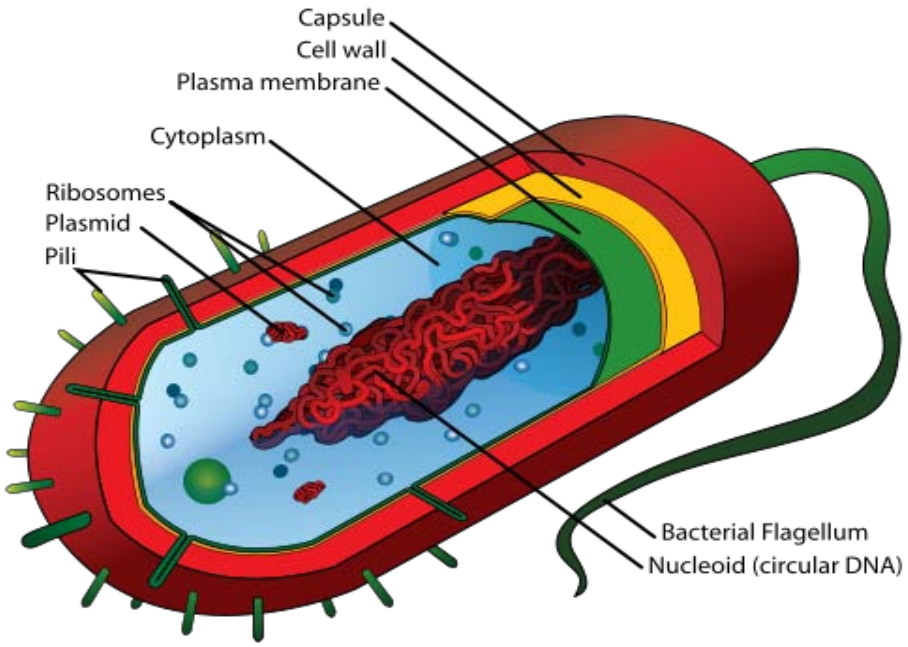


The range of sizes shown by prokaryotes, relative to those of other organisms and biomolecules

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



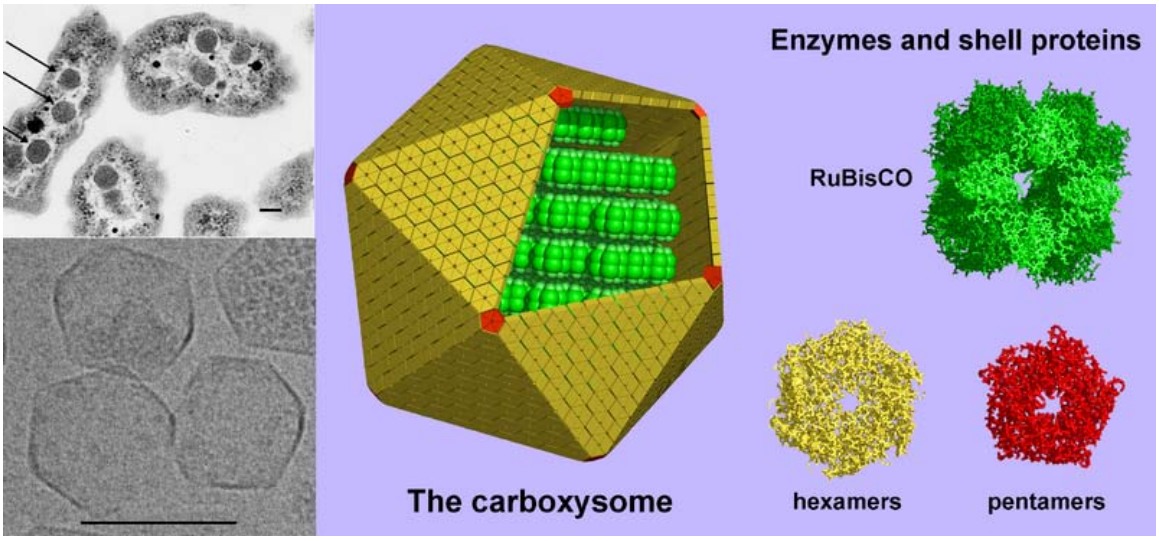
Structure and contents of a typical Gram positive bacterial cell

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.

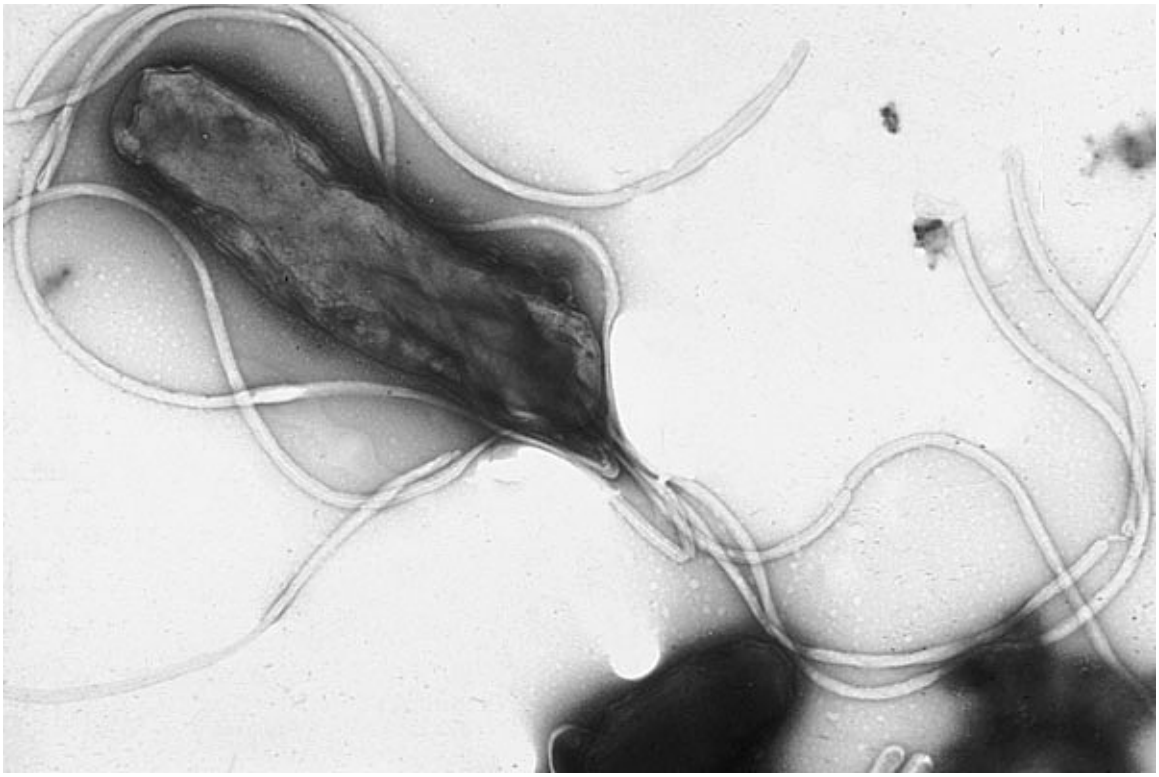
Extracellular structures

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.

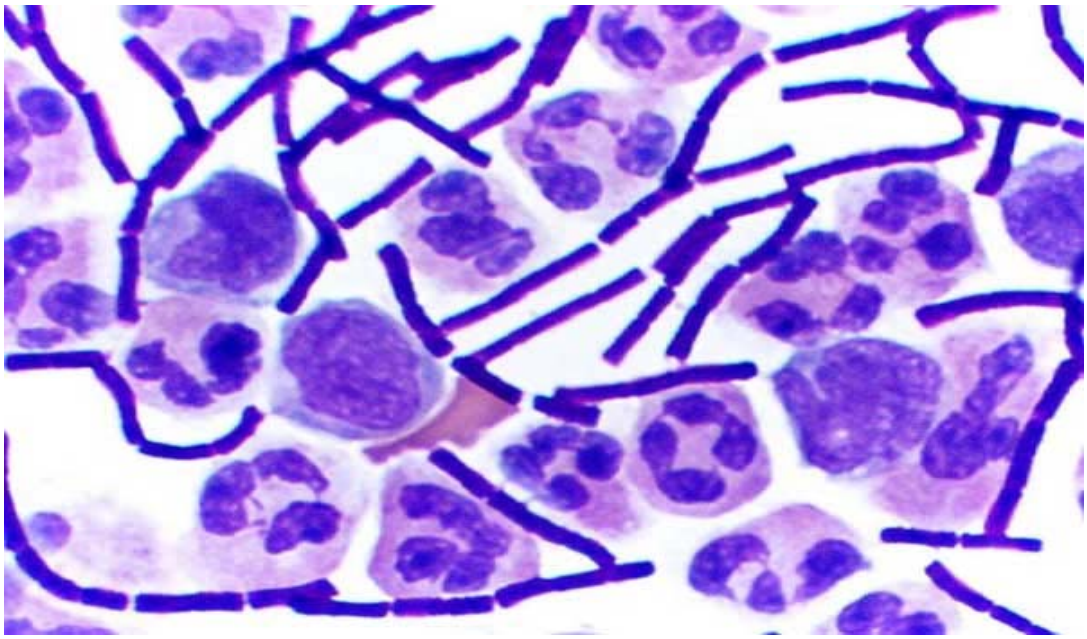
Fimbriae are fine filaments of protein, just 2–10 nanometres in diameter and up to several micrometers in length. They are distributed over the surface of the cell, and resemble fine hairs when seen under the electron microscope. Fimbriae are believed to be involved in attachment to solid surfaces or 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.

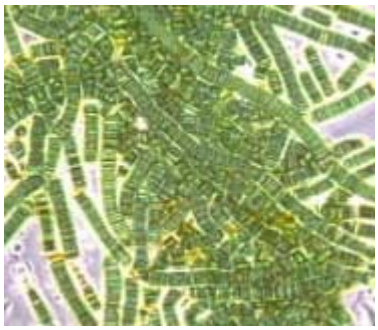
Metabolism

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.

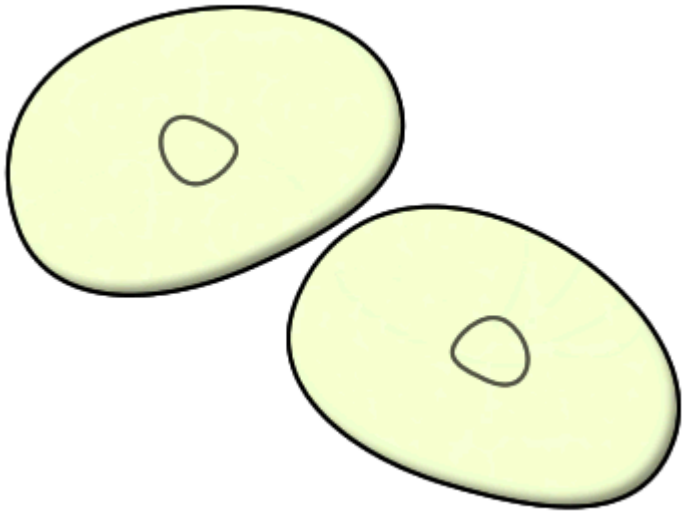
Another way of life of chemotrophs in the absence of possible electron acceptors is fermentation, where the electrons taken from the reduced substrates are transferred to oxidised intermediates to generate reduced fermentation products (e.g. lactate, ethanol, hydrogen, butyric acid). Fermentation is 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.

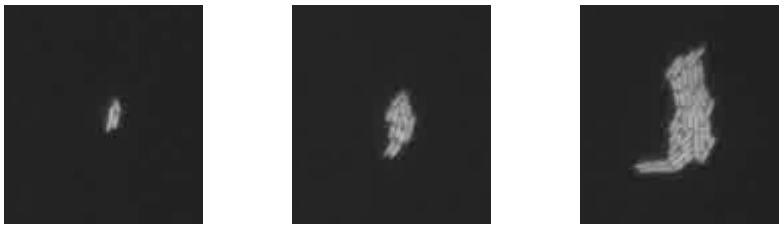
In addition to fixing carbon dioxide in photosynthesis, some bacteria also fix nitrogen gas (nitrogen fixation) using the enzyme nitrogenase. This environmentally important trait can be found in bacteria of nearly all the metabolic types listed above, but is not universal.

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.

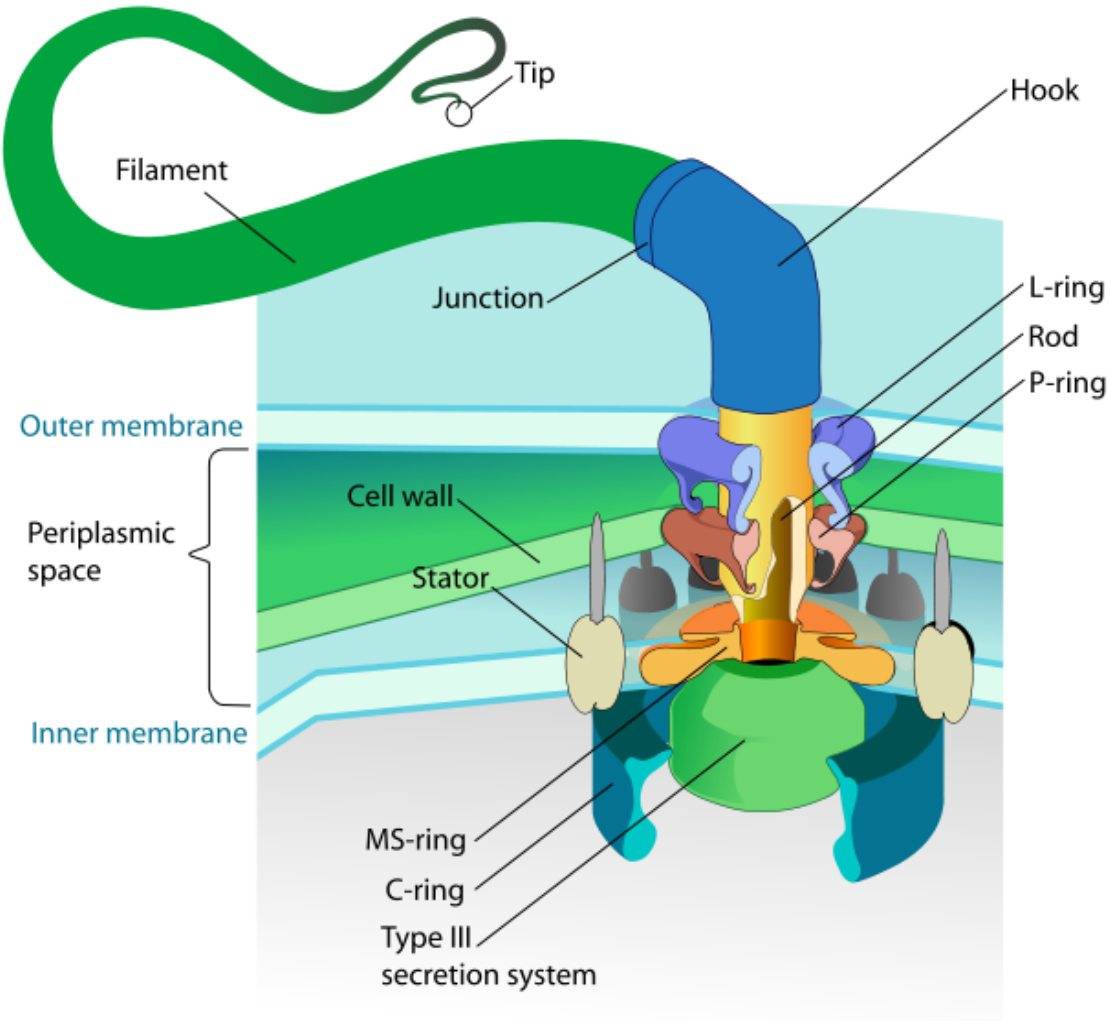
The communal benefits of multicellular cooperation include a cellular division of labor, accessing resources that cannot effectively be utilized by single cells, collectively defending against antagonists, and optimizing population survival by differentiating into distinct cell types. For example, bacteria 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-

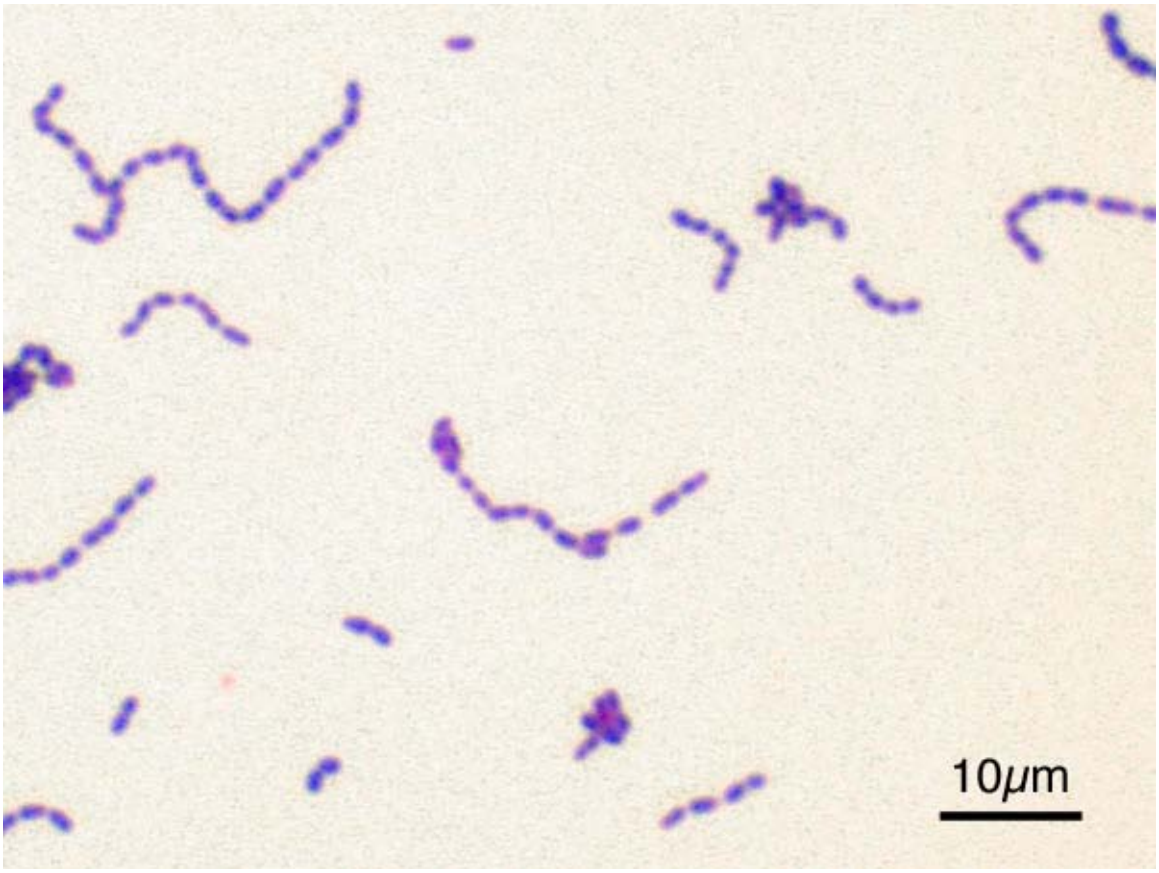
understood motility structure in any organism and is made of about 20 proteins, with approximately another 30 proteins required for its regulation and assembly. The flagellum is a rotating structure driven by a reversible motor at the base that uses the electrochemical gradient across the membrane for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

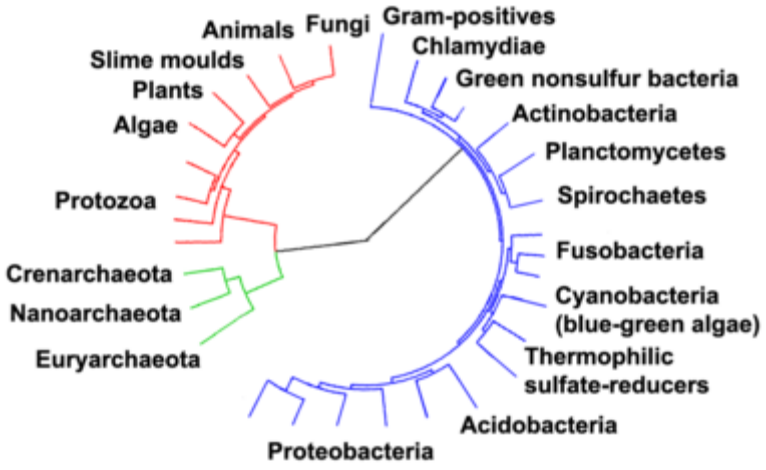


Streptococcus mutans visualized with a Gram stain

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

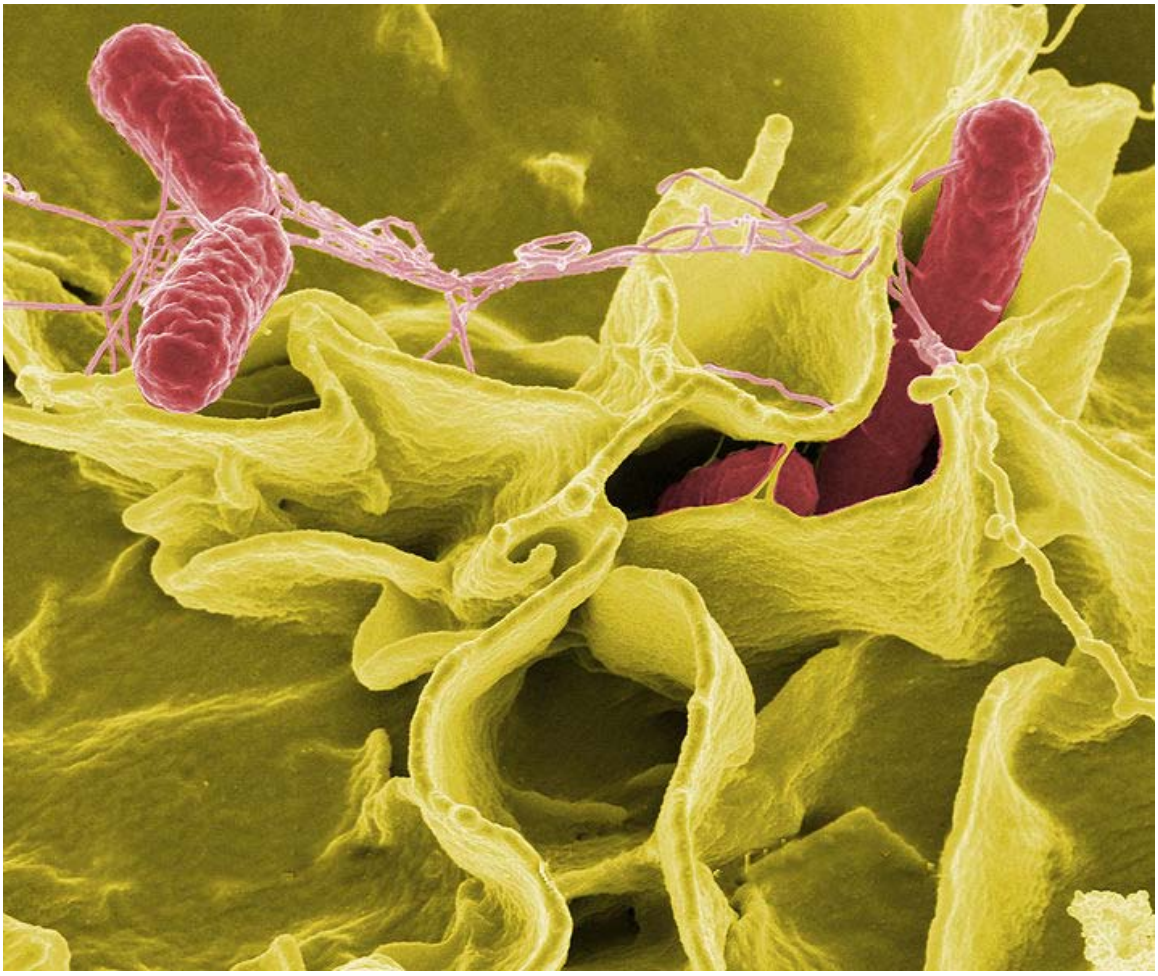
Some species of bacteria kill and then consume other microorganisms, these species called *predatory bacteria*. These include organisms such as *Myxococcus xanthus*, which forms swarms of cells that kill and digest any bacteria they encounter. Other bacterial predators either attach to their prey in 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.

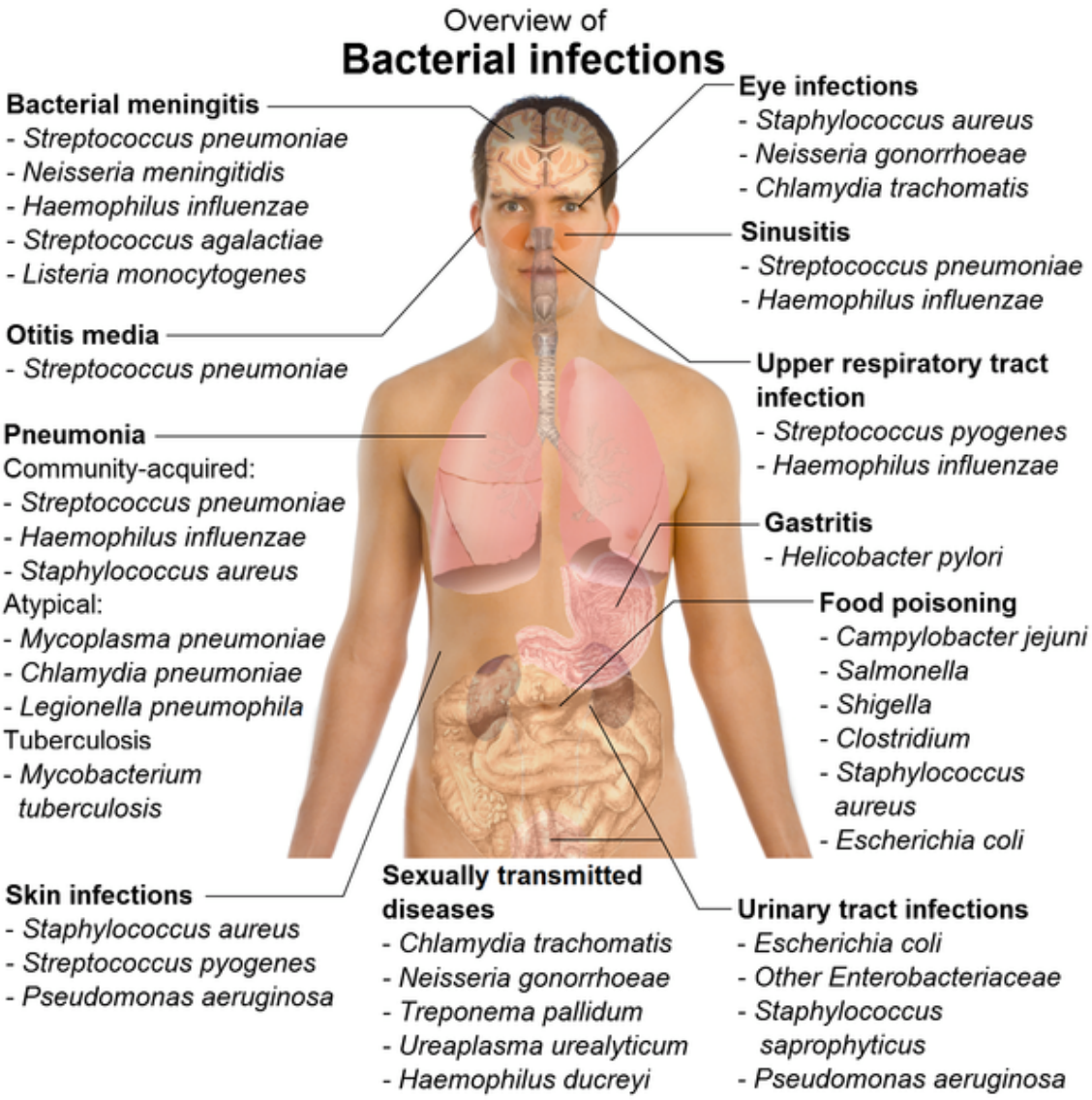


Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells

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 6

Oral Microbiology

Oral microbiology is the study of the microorganisms of the oral cavity and the interactions between the oral microorganisms with each other and with the host. Of particular interest is the role of oral microorganisms in the two major dental diseases: dental caries and periodontal disease.

The mouth harbors a diverse, abundant and complex microbial community. This highly diverse microflora inhabits the various surfaces of the normal mouth. Bacteria accumulate on both the hard and soft oral tissues in biofilms. Bacterial adhesion is particularly important for oral bacteria.

Oral bacteria have evolved mechanisms to sense their environment and evade or modify the host. Bacteria occupy the ecological niche provided by both the tooth surface and gingival epithelium. However, a highly efficient innate host defense system constantly monitors the bacterial colonization and prevents bacterial invasion of local tissues. A dynamic equilibrium exists between dental plaque bacteria and the innate host defense system.

Oral bacteria

Oral bacteria include streptococci, lactobacilli, staphylococci, corynebacteria, and various anaerobes in particular bacteroides. The oral cavity of the new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as *Streptococcus salivarius*. With the appearance of the teeth during the first year colonization by *Streptococcus mutans* and *Streptococcus sanguis* occurs as these organisms colonise the dental surface and gingiva. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat for a variety of anaerobic species. Bacteroides and spirochetes colonize the mouth around puberty.

Treponema denticola

The levels of oral spirochetes are elevated in patients with periodontal diseases. Among this group, *Treponema denticola* is the most studied and is considered as one of the main etiological bacteria of periodontitis. *Treponema denticola* is a motile and highly proteolytic bacterium.

Fusospirochetes

Spirochetes and fusi-form bacilli live as normal flora in the mouth, but in the case of bleeding in the oral cavity, the bacteria can cause infection and diseases to oral cavity: 1/ Acute necrotizing ulcerative gingivitis (ANUG) 2/ Vincent angina with a membrane covering the throat area

Veillonella

Veillonella are gram-negative anaerobic cocci. It is thought that this species thrives in the acidic environment of caries and is thought to slow the development of dental caries. It converts the acidic products of other species to less acidic products.

Porphyromonas gingivalis

Porphyromonas gingivalis is a Gram-negative oral anaerobe strongly associated with chronic adult periodontitis. The bacterium produces a number of well-characterized virulence factors and can be manipulated genetically. The availability of the genome sequence is aiding our understanding of the biology of *P. gingivalis* and how it interacts with the environment, other bacteria and the human host.

Aggregatibacter actinomycetemcomitans

Aggregatibacter actinomycetemcomitans is considered an oral pathogen due to its virulence factors, its association with localized aggressive periodontitis in young adolescents, and studies indicating that it can cause bone loss.

Lactobacillus

Some *Lactobacillus* species have been associated with dental caries although these bacteria are normally symbiotic in humans and are found in the gut flora.

Dental plaque

Dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly *S. mutans* and *S. sanguis*), 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. If not taken care of, via brushing or flossing, the plaque can turn into tartar (its hardened form)and lead to gingivitis or periodontal disease.

Cell-cell communication

Most of the bacterial species found in the mouth belong to microbial communities, called biofilms, a feature of which is inter-bacterial communication. Cell-cell contact, is mediated by specific protein adhesins and often, as in the case of inter-species aggregation, by complementary polysaccharide receptors. Another method of communication invoves cell-cell signalling molecules, which are of two classes: those used for intra-species and those used for inter-species signalling. An example of intra-species communication is quorum sensing. Oral bacteria have been shown to produce small peptides, such as competence stimulating peptides, which can help promote single-species biofilm formation. A common form of inter-species signalling is mediated by 4, 5-dihydroxy-2, 3-pentanedione (DPD) or Autoinducer-2 (Al-2).

Vaccination against oral infections

Dental caries and periodontitis have an infectious etiology and immunization has been proposed as a means of controlling them. However, the approaches vary according to the nature of the bacteria involved and the mechanisms of pathogenesis for these two very different diseases. In the case of dental caries, proteins involved in colonization of teeth by *Streptococcus mutans* can produce antibodies that inhibit the cariogenic process. Periodontal vaccines are less well developed, but some antigenic targets have been identified.

Chapter 7

Pathogen

A **pathogen**, (from Greek: πάθος pathos "suffering, passion", and γἰγνομαι (γεν-) gignomai (gen-) "I give birth to") an **infectious agent**, or more commonly **germ**, is a biological agent such as a virus, bacteria, prion, or fungus that causes disease to its host. There are several substrates including *pathways* whereby pathogens can invade a host; the principal pathways have different episodic time frames, but soil contamination has the longest or most persistent potential for harboring a pathogen.

The body contains many natural orders of defense against some of the common pathogens (such as *Pneumocystis*) in the form of the human immune system and by some "helpful" bacteria present in the human body's normal flora. However, if the immune system or "good" bacteria is damaged in any way (such as by chemotherapy, human immunodeficiency virus (HIV), or antibiotics being taken to kill other pathogens), pathogenic bacteria that were being held at bay can proliferate and cause harm to the host. Such cases are called opportunistic infection.

Some pathogens (such as the bacterium *Yersinia pestis* which may have caused the Black Plague, the *Variola* virus, and the Malaria protozoa) have been responsible for massive numbers of casualties and have had numerous effects on afflicted groups. Of particular note in modern times is HIV, which is known to have infected several million humans globally, along with the Influenza virus. Today, while many medical advances have been made to safeguard against infection by pathogens, through the use of vaccination, antibiotics, and fungicide, pathogens continue to threaten human life. Social advances such as food safety, hygiene, and water treatment have reduced the threat from some pathogens. Not all pathogens are negative. In entomology, pathogens are one of the "Three P's" (predators, pathogens, and parasitoids) that serve as natural or introduced biological controls to suppress arthropod pest populations.

Types of pathogen

Viral

Pathogenic viruses are mainly those of the families of: Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, Togaviridae. Some notable pathogenic viruses cause: smallpox, influenza, mumps, measles, chickenpox, ebola, and rubella. Viruses typically range between 20-300 nanometers in length.

Bacterial

Although the vast majority of bacteria are harmless or beneficial, a few pathogenic bacteria can cause infectious diseases. The most common bacterial disease is tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, which affects about 2 million people mostly in sub-Saharan Africa. Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas*, and foodborne illnesses, which can be caused by bacteria such as *Shigella*, *Campylobacter* and *Salmonella*. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis and Hansen's disease. Bacteria can often be killed by antibiotics because the cell wall in the outside is destroyed and then the D.N.A. They typically range between 1-5 micrometers in length.

Fungal

Fungi comprise a eukaryotic kingdom of microbes that are usually saprophytes but can cause diseases in humans, animals and plants. Fungi are the most common cause of diseases in crops and other plants. Life threatening fungal infections in humans most often occur in immunocompromised patients or vulnerable people with a weakened immune system, although fungi are common problems in the immunocompetent population as the causative agents of skin, nail or yeast infections. Most antibiotics that function on bacterial pathogens cannot be used to treat fungal infections because fungi and their hosts both have eukaryotic cells. Most clinical fungicides belong to the azole group. The typical fungal spore size is 1-40 micrometer in length.

Other parasites

Some eukaryotic organisms, such as protists and helminths, cause disease. One of the best known diseases caused by protists in the genus *Plasmodium* is malaria. These can range from 3-200 micrometers in length.

Prionic

Prions are infectious pathogens that do not contain nucleic acids. Prions are abnormal proteins whose presence causes some diseases such as scrapie, bovine spongiform

encephalopathy (mad cow disease) and Creutzfeldt–Jakob disease.. The discovery of prion as a new class of pathogen has lead Stanley B. Prusiner to receive Nobel Prize in Physiology or Medicine in 1997.

Potency

One hypothesis regarding pathogens states that the longer a pathogen can survive outside of the body, the more dangerous it can be to a potential host. For example, the smallpox virus (*variola virus*) can survive outside the human body for approximately 885 days. It is also one of the most deadly pathogenic viruses, as it kills between 20-50% of the people it infects. The tuberculosis bacterium kills 1 in 5 of the people it infects, but only survives 244 days outside of its host. However, research into the basis of the ability of pathogens to cause disease provides evidence from multiple and diverse species of the existence of pathogenicity or virulence factors, encoded within the pathogens' genetic material, that facilitate microbes to cause disease.

In countries that have higher sanitation standards, pathogens cannot survive for as long outside of the human. This is seen as encouragement to mutations to the pathogen which would make it less deadly, as such mutations would allow the pathogen to survive in the host for longer periods of time.

Transmission

One of the primary pathways by which food or water become contaminated is from the release of untreated sewage into a drinking water supply or onto cropland, with the result that people who eat or drink contaminated sources become infected. In developing countries most sewage is discharged into the environment or on cropland; even in developed countries there are periodic system failures resulting in a sanitary sewer overflow.

Examples of pathogens

Major human pathogens

- *Mycobacterium tuberculosis* — the causative agent of most cases of tuberculosis
- *Mycobacterium leprae* — the bacterium that causes leprosy (Hansen's disease)
- *Yersinia pestis* — pneumonic, septicemic, and the notorious bubonic plagues (aka "Black Death")
- *Rickettsia prowazekii* — the etiologic agent of typhus fever
- *Bartonella* spp.
- Spanish influenza virus

Chapter 8

Microbiology

An agar plate streaked with microorganisms

Microbiology (from Greek μῑκρος, *mīkros*, "small"; βίος, *bios*, "life"; and -λογία, *-logia*) is the study of *microorganisms*, which are microscopic, unicellular, and cell-cluster organisms. This includes eukaryotes such as fungi and protists, and prokaryotes. Viruses

and prions, though not strictly classed as living organisms, are also studied. Microbiology typically includes the study of the immune system, or Immunology. Generally, immune systems interact with pathogenic microbes; these two disciplines often intersect which is why many colleges offer a paired degree such as "Microbiology and Immunology".

Microbiology is a broad term which includes virology, mycology, parasitology, bacteriology and other branches. A microbiologist is a specialist in microbiology and these other topics.

Microbiology is researched actively, and the field is advancing continually. It is estimated only about one percent of all of the microbe species on Earth have been studied. Although microbes were directly observed over three hundred years ago, the field of microbiology can be said to be in its infancy relative to older biological disciplines such as zoology and botany.

History

Ancient

The existence of microorganisms was hypothesized for many centuries before their actual discovery. The existence of unseen microbiological life was postulated by Jainism which is based on Mahavira's teachings as early as 6th century BCE.. Paul Dundas notes that Mahavira asserted existence of unseen microbiological creatures living in earth, water, air and fire. Jain scriptures also describe nigodas which are sub-microscopic creatures living in large clusters and having a very short life and are said to pervade each and every part of the universe, even in tissues of plants and flesh of animals. The Roman Marcus Terentius Varro made references to microbes when he warned against locating a homestead in the vicinity of swamps "because there are bred certain minute creatures which cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose and there cause serious diseases."

In 1546 Girolamo Fracastoro proposed that epidemic diseases were caused by transferable seedlike entities that could transmit infection by direct or indirect contact, or even without contact over long distances.

However, early claims about the existence of microorganisms were speculative, and not based on any data or observation. Actual observation and discovery of microbes had to await the invention of the microscope in the 17th century.

Modern

Antonie van Leeuwenhoek, was considered to be the first to observe microorganisms using a microscope.

in 1676, Antonie van Leeuwenhoek observed bacteria and other microorganisms, using a single-lens microscope of his own design. While Van Leeuwenhoek is often cited as the first to observe microbes, Robert Hooke made the first recorded microscopic observation, of the fruiting bodies of molds, in 1665.. The first observation of microbes using a microscope is generally credited to the Dutch draper and haberdasher, Antonie van Leeuwenhoek, who lived for most of his life in Delft, Holland. It has, however, been suggested that a Jesuit priest called Athanasius Kircher was the first to observe microorganisms. He was among the first to design magic lanterns for projection purposes,

so he must have been well acquainted with the properties of lenses. One of his book contains a chapter in Latin, which reads in translation – 'Concerning the wonderful structure of things in nature, investigated by Microscope. Here, he wrote 'who would believe that vinegar and milk abound with an innumerable multitude of worms.' He also noted that putrid material is full of innumerable creeping animalculae. These observations antedate Robert Hooke's Micrographia by nearly 20 years and were published some 29 years before van Leeuwenhoek saw protozoa and 37 years before he described having seen bacteria.

The field of bacteriology (later a subdiscipline of microbiology) was founded in the 19th century by Ferdinand Cohn, a botanist whose studies on algae and photosynthetic bacteria led him to describe several bacteria including *Bacillus* and *Beggiatoa*. Cohn was also the first to formulate a scheme for the taxonomic classification of bacteria and discover spores. Louis Pasteur and Robert Koch were contemporaries of Cohn's and are often considered to be the father of Microbiology and **medical microbiology**, respectively. Pasteur is most famous for his series of experiments designed to disprove the then widely held theory of spontaneous generation, thereby solidifying microbiology's identity as a biological science. Pasteur also designed methods for food preservation (pasteurization) and vaccines against several diseases such as anthrax, fowl cholera and rabies. Koch is best known for his contributions to the germ theory of disease, proving that specific diseases were caused by specific pathogenic microorganisms. He developed a series of criteria that have become known as the Koch's postulates. Koch was one of the first scientists to focus on the isolation of bacteria in pure culture resulting in his description of several novel bacteria including *Mycobacterium tuberculosis*, the causative agent of tuberculosis.

While Pasteur and Koch are often considered the founders of microbiology, their work did not accurately reflect the true diversity of the microbial world because of their exclusive focus on microorganisms having direct medical relevance. It was not until the late 19th century and the work of Martinus Beijerinck and Sergei Winogradsky, the founders of **general microbiology** (an older term encompassing aspects of microbial physiology, diversity and ecology), that the true breadth of microbiology was revealed. Beijerinck made two major contributions to microbiology: the discovery of viruses and the development of enrichment culture techniques. While his work on the Tobacco Mosaic Virus established the basic principles of virology, it was his development of enrichment culturing that had the most immediate impact on microbiology by allowing for the cultivation of a wide range of microbes with wildly different physiologies. Winogradsky was the first to develop the concept of chemolithotrophy and to thereby reveal the essential role played by microorganisms in geochemical processes. He was responsible for the first isolation and description of both nitrifying and nitrogen-fixing bacteria.

Fields

The field of microbiology can be generally divided into several subdisciplines:

- **Microbial physiology**: The study of how the microbial cell functions biochemically. Includes the study of microbial growth, microbial metabolism and microbial cell structure.
- **Microbial genetics**: The study of how genes are organized and regulated in microbes in relation to their cellular functions. Closely related to the field of molecular biology.
- **Cellular microbiology**: A discipline bridging microbiology and cell biology.
- **Medical microbiology**: The study of the pathogenic microbes and the role of microbes in human illness. Includes the study of microbial pathogenesis and epidemiology and is related to the study of disease pathology and immunology.
- **Veterinary microbiology**: The study of the role in microbes in veterinary medicine or animal taxonomy.
- **Environmental microbiology**: The study of the function and diversity of microbes in their natural environments. Includes the study of microbial ecology, microbially-mediated nutrient cycling, geomicrobiology, microbial diversity and bioremediation. Characterization of key bacterial habitats such as the rhizosphere and phyllosphere, soil and groundwater ecosystems, open oceans or extreme environments (extremophiles).
- **Evolutionary microbiology**: The study of the evolution of microbes. Includes the study of bacterial systematics and taxonomy.
- **Industrial microbiology**: The exploitation of microbes for use in industrial processes. Examples include industrial fermentation and wastewater treatment. Closely linked to the biotechnology industry. This field also includes brewing, an important application of microbiology.
- **Aeromicrobiology**: The study of airborne microorganisms.
- **Food microbiology**: The study of microorganisms causing food spoilage and foodborne illness. Using microorganisms to produce foods, for example by fermentation.
- **Pharmaceutical microbiology**: the study of microorganisms causing pharmaceutical contamination and spoil
- **Agricultural microbiology**: The study of agriculturaly important microorganisms.

(Jobs with the Center For Disease Control and Prevention requires a degree in microbiology for most positions)

- **Soil Microbiology**: The study of those microorganisms that are found in soil.
- **Water microbiology**: The study of those microorganisms that are found in water.
- **Generation microbiology**: The study of those microorganisms that have the same characters as their parents.
- **Nano microbiology**: The study of those microorganisms at nano level.

Benefits

Fermenting tanks with yeast being used to brew beer

Whilst there are undoubtedly some who fear all microbes due to the association of some microbes with various human illnesses, many microbes are also responsible for numerous beneficial processes such as industrial fermentation (e.g. the production of alcohol, vinegar and dairy products), antibiotic production and as vehicles for cloning in more complex organisms such as plants. Scientists have also exploited their knowledge of microbes to produce biotechnologically important enzymes such as Taq polymerase, reporter genes for use in other genetic systems and novel molecular biology techniques such as the yeast two-hybrid system.

Bacteria can be used for the industrial production of amino acids. *Corynebacterium glutamicum* is one of the most important bacterial species with an annual production of more than two million tons of amino acids, mainly L-glutamate and L-lysine.

A variety of biopolymers, such as polysaccharides, polyesters, and polyamides, are produced by microorganisms. Microorganisms are used for the biotechnological production of biopolymers with tailored properties suitable for high-value medical application such as tissue engineering and drug delivery. Microorganisms are used for the biosynthesis of xanthan, alginate, cellulose, cyanophycin, poly(gamma-glutamic acid), levan, hyaluronic acid, organic acids, oligosaccharides and polysaccharide, and polyhydroxyalkanoates.

Microorganisms are beneficial for microbial biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The ability of each microorganism to degrade toxic waste depends on the nature of each contaminant. Since sites typically have multiple pollutant types, the most effective approach to microbial biodegradation is to use a mixture of bacterial species and strains, each specific to the biodegradation of one or more types of contaminants.

There are also various claims concerning the contributions to human and animal health by consuming probiotics (bacteria potentially beneficial to the digestive system) and/or prebiotics (substances consumed to promote the growth of probiotic microorganisms).

Recent research has suggested that microorganisms could be useful in the treatment of cancer. Various strains of non-pathogenic clostridia can infiltrate and replicate within solid tumors. Clostridial vectors can be safely administered and their potential to deliver therapeutic proteins has been demonstrated in a variety of preclinical models.

Chapter 9

Biological Warfare

Biological warfare (BW), also known as **germ warfare**, is the deliberate use of disease-causing biological agents such as dumb, intelligent sedatives, tranquilizers (with delayed release pediatric doses for non lethal/fatal fallout) protozoa, fungi, bacteria, protists, or viruses, to kill or incapacitate humans, other animals or plants. Biological weapons (often referred to as *bioweapons*) are living organisms or replicating entities (virus) that reproduce or replicate within their host victims.

Biological weapons may be employed in various ways to gain a strategic or tactical advantage over an adversary, either by threat or by actual deployment. Like some of the chemical weapons, biological weapons may also be useful as area denial weapons. These agents may be lethal or non-lethal, and may be targeted against a single individual, a group of people, or even an entire population. They may be developed, acquired, stockpiled or deployed by nation states or by non-national groups. In the latter case, or if a nation-state uses it clandestinely, it may also be considered bioterrorism.

There is an overlap between biological warfare and chemical warfare, as the use of toxins produced by living organisms is considered under the provisions of both the Biological Weapons Convention and the Chemical Weapons Convention. Toxins and Psychochemical weapons are often referred to as *midspectrum agents*. Unlike bioweapons, these midspectrum agents do not reproduce in their host and are typically characterized by shorter incubation periods.

Overview

Offensive biological warfare, including mass production, stockpiling and use of biological weapons, was outlawed by the 1972 Biological Weapons Convention (BWC). The rationale behind this treaty, which has been ratified or acceded to by 163 countries as of 2009, is to prevent a biological attack which could conceivably result in large numbers of civilian fatalities and cause severe disruption to economic and societal infrastructure.

Many countries, including signatories of the BWC, currently pursue research into the defense or protection against BW, which is not prohibited by the BWC.

A nation or group that can pose a credible threat of mass casualty has the ability to alter the terms on which other nations or groups interact with it. Biological weapons allow for the potential to create a level of destruction and loss of life far in excess of nuclear, chemical or conventional weapons, relative to their mass and cost of development and storage. Therefore, biological agents may be useful as strategic deterrents in addition to their utility as offensive weapons on the battlefield.

As a tactical weapon for military use, a significant problem with a BW attack is that it would take days to be effective, and therefore might not immediately stop an opposing force. Some biological agents (especially smallpox, plague, and tularemia) have the capability of person-to-person transmission via aerosolized respiratory droplets. This feature can be undesirable, as the agent(s) may be transmitted by this mechanism to unintended populations, including neutral or even friendly forces. While containment of BW transmission is less of a concern for certain criminal or terrorist organizations, it remains a significant concern for the military and civilian populations of virtually all nations.

Characteristics of biological weapons

Anti-personnel

The international biological hazard symbol

Ideal characteristics of a biological agent to be used as a weapon against humans are high infectivity, high virulence, non-availability of vaccines, and availability of an effective and efficient delivery system. Stability of the weaponized agent (ability of the agent to retain its infectivity and virulence after a prolonged period of storage) may also be desirable, particularly for military applications.

The primary difficulty is not the production of the biological agent, as many biological agents used in weapons can often be manufactured relatively quickly, cheaply and easily. Rather, it is the weaponization, storage and delivery in an effective vehicle to a vulnerable target that pose significant problems.

For example, *Bacillus anthracis* is considered an effective agent for several reasons. First, it forms hardy spores, perfect for dispersal aerosols. Second, this organism is not considered transmissible from person to person, and thus rarely if ever causes secondary infections. A pulmonary anthrax infection starts with ordinary influenza-like symptoms and progresses to a lethal hemorrhagic mediastinitis within 3–7 days, with a fatality rate that is 90% or higher in untreated patients. Finally, friendly personnel can be protected with suitable antibiotics.

A large-scale attack using anthrax would require the creation of aerosol particles of 1.5 to 5 microns. Too large and the particles would not reach the lower respiratory tract. Too small and the particles would be exhaled back out into the atmosphere. At this size, conductive powders tend to aggregate because of electrostatic charges, hindering dispersion. So the material must be treated to insulate and neutralize the charges. The weaponized agent must be resistant to degradation by rain and ultraviolet radiation from sunlight, while retaining the ability to efficiently infect the human lung. There are other technological difficulties as well, chiefly relating to storage of the weaponized agent.

Agents considered for weaponization, or known to be weaponized, include bacteria such as *Bacillus anthracis*, *Brucella spp.*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Chlamydophila psittaci*, *Coxiella burnetii*, *Francisella tularensis*, some of the Rickettsiaceae (especially *Rickettsia prowazekii* and *Rickettsia rickettsii*), *Shigella spp.*, *Vibrio cholerae*, and *Yersinia pestis*. Many viral agents have been studied and/or weaponized, including some of the Bunyaviridae (especially Rift Valley fever virus), Ebolavirus, many of the Flaviviridae (especially Japanese encephalitis virus), Machupo virus, Marburg virus, Variola virus, and Yellow fever virus. Fungal agents that have been studied include *Coccidioides spp.*.

Toxins that can be used as weapons include ricin, staphylococcal enterotoxin B, botulinum toxin, saxitoxin, and many mycotoxins. These toxins and the organisms that produce them are sometimes referred to as select agents. In the United States, their possession, use, and transfer are regulated by the Centers for Disease Control and Prevention's Select Agent Program.

Anti-agriculture

Biological warfare can also specifically target plants to destroy crops or defoliate vegetation. The United States and Britain discovered plant growth regulators (i.e., herbicides) during the Second World War, and initiated an herbicidal warfare program that was eventually used in Malaya and Vietnam in counter insurgency. Though herbicides are chemicals, they are often grouped with biological warfare as bioregulators in a similar manner as biotoxins. Scorched earth tactics or destroying livestock and farmland were carried out in the Vietnam war (cf. Agent Orange) and Eelam War in Sri Lanka.

The United States developed an anti-crop capability during the Cold War that used plant diseases (bioherbicides, or mycoherbicides) for destroying enemy agriculture. It was believed that destruction of enemy agriculture on a strategic scale could thwart Sino-Soviet aggression in a general war. Diseases such as wheat blast and rice blast were weaponized in aerial spray tanks and cluster bombs for delivery to enemy watersheds in agricultural regions to initiate epiphytotics (epidemics among plants). When the United States renounced its offensive biological warfare program in 1969 and 1970, the vast majority of its biological arsenal was composed of these plant diseases.

In 1980s Soviet Ministry of Agriculture had successfully developed variants of foot-and-mouth disease, and rinderpest against cows, African swine fever for pigs, and psittacosis to kill chicken. These agents were prepared to spray them down from tanks attached to airplanes over hundreds of miles. The secret program was code-named "Ecology".

Attacking animals is another area of biological warfare intended to eliminate animal resources for transportation and food. In the First World War, German agents were arrested attempting to inoculate draft animals with anthrax, and they were believed to be responsible for outbreaks of glanders in horses and mules. The British tainted small feed cakes with anthrax in the Second World War as a potential means of attacking German cattle for food denial, but never employed the weapon. In the 1950s, the United States had a field trial with hog cholera. During the Mau Mau Uprising in 1952, the poisonous latex of the African milk bush was used to kill cattle.

Unconnected with inter-human wars, humans have deliberately introduced the rabbit disease Myxomatosis, originating in South America, to Australia and Europe, with the intention of reducing the rabbit population - which had devastating but temporary results, with wild rabbit populations reduced to a fraction of their former size but survivors developing immunity and increasing again.

Biodefense

Role of public health departments and disease surveillance

It is important to note that all of the classical and modern biological weapons organisms are animal diseases, the only exception being smallpox. Thus, in any use of biological

weapons, it is highly likely that animals will become ill either simultaneously with, or perhaps earlier than humans.

Indeed, in the largest biological weapons accident known– the anthrax outbreak in Sverdlovsk (now Yekaterinburg) in the Soviet Union in 1979, sheep became ill with anthrax as far as 200 kilometers from the release point of the organism from a military facility in the southeastern portion of the city.

Thus, a robust surveillance system involving human clinicians and veterinarians may identify a bioweapons attack early in the course of an epidemic, permitting the prophylaxis of disease in the vast majority of people (and/or animals) exposed but not yet ill.

For example in the case of anthrax, it is likely that by 24 – 36 hours after an attack, some small percentage of individuals (those with compromised immune system or who had received a large dose of the organism due to proximity to the release point) will become ill with classical symptoms and signs (including a virtually unique chest X-ray finding, often recognized by public health officials if they receive timely reports). By making these data available to local public health officials in real time, most models of anthrax epidemics indicate that more than 80% of an exposed population can receive antibiotic treatment before becoming symptomatic, and thus avoid the moderately high mortality of the disease.

Identification of bioweapons

The goal of biodefense is to integrate the sustained efforts of the national and homeland security, medical, public health, intelligence, diplomatic, and law enforcement communities. Health care providers and public health officers are among the first lines of defense. In some countries private, local, and provincial (state) capabilities are being augmented by and coordinated with federal assets, to provide layered defenses against biological weapons attacks. During the first Gulf War the United Nations activated a biological and chemical response team, Task Force Scorpio, to respond to any potential use of weapons of mass destruction on civilians.

The traditional approach toward protecting agriculture, food, and water: focusing on the natural or unintentional introduction of a disease is being strengthened by focused efforts to address current and anticipated future biological weapons threats that may be deliberate, multiple, and repetitive.

The growing threat of biowarfare agents and bioterrorism has led to the development of specific field tools that perform on-the-spot analysis and identification of encountered suspect materials. One such technology, being developed by researchers from the Lawrence Livermore National Laboratory (LLNL), employs a "sandwich immunoassay", in which fluorescent dye-labeled antibodies aimed at specific pathogens are attached to silver and gold nanowires.

In the Netherlands, the company TNO has designed Bioaerosol Single Particle Recognition eQuipment (BiosparQ). This system would be implemented into the national response plan for bioweapons attacks in the Netherlands.

Researchers at Ben Gurion University in Israel are developing a different device called the BioPen, essentially a "Lab-in-a-Pen", which can detect known biological agents in under 20 minutes using an adaptation of the ELISA, a similar widely employed immunological technique, that in this case incorporates fiber optics.

List of BW institutions and programs by country

According to the United States Office of Technology Assessment, since disbanded, seventeen countries were believed to possess biological weapons in 1995: Libya, North Korea, South Korea, Iraq, Taiwan, Syria, Israel, Iran, China, Egypt, Vietnam, Laos, Cuba, Bulgaria, India, South Africa, and Russia.

United States

- Fort Detrick, Maryland
  - U.S. Army Biological Warfare Laboratories (1943–69)
    - Building 470
    - One-Million-Liter Test Sphere
    - Operation Whitecoat
  - United States Army Medical Unit (1954–69)
  - U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID; 1969–present)
  - National Biodefense Analysis and Countermeasures Center (NBACC; Projected: 2008)

- Project Bacchus
- Project Clear Vision
- Project SHAD
- Project 112

United Kingdom

- Porton Down
- Gruinard Island
- Nancekuke

Former Soviet Union and Russia

- Biopreparat (18 labs and production centers)
  - Stepnagorsk Scientific and Technical Institute for Microbiology, Stepnogorsk, northern Kazakhstan

    - Institute of Ultra Pure Biochemical Preparations, Leningrad, a weaponized plague center
    - Vector State Research Center of Virology and Biotechnology (VECTOR), a weaponized smallpox center
    - Institute of Applied Biochemistry, Omutninsk
    - Kirov bioweapons production facility, Kirov, Kirov Oblast
    - Zagorsk smallpox production facility, Zagorsk
    - Berdsk bioweapons production facility, Berdsk
    - Sverdlovsk bioweapons production facility (Military Compound 19), Sverdlovsk, a weaponized anthrax center

- Poison laboratory of the Soviet secret services
- Vozrozhdeniya

Japan

- Unit 731
- Zhongma Fortress
- Unit 100
- Unit 2646
- Unit 8604
- Unit Ei 1644

Iraq

- Al Hakum
- Salman Pak facility
- Al Manal facility

Treaties banning or restricting BW

- Geneva Protocol
- Biological Weapons Convention

List of people associated with Biological Weapons

Bioweaponeers:

- Anton Dilger
- Ira Baldwin
- Paul Fildes
- Rihab Rashid Taha
- William C. Patrick III
- Kanatjan Alibekov, known as Ken Alibek
- Vladimir Pasechnik

- Kurt Blome
- Eugen von Haagen
- Kurt Gutzeit
- Erich Traub
- Shiro Ishii

Writers and activists:

- Matthew Meselson
- Jeanne Guillemin
- Sheldon H. Harris

History

Biological warfare has been practiced repeatedly throughout history. Before the 20th century, the use of biological agents took three major forms:

- Deliberate poisoning of food and water with infectious material
- Use of microorganisms, toxins or animals, living or dead, in a weapon system
- Use of biologically inoculated fabrics

Antiquity

The earliest documented incident of the intention to use biological weapons is recorded in Hittite texts of 1500–1200 B.C, in which victims of plague were driven into enemy lands. Although the Assyrians knew of ergot, a parasitic fungus of rye which produces ergotism when ingested, there is no evidence that they poisoned enemy wells with the fungus, as has been claimed.

According to Homer's epic poems about the legendary Trojan War, the *Iliad* and the *Odyssey,* spears and arrows were tipped with poison. During the First Sacred War in Greece, in about 590 BC, Athens and the Amphictionic League poisoned the water supply of the besieged town of Kirrha (near Delphi) with the toxic plant hellebore. The Roman commander Manius Aquillus poisoned the wells of besieged enemy cities in about 130 BC.

During the 4th century BC Scythian archers tipped their arrow tips with snake venom, human blood, and animal feces to cause wounds to become infected. There are numerous other instances of the use of plant toxins, venoms, and other poisonous substances to create biological weapons in antiquity.

In 184 B.C, Hannibal of Carthage had clay pots filled with venomous snakes and instructed his soldiers to throw the pots onto the decks of Pergamene ships. In about AD 198, the Parthian city of Hatra (near Mosul, Iraq) repulsed the Roman army led by Septimius Severus by hurling clay pots filled with live scorpions at them.

Middle Ages

The Mongol Empire established commercial and political connections between the Eastern and Western areas of the world, but it did so through the most mobile army ever seen. The armies, being the most rapidly moving travelers who had ever moved between the steppes of East Asia (where bubonic plague was and remains endemic among small rodents) and managed to keep the chain of infection without a break until they reached, and infected, peoples and rodents who had never encountered it. The ensuing Black Death may have killed almost half of the population of Europe in the next decades, changing the course of Asian and European history.

During the Middle Ages, victims of the bubonic plague were used for biological attacks, often by flinging the infected corpses (formites)and excrement over castle walls using catapults. In 1346, during the siege of Kafa (now Feodossia Ukraine) the attacking Tartar Forces which were subjugated by the Mongol empire under Genghis Khan, used the bodies of Mongol warriors of the Golden Horde who had died of plague, as weapons. The dead warriors were thrown over the walls of the besieged city of Crimean. An outbreak of plague followed and the defending forces retreated, followed by the conquest of the city by the Mongol army. It has been speculated that this operation may have been responsible for the advent of the Black Death in Europe.

At the siege of Thun-l'Évêque in 1340, during the Hundred Years' War, the attackers catapulted decomposing animals into the besieged area.

In 1422, during the siege of Karlstein Castle in Bohemia, Hussite attackers used catapults to throw dead (but not plague-infected) bodies and 2000 carriage-loads of dung over the walls.

The last known incident of using plague corpses for biological warfare occurred in 1710, when Russian forces attacked the Swedes by flinging plague-infected corpses over the city walls of Reval (Tallinn). However, during the 1785 siege of La Calle, Tunisian forces flung diseased clothing into the city.

18th century

North America
The Native American population was devastated after contact with the Old World due to the introduction of many different fatal diseases. There are two documented cases of alleged and attempted germ warfare. The first, during a parley at Fort Pitt on June 24, 1763, Ecuyer gave representatives of the besieging Delawares two blankets and a handkerchief that had been exposed to smallpox, hoping to spread the disease to the Natives in order to end the siege. William Trent, the militia commander, left records that clearly indicated that the purpose of giving the blankets was "to Convey the Smallpox to the Indians."

British commander Lord Jeffrey Amherst and Swiss-British officer Colonel Henry Bouquet certainly discussed this, in the course of Pontiac's Rebellion; there still exists correspondence referencing the idea of giving smallpox-infected blankets to enemy Indians. Historian Francis Parkman verifies four letters from June 29, July 13, 16 and 26th, 1763. Excerpts: Commander Lord Jeffrey Amherst writes July 16, 1763, "P.S. You will Do well to try to Inocculate the Indians by means of Blankets, as well as to try Every other method that can serve to Extirpate this Execrable Race. I should be very glad your Scheme for Hunting them Down by Dogs could take Effect,..." Colonel Henry Bouquet replies July 26, 1763, "I received yesterday your Excellency's letters of 16th with their Inclosures. The signal for Indian Messengers, and all your directions will be observed."

While the intent of carrying out biological warfare is clear, there is debate among historians as to whether this actually took place despite Bouquet's affirmative reply to Amherst, and the continuing correspondence on the point. Smallpox is highly infectious and does not require contaminated blankets to spread uncontrollably, and together with measles, influenza, chicken pox, etc. had been doing so since the arrival of Europeans and their animals. Historians have been unable to establish whether or not the Amherst plan was implemented, particularly in light of the fact that smallpox was already present in the region, and that scientific knowledge of disease at that time had yet to develop an understanding of infection vectors, nor in the case of smallpox a full acknowledgment of the protective effect of a cowpox infection.

Regardless of whether the plan was carried out, trade and combat provided ample opportunity for transmission of the disease.

The diseases that struck indigenous Americans can be traced to Eurasia where people had long lived with them and developed some immunological ability to survive their presence. Without similarly long ancestral exposure, indigenous Americans were immunologically naive and extremely vulnerable.

New South Wales

Australian aborigines (Kooris) have always maintained that the British deliberately spread smallpox in 1789, but this fact has only been apparent to historians from the 1980's when Dr Noel Butlin suggested; "there are some possibilities that ... disease could have been used deliberately as an exterminating agent".

In 1997, David Day claimed there "remains considerable circumstantial evidence to suggest that officers other than Phillip, or perhaps convicts or soldiers … deliberately spread smallpox among aborigines" and in 2000 Dr John Lambert argued that "strong circumstantial evidence suggests the smallpox epidemic which ravaged Aborigines in 1789, may have resulted from deliberate infection".

These claims were controversial as it was argued that any smallpox virus brought to New South Wales would have been sterilised during the voyage of the First Fleet from England and incapable of biological warfare. However, in 2007, Christopher Warren

demonstrated conclusively that the British smallpox was still viable. Since then most scholars have recognised that the British committed biological warfare in 1789 near their new convict settlement at Port Jackson.

Some earlier writers, misunderstanding that British stocks of virus had been sterilised, proposed that the 1789 outbreak was caused by a hypothetical transmission from Macassar in Sulawesi. However the available records for smallpox in Macassar only show an outbreak in 1789, too late and inconvenient to be associated with the First Fleet outbreak.

19th century

In 1834, Massachusetts diarist Richard Henry Dana visited San Francisco on a merchant ship. His ship traded many items including blankets with Mexicans and Russians who had established outposts on the northern side of the San Francisco Bay. Local histories document that the California plague epidemic began at the Russian fort soon after they left. It is possible that the blankets were the source of the contamination (hidden fleas, or rats, perhaps), but another possible source was a Chinese ship making port in San Francisco at the same time. Plague became established in California and has since become endemic throughout much of the North American West. Native rodents have suffered a severe population decline, only partly due to human eradication action.

During the American Civil War, General Sherman reported that Confederate forces shot farm animals in ponds upon which the Union troops depended for drinking water. This would have made the water unpleasant to drink, though perhaps the death caused might not have been that desired. A Confederate doctor planned and may have carried out a bacteriological attack on Northern populations across the Canadian border.

Jack London, in his story "'Yah! Yah! Yah!'", described a punitive European expedition to a South Pacific island deliberately exposing the Polynesian population to measles, of which many of them died. While much of the material for London's *South Sea Tales* is derived from his personal experience in the region, it is not known whether this particular incident is historical.

20th century

During the First World War, the Empire of Germany pursued an ambitious biological warfare program. Using diplomatic pouches and couriers, the German General Staff supplied small teams of saboteurs in the Russian Duchy of Finland, and in the then-neutral countries of Romania, the United States, and Argentina.

In Finland, saboteurs mounted on reindeer placed ampoules of anthrax in stables of Russian horses in 1916 . Anthrax was also supplied to the German military attaché in Bucharest, as was glanders, which was employed against livestock destined for Allied service.

German intelligence officer and US citizen Dr. Anton Casimir Dilger established a secret lab in the basement of his sister's home in Chevy Chase, Maryland, that produced glanders which was used to infect livestock in ports and inland collection points including, at least, Newport News, Norfolk, Baltimore, and New York, and probably St. Louis and Covington, Kentucky. In Argentina, German agents also employed glanders in the port of Buenos Aires and also tried to ruin wheat harvests with a destructive fungus.

The Geneva Protocol of 1925 prohibited the use of chemical weapons and biological weapons, but said nothing about experimentation, production, storage, or transfer; later treaties did cover these aspects. Twentieth-century advances in microbiology enabled the first pure-culture biological agents to be developed by World War II.

The interwar period was a period of development by many nations, most notably the Empire of Japan. Secret Imperial Japanese Army Unit 731, based primarily at Pingfan in Manchuria commanded by Lieutenant General Shirō Ishii, did research on BW, conducted often fatal human experiments on prisoners, and produced biological weapons for combat use during the Second Sino-Japanese War.

Biological experiments, often using twins with one subject to the procedure and the other as a control, were carried out by Nazi Germany on concentration camp inmates, particularly by Joseph Mengele.

1937–1945

During the Sino-Japanese War (1937–1945) and World War II, the Imperial Japanese Army made use of biological weapons against both Chinese soldiers and civilians in several military campaigns. Three veterans of Unit 731 testified, in a 1989 interview to the Asahi Shimbun, that they were part of a mission to contaminate the Horustein river with typhoid near the Soviet troops during the Battle of Khalkhin Gol. In 1940, the Imperial Japanese Army Air Force bombed Ningbo with ceramic bombs full of fleas carrying the bubonic plague. A film showing this operation was seen by the imperial princes Tsuneyoshi Takeda and Takahito Mikasa during a screening made by mastermind Shiro Ishii. During the Khabarovsk War Crime Trials the accused, such as Major General Kiyashi Kawashima, testified that as early as 1941 some 40 members of Unit 731 air-dropped plague-contaminated fleas on Changde. These operations caused epidemic plague outbreaks.

Many operations were ineffective due to inefficient delivery systems, using disease-bearing insects rather than dispersing the agent as an bioaerosol cloud. Nevertheless, some modern Chinese historians estimate that 400,000 Chinese died as a direct result of Japanese field testing and operational use of biological weapons.

In 1943, following the Allied invasion at Anzio, German forces flooded The Pontine Marshes to reintroduce Malaria to the area. Perhaps 100,000 cases of the disease were noted in the region in 1944 and 43,000 in 1945. German forces withheld medical care to the civilian population.

In response to biological weapons development in Japan, and at the time suspected in Nazi Germany, the United States, United Kingdom, and Canada initiated a BW development programs in 1941 that resulted in the weaponization of tularemia, anthrax, brucellosis, and botulism toxin.

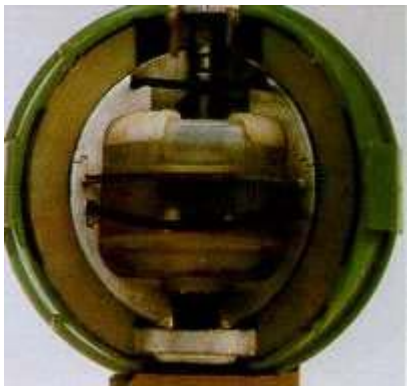
The center for United States military BW research was Fort Detrick, Maryland, where USAMRIID is currently based; the first director was pharmaceutical executive George W. Merck. Some biological and chemical weapons research and testing was also conducted at Dugway Proving Grounds in Utah, at a munition manufacturing complex in Terre Haute, Indiana, and at a tract on Horn Island, Mississippi.

Much of the British work was carried out at Porton Down. Field testing carried out in the United Kingdom during World War II left Gruinard island in Scotland contaminated with anthrax for the next 48 years.

1946 to 1972

During the 1948 Israel War of Independence, International Red Cross reports raised suspicion that the Jewish Haganah militia had released Salmonella typhi bacteria into the water supply for the city of Acre, causing an outbreak of typhoid among the inhabitants. Egyptian troops later claimed to have captured disguised Haganah soldiers near wells in Gaza, whom they executed for allegedly attempting another attack. Israel denies these allegations.

During the Cold War, US conscientious objectors were used as consenting test subjects for biological agents in a program known as Operation Whitecoat. There were also many unpublicized tests carried out on the public during the Cold War.



E120 biological bomblet, developed before the U.S. signed the Biological and Toxic Weapons Convention.

Considerable research on the topic was performed by the United States, the Soviet Union, and probably other major nations throughout the Cold War era, though it is generally believed that biological weapons were never used after World War II. This view was

challenged by China and North Korea, who accused the United States of germ warfare in the Korean War (1950–1953).

Cuba has also accused the United States of spreading human and animal disease on their island nation.

At the time of the Korean War the United States had only weaponized one agent, brucellosis ("Agent US"), which is caused by *Brucella suis*. The original weaponized form used the M114 bursting bomblet in M33 cluster bombs.

While the specific form of the biological bomb was classified until some years after the Korean War, in the various exhibits of biological weapons that Korea alleged were dropped on their country nothing resembled an M114 bomblet. There were ceramic containers that had some similarity to Japanese weapons used against the Chinese in World War II, developed by Unit 731.

Some of the Unit 731 personnel were imprisoned by the Soviets, and would have been a potential source of information on Japanese weaponization. The head of Unit 731, Lieutenant General Shiro Ishii, was granted immunity from war crimes prosecution in exchange for providing information to the United States on the Unit's activities.

The Korean War allegations also stressed the use of disease vectors, such as fleas, which, again, were probably a legacy of Japanese biological warfare efforts. The United States initiated its weaponization efforts with disease vectors in 1953, focused on Plague-fleas, EEE-mosquitoes, and yellow fever - mosquitoes (OJ-AP). However, US medical scientists in occupied Japan undertook extensive research on insect vectors, with the assistance of former Unit 731 staff, as early as 1946.

The United States Air Force was not satisfied with the operational qualities of the M114/US and labeled it an interim item until the United States Army Chemical Corps could deliver a superior weapon. The Air Force also changed its plans and wanted lethal biologicals.

The Chemical Corps then initiated a crash program to weaponize anthrax (N) in the E61 1/2-lb hour-glass bomblet. Though the program was successful in meeting its development goals, the lack of validation on the infectivity of anthrax stalled standardization.

Around 1950 the Chemical Corps also initiated a program to weaponize tularemia (UL). Shortly after the E61/N failed to make standardization, tularemia was standardized in the 3.4" M143 bursting spherical bomblet. This was intended for delivery by the MGM-29 Sergeant missile warhead and could produce 50% infection over a 7-square-mile (18 km^2) area.

Unlike anthrax, tularemia had a demonstrated infectivity with human volunteers (Operation Whitecoat). Furthermore, although tularemia is treatable by antibiotics, treatment does not shorten the course of the disease.

In addition to the use of bursting bomblets for creating biological aerosols, the Chemical Corps started investigating aerosol-generating bomblets in the 1950s. The E99 was the first workable design, but was too complex to be manufactured. By the late 1950s the 4.5" E120 spraying spherical bomblet was developed; a B-47 bomber with a SUU-24/A dispenser could infect 50% or more of the population of a 16-square-mile (41 km^2) area with tularemia with the E120. The E120 was later superseded by dry-type agents.

Dry-type biologicals resemble talcum powder, and can be disseminated as aerosols using gas expulsion devices instead of a burster or complex sprayer. The Chemical Corps developed Flettner rotor bomblets and later triangular bomblets for wider coverage due to improved glide angles over Magnus-lift spherical bomblets. Weapons of this type were in advanced development by the time the program ended.

United States President Richard Nixon signed an executive order on November 1969, which stopped production of biological weapons in the United States and allowed only scientific research of lethal biological agents and defensive measures such as immunization and biosafety. The biological munition stockpiles were destroyed, and approximately 2,200 researchers became redundant.

United States Special Forces and the CIA also had an interest in biological warfare, and a series of special munitions was created for their operations. The covert weapons developed for the military (M1, M2, M4, M5, and M32 - or Big Five Weapons) were destroyed in accordance with Nixon's executive order to end the offensive program. The CIA maintained its collection of biologicals well into 1975 when it became the subject of the senate Church Committee.

The Biological Weapons Convention

In 1972, the United States signed the Biological and Toxic Weapons Convention, which banned the "development, production and stockpiling of microbes or their poisonous products except in amounts necessary for protective and peaceful research." By 1996, 137 countries had signed the treaty; however it is believed that since the signing of the Convention the number of countries capable of producing such weapons has increased.

The Soviet Union continued research and production of offensive biological weapons in a program called Biopreparat, despite having signed the convention. The United States was unaware of the program until Dr. Vladimir Pasechnik defected in 1989, and Dr. Kanatjan Alibekov, the first deputy director of Biopreparat defected in 1992.

After the 1991 Persian Gulf War, Iraq admitted to the United Nations inspection team to having produced 19,000 liters of concentrated botulinum toxin, of which approximately 10,000 L were loaded into military weapons; the 19,000 liters have never been fully

accounted for. This is approximately three times the amount needed to kill the entire current human population by inhalation, although in practice it would be impossible to distribute it so efficiently, and, unless it is protected from oxygen, it deteriorates in storage.

On September 18, 2001 and for a few days after several letters were received by members of the United States Congress and media outlets containing anthrax spores: the attack killed five people. The identity of the perpetrator remained unknown until 2008, when a primary suspect was named.

Chapter 10

Algae Fuel

Algae fuel is an alternative to fossil fuel and uses algae as its source of natural deposits. Several companies and government agencies are funding efforts to reduce capital and operating costs and make algae fuel production commercially viable. The production of biofuels from algae does not reduce atmospheric carbon dioxide (CO_2), because any CO_2 taken out of the atmosphere by the algae is returned when the biofuels are burned. They do however potentially reduce the introduction of new CO_2 by displacing fossil hydrocarbon fuels.

High oil prices, competing demands between foods and other biofuel sources, and the world food crisis, have ignited interest in algaculture (farming algae) for making vegetable oil, biodiesel, bioethanol, biogasoline, biomethanol, biobutanol and other biofuels, using land that is not suitable for agriculture. Among algal fuels' attractive characteristics: they do not affect fresh water resources, can be produced using ocean and wastewater, and are biodegradable and relatively harmless to the environment if spilled. Algae cost more per unit mass (as of 2010, food grade algae costs ~$5000/tonne), due to high capital and operating costs, yet can theoretically yield between 10 and 100 times more energy per unit area than other second-generation biofuel crops. One biofuels company has claimed that algae can produce more oil in an area the size of a two car garage than a football field of soybeans, because almost the entire algal organism can use sunlight to produce lipids, or oil. The United States Department of Energy estimates that if algae fuel replaced all the petroleum fuel in the United States, it would require 15,000 square miles (39,000 km^2) which is only 0.42% of the U.S. map. This is less than $^1/_7$ the area of corn harvested in the United States in 2000. However, these claims remain unrealized, commercially. According to the head of the Algal Biomass Organization algae fuel can reach price parity with oil in 2018 if granted production tax credits.

Factors

Dry mass factor is the percentage of dry biomass in relation to the fresh biomass; e.g. if the dry mass factor is 5%, one would need 20 kg of wet algae (algae in the media) to get 1 kg of dry algae cells.

Lipid content is the percentage of oil in relation to the dry biomass needed to get it, i.e. if the algae lipid content is 40%, one would need 2.5 kg of dry algae to get 1 kg of oil.

Fuels

The vegoil algae product can then be harvested and converted into biodiesel or green-colored crude oil. The algae's carbohydrate content can be fermented into bioethanol and biobutanol.

Biodiesel

Currently most research into efficient algal-oil production is being done in the private sector, but predictions from small scale production experiments bear out that using algae to produce biodiesel may be the only viable method by which to produce enough automotive fuel to replace current world diesel usage.

Microalgae have much faster growth rates than terrestrial crops. The per unit area yield of oil from algae is estimated to be from between 5,000 to 20,000 US gallons per acre per year (4,700 to 18,000 $m^3/km^2 \cdot a$). This is 7 to 30 times greater than the next best crop, Chinese tallow (700 US gal/acre·a or 650 $m^3/km^2 \cdot a$).

Studies show that some species of algae can produce up to 60% of their dry weight in the form of oil. Because the cells grow in aqueous suspension, where they have more efficient access to water, CO_2 and dissolved nutrients, microalgae are capable of producing large amounts of biomass and usable oil in either high rate algal ponds or photobioreactors. This oil can then be turned into biodiesel which could be sold for use in automobiles. Regional production of microalgae and processing into biofuels will provide economic benefits to rural communities.

Biobutanol

Butanol can be made from algae or diatoms using only a solar powered biorefinery. This fuel has an energy density 10% less than gasoline, and greater than that of either ethanol or methanol. In most gasoline engines, butanol can be used in place of gasoline with no modifications. In several tests, butanol consumption is similar to that of gasoline, and when blended with gasoline, provides better performance and corrosion resistance than that of ethanol or E85.

The green waste left over from the algae oil extraction can be used to produce butanol.

Biogasoline

Biogasoline is gasoline produced from biomass such as algae. Like traditionally produced gasoline, it contains between 6 (hexane) and 12 (dodecane) carbon atoms per molecule and can be used in internal-combustion engines.

Methane

Methane a form of natural gas can be produced from algae in various methods, namely Gasification, Pyrolysis and Anaerobic Digestion. In Gasification and Pyrolysis methods methane is extracted under high temperature and pressure. Anaerobic Digestion is a straight forward method involves in decomposition of algae in to simple components then transforming it in to fatty acids using microbes like acidific bacteria followed by removing any solid particles and finally adding methanogenic bacteria to release a gas mixture containing methane.

Ethanol

The Algenol system which is being commercialized by BioFields in Puerto Libertad, Sonora, Mexico utilizes seawater and industrial exhaust to produce ethanol.

SVO

The algal-oil feedstock that is used to produce biodiesel can also be used for fuel directly as "Straight Vegetable Oil", (SVO). The benefit of using the oil in this manner is that it doesn't require the additional energy needed for transesterification, (processing the oil with an alcohol and a catalyst to produce biodiesel). The drawback is that it does require modifications to a normal diesel engine. Transesterified biodiesel can be run in an unmodified modern diesel engine, provided the engine is designed to use ultra-low sulfur diesel, which, as of 2006, is the new diesel fuel standard in the United States.

Hydrocracking to traditional transport fuels

Vegetable oil can be used as feedstock for an oil refinery where methods like hydrocracking or hydrogenation can be used to transform the vegetable oil into standard fuels like gasoline and diesel.

Jet fuel

Rising jet fuel prices are putting severe pressure on airline companies, creating an incentive for algal jet fuel research. The International Air Transport Association, for example, supports research, development and deployment of algal fuels. IATA’s goal is for its members to be using 10% alternative fuels by 2017.

Trials have been carried with aviation biofuel by Air New Zealand, and Virgin Airlines.

In February 2010, the Defense Advanced Research Projects Agency announced that the U.S. military was about to begin large-scale production oil from algal ponds into jet fuel. After extraction at a cost of $2 per gallon, the oil will be refined at less than $3 a gallon. A larger-scale refining operation, producing 50 million gallons a year, is expected to go into production in 2013, with the possibility of lower per gallon costs so that algae-based fuel would be competitive with fossil fuels. The projects, run by the companies SAIC and General Atomics, are expected to produce 1,000 gallons of oil per acre per year from algal ponds.

Algae cultivation

Algae can produce up to 300 times more oil per acre than conventional crops, such as rapeseed, palms, soybeans, or jatropha. As Algae has a harvesting cycle of 1–10 days, it permits several harvests in a very short time frame, a differing strategy to yearly crops (Chisti 2007). Algae can also be grown on land that is not suitable for other established crops, for instance, arid land, land with excessively saline soil, and drought-stricken land. This minimizes the issue of taking away pieces of land from the cultivation of food crops (Schenk et al. 2008). Algae can grow 20 to 30 times faster than food crops.

Photobioreactors

Most companies pursuing algae as a source of biofuels are pumping nutrient-laden water through plastic tubes (called "bioreactors") that are exposed to sunlight (and so called photobioreactors or PBR).

Running a PBR is more difficult than an open pond, and more costly.

Algae can also grow on marginal lands, such as in desert areas where the groundwater is saline, rather than utilize fresh water.

Because algae strains with lower lipid content may grow as much as 30 times faster than those with high lipid content, the difficulties in efficient biodiesel production from algae lie in finding an algal strain with a combination of high lipid content and fast growth rate, that isn't too difficult to harvest; and a cost-effective cultivation system (i.e., type of photobioreactor) that is best suited to that strain. There is also a need to provide concentrated CO_2 to increase the rate of production.

Closed loop system

Another obstacle preventing widespread mass production of algae for biofuel production has been the equipment and structures needed to begin growing algae in large quantities. Maximum use of existing agriculture processes and hardware is the goal.

In a closed system (not exposed to open air) there is not the problem of contamination by other organisms blown in by the air. The problem for a closed system is finding a cheap source of sterile CO_2. Several experimenters have found the CO_2 from a smokestack

works well for growing algae. To be economical, some experts think that algae farming for biofuels will have to be done as part of cogeneration, where it can make use of waste heat, and help soak up pollution.

Open pond

Open-pond systems for the most part have been given up for the cultivation of algae with high-oil content. Many believe that a major flaw of the Aquatic Species Program was the decision to focus their efforts exclusively on open-ponds; this makes the entire effort dependent upon the hardiness of the strain chosen, requiring it to be unnecessarily resilient in order to withstand wide swings in temperature and pH, and competition from invasive algae and bacteria. Open systems using a monoculture are also vulnerable to viral infection. The energy that a high-oil strain invests into the production of oil is energy that is not invested into the production of proteins or carbohydrates, usually resulting in the species being less hardy, or having a slower growth rate. Algal species with a lower oil content, not having to divert their energies away from growth, have an easier time in the harsher conditions of an open system.

Some open sewage ponds trial production has been done in Marlborough, New Zealand.

Algae types

Research into algae for the mass-production of oil is mainly focused on microalgae; organisms capable of photosynthesis that are less than 0.4 mm in diameter, including the diatoms and cyanobacteria; as opposed to macroalgae, such as seaweed. The preference towards microalgae is due largely to its less complex structure, fast growth rate, and high oil content (for some species). However, some research is being done into using seaweeds for biofuels, probably due to the high availability of this resource.

The following species listed are currently being studied for their suitability as a mass-oil producing crop, across various locations worldwide:

- *Botryococcus braunii*
- *Chlorella*
- *Dunaliella tertiolecta*
- *Gracilaria*
- *Pleurochrysis carterae* (also called CCMP647).
- *Sargassum*, with 10 times the output volume of *Gracilaria*.

In addition, due to its high growth rate, *Ulva* has been investigated as a fuel for use in the *SOFT cycle*, (SOFT stands for Solar Oxygen Fuel Turbine), a closed-cycle power generation system suitable for use in arid, subtropical regions.

Specific research

Companies such as Sapphire Energy are using genetic engineering and chemically induced mutations to produce algae suitable for use as a crop.

Some commercial interests into large scale algal-cultivation systems are looking to tie in to existing infrastructures, such as cement factories, coal power plants, or sewage treatment facilities. This approach changes wastes into resources to provide the raw materials, CO_2 and nutrients, for the system.

Aquaflow Bionomic Corporation of New Zealand announced that it has produced its first sample of homegrown bio-diesel fuel with algae sourced from local sewerage ponds. A small quantity of laboratory produced oil was mixed with 95% regular diesel.

A feasibility study using marine microalgae in a photobioreactor is being done by The International Research Consortium on Continental Margins at the Jacobs University Bremen.

The Department of Environmental Science at Ateneo de Manila University in the Philippines, is working on producing biofuel from a local species of algae.

NBB's Feedstock Development program is addressing production of algae on the horizon to expand available material for biodiesel in a sustainable manner.

Nutrients

Nutrients like nitrogen (N), phosphorus (P), and potassium (K), are important for plant growth and are essential parts of fertilizer. Silica and iron, as well as several trace elements, may also be considered important marine nutrients as the lack of one can limit the growth of, or productivity in, an area.

Carbon Dioxide

The Glenturret Distillery in Perthshire, UK – home to The Famous Grouse Whisky – percolate CO2 made during the whisky distillation through a microalgae bioreactor. Each tonne of microalgae absorbs two tonnes of CO2. Scottish Bioenergy, who run the project, sell the microalgae as high value, protein-rich food for fisheries. In the future, they will use the algae residues to produce renewable energy through anaerobic digestion.

Wastewater

A possible nutrient source is waste water from the treatment of sewage, agricultural, or flood plain run-off, all currently major pollutants and health risks. However, this waste water cannot feed algae directly and must first be processed by bacteria, through anaerobic digestion. If waste water is not processed before it reaches the algae, it will

contaminate the algae in the reactor, and at the very least, kill much of the desired algae strain. In biogas facilities, organic waste is often converted to a mixture of carbon dioxide, methane, and organic fertilizer. Organic fertilizer that comes out of the digester is liquid, and nearly suitable for algae growth, but it must first be cleaned and sterilized.

The utilization of wastewater and ocean water instead of freshwater is strongly advocated due to the continuing depletion of freshwater resources. However, heavy metals, trace metals, and other contaminants in wastewater can decrease the ability of cells to produce lipids biosynthetically and also impact various other workings in the machinery of cells. The same is true for ocean water, but the contaminants are found in different concentrations. Thus, agricultural-grade fertilizer is the preferred source of nutrients, but heavy metals are again a problem, especially for strains of algae that are susceptible to these metals. In open pond systems the use of strains of algae that can deal with high concentrations of heavy metals could prevent other organisms from infesting these systems (Schenk et al. 2008). In some instances it has even been shown that strains of algae can remove over 90% of nickel and zinc from industrial wastewater in relatively short periods of time (Chong, Wong et al. 1998).

Investment and economic viability

There is always uncertainty about the success of new products and investors have to consider carefully the proper energy sources in which to invest. A drop in fossil fuel oil prices might make consumers and therefore investors lose interest in renewable energy. Algal fuel companies are learning that investors have different expectations about returns and length of investments. AlgaePro Systems found in its talks with investors that while one wants at least 5 times the returns on their investment, others would only be willing to invest in a profitable operation over the long term. Every investor has its own unique stipulations that are obstacles to further algae fuel development. Additional concerns consider the potential environmental impact of Algal fuel development, as well as secondary impacts on wildlife such as bears and fish.

Whereas technical problems, such as harvesting, are being addressed successfully by the industry, the high up-front investment of algae-to-biofuels facilities is seen by many as a major obstacle to the success of this technology. Only few studies on the economic viability are publicly available, and must often rely on the little data (often only engineering estimates) available in the public domain. Dmitrov examined the GreenFuels photobioreactor and estimated that algae oil would only be competitive at an oil price of $800 per barrel. A study by Alabi at al. examined raceways, photobioreactors and anaerobic fermenters to make biofuels from algae and found that photobioreactors are too expensive to make biofuels. Raceways might be cost-effective in warm climates with very low labor costs, and fermenters may become cost-effective subsequent to significant process improvements. The group found that capital cost, labor cost and operational costs (fertilizer, electricity, etc.) by themselves are too high for algae biofuels to be cost-competitive with conventional fuels. Similar results were found by others, suggesting that unless new, cheaper ways of harnessing algae for biofuels production are found, their great technical potential may never become economically accessible.

Algae fuel by country

Europe

Universities in the United Kingdom which are working on producing oil from algae include:University of Glasgow, University of Brighton, Cambridge University, University College London, Imperial College London, Cranfield University and Newcastle University. In Spain, it is also relevant the research carried out by the CSIC´s Instituto de Bioquímica Vegetal y Fotosíntesis (Microalgae Biotechnology Group, Seville).

Ukraine plans to produce biofuel using a special type of algae.

United States

The Aquatic Species Program, launched in 1978, was a research program funded by the United States Department of Energy (DoE) which was tasked with investigating the use of algae for the production of energy. The program initially focused efforts on the production of hydrogen, shifting primary research to studying oil production in 1982. From 1982 until its end in 1996, the majority of the program research was focused on the production of transportation fuels, notably biodiesel, from algae. In 1995, as part of overall efforts to lower budget demands, the DoE decided to end the program. Research stopped in 1996 and staff began compiling their research for publication.

US universities which are working on producing oil from algae include: University of California San Diego, University of Texas at Austin, University of Maine, University of Kansas, Old Dominion University, Utah State University, and New Mexico State University.

At the Woods Hole Oceanographic Institution and the Harbor Branch Oceanographic Institution the wastewater from domestic and industrial sources contain rich organic compounds that are being used to accelerate the growth of algae. The Department of Biological and Agricultural Engineering at University of Georgia is exploring microalgal biomass production using industrial wastewater. Algaewheel, based in Indianapolis, Indiana, presented a proposal to build a facility in Cedar Lake, Indiana that uses algae to treat municipal wastewater, using the sludge byproduct to produce biofuel.

Sapphire Energy (San Diego) has produced green crude from algae.

Solazyme (South San Francisco, California) has produced a fuel suitable for powering jet aircraft from algae.

Other

The Algal Biomass Organization (ABO) is formed by Boeing Commercial Airplanes, A2BE Carbon Capture Corporation, National Renewable Energy Labs, Scripps Institution of Oceanography, Benemann Associates, Mont Vista Capital and Montana State University.

Global air carriers Air New Zealand, Continental, Virgin Atlantic Airways, and biofuel technology developer UOP, a Honeywell company, will be the first wave of aviation-related members, together with Boeing, to join Algal Biomass Organization.

The National Algae Association (NAA) is a non-profit organization of algae researchers, algae production companies and the investment community who share the goal of commercializing algae oil as an alternative feedstock for the biofuels markets. The NAA gives its members a forum to efficiently evaluate various algae technologies for potential early stage company opportunities.

The European Algae Biomass Association (EABA) is the European association representing both research and industry in the field of algae technologies, currently with 79 members. The association is headquartered in Florence, Italy. The general objective of the European Algae Biomass Association (EABA) is to promote mutual interchange and cooperation in the field of biomass production and use, including biofuels uses and all other utilisations. It aims at creating, developing and maintaining solidarity and links between its Members and at defending their interests at European and international level. Its main target is to act as a catalyst for fostering synergies among scientists, industrialists and decision makers in order to promote the development of research, technology and industrial capacities in the field of Algae.

Pond Biofuels Inc. in Canada has grown algae directly off of a cement plant smokestack emissions, and used waste heat to dry the algae, as well.

Ocean Nutrition Canada in Halifax, Nova Scotia, Canada has found a new strain of algae that appears capable of producing oil at a rate 60 times greater than other types of algae being used for the generation of biofuels.

Algae production from the warm water discharge of a nuclear power plant has been piloted by Patrick C. Kangas at Peach Bottom Atomic Power Station, owned by Exelon Corporation. This process takes advantage of the relatively high temperature water to sustain algae growth even during winter months.