UNIT – II MICROBIAL STRUCTURE AND REPRODUCTION

SATHYABAMA UNIVERSITY **FACULTY OF BIO AND CHEMICAL ENGINEERING**

SBT1103	MICROBIOLOGY	L	T	P	Credits	Total Marks
		3	0	0	3	100

COURSE OBJECTIVES

- To enable students to learn about the principles of Microbiology to emphasize structure and biochemical aspects of various micro organisms.
- To know the control and preventive measures of microbial infections and environmental pollutions.

UNIT 1 INTRODUCTION TO MICROBIOLOGY **11 Hrs.**

Introduction, History and scope of microbiology, Contributions of Leewenhoek, Pasteur, Koch, Jenner and Fleming, Microbial classification: Classical and Current systems, Methods of identifying microbes.

Basics of Microscopy, Staining: simple, differential (Gram staining, Acid fast staining), special staining (flagella,capsule,endospore)

UNIT 2 MICROBIAL STRUCTURE AND REPRODUCTION **9 Hrs.**

Morphology and Reproduction: Bacteria - General structure and forms, Reproduction methods - Fission, budding and sporulation, Virus - TMV, HIV & T4 bacteriophage - lytic, lysogenic cycle, Fungi - Fungal morphology - Mycelial and yeast forms - sexual and asexual Reproduction, Actinomycete

UNIT 3 MICROBIAL GROWTH AND PHYSIOLOGY **7 Hrs.**

Microbial Growth and Nutrition, Types of media - Based on Consistency, Nutritional components, Funtional uses and application, Microbial types based on nutrition, Growth of microbes in culture - Pure culture techniques, Batch & Continous - Growth curve - Enumeration methods , Types of fungal growth media. Aerobic and Anaerobic metabolism of sugars, mixed acid fermentation.

UNIT 4 CONTROL OF MICROORGANISMS **9 Hrs**

Definitions of frequently used terms - Pattern or Rate of Microbial Death, Physical methods of Microbial Control: Heat (Moist & Dry), Low temperature, Filtration, High pressure, Desiccation, Osmotic pressure, Radiation. Chemical methods of Microbial Control: Liquids - Alcholos, Aldehydes, Phenolics, Halogens - Heavy metals, Surface active agents & Dyes, Gases - Formaldehyde, Ethylene Oxide, Plasma - Physico-chemical methods - Chemotherapeutic agents - Evaluation of effectiveness of antimicrobial agents. Difference between cleaning - sanitizing - sterilizing agents. Moist heat sterilization: D, Z and F Values and significance.

UNIT 5 APPLICATIONS OF MICROBIOLOGY **9 Hrs.**

Microbial ecology: Microbe-Microbe interaction - Mutualism, Commensalism, Altruism, Microbe - host interactions - Colonization and Infection- Causes and Transmission of Infectious Diseases, Emerging and re-emerging infectious diseases - Mechanism and examples, Multidrug resistance - MRSA, Diagnostic Microbiology, Childhood and adult vaccinations - MMR, Polio, Rabies etc, bioterrorism agents, Biofilm - Quorum sensing,

Max. 45 Hours.

TEXT / REFERENCE BOOKS

1. Pelczar, Jr E.C.S Chan and noel R.Krieg, Microbiology, 5th edition Tata McGrawHill -2006
2. Joanne M. Willey, Linda Sherwood, Christopher J. Woolverton, Prescott's Microbiology, 8th Edition, McGraw-Hill Higher Education, 2008
3. Jawetz, Melnick and Adelberg's Medical Microbiology . McGraw-Hill Medical, 2007
4. University of South Carolina School of Medicine (http://pathmicro.med.sc.edu/book/bact-sta.htm)

END SEMESTER EXAMINATION QUESTION PAPER PATTERN

Max Marks : 80 **Exam Duration : 3 Hrs.**

PART A : 10 questions of 2 marks each - No choice **20 Marks**

PART B : 2 questions from each unit of internal choice; each carrying 12 marks **60 Marks**

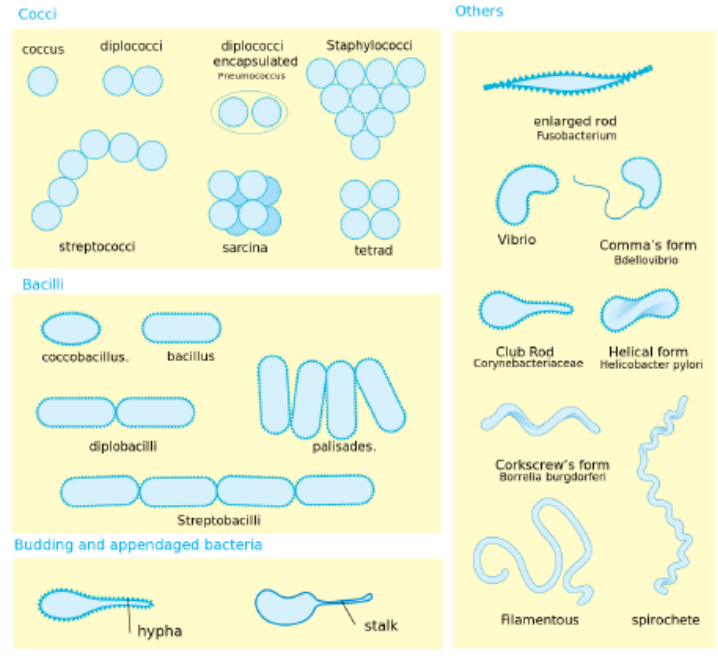
Bacterial cell structure

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

Cell morphology

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

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



Bacteria come in a wide variety of shapes

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

ANATOMY OF BACTERIA CELL

Any bacterial cell whether it is a coccus or a bacillus will have some structures common. These structures are cell wall, cell membrane, cytoplasm, ribosomes and the chromosome. Other intra-cellular structures such as plasmid, inclusion bodies and extra-cellular structures such as capsule, fimbriae and flagella are possessed only by some bacteria.

Glycocalyx/Capsule/Slime:
A gelatinous polysaccharide or polypeptide outer covering of certain bacteria is called glycocalyx. These are the structures that surround the outside of the cell envelope. The glycocalyx is referred to as a capsule if it is firmly attached to the cell wall, or as a slime layer if loosely attached.

The chemical nature of bacterial capsules is diverse but majority of them are polysaccharides. These polymers are composed of repeating oligosaccharide units. However, the capsule of *Bacillus anthracis* is composed of a polypeptide (polyglutamic acid). *Yersinia pestis* produces a capsule of mixed amino acids. Capsules may be weakly antigenic to strongly antigenic, depending on their chemical complexity. Capsules may be covalently linked to the underlying cell wall or just loosely bound to it. They have no net charge and will not bind charged dye particles, hence they can't be stained. Bacteria with capsules form smooth (S) colonies while those without capsules form rough (R) colonies. A given species may undergo a phenomenon called S-R variation whereby the cell loses the ability to form a capsule. Some capsules are very large and absorb water (e.g., *Klebsiella pneumoniae*) forming mucoid (M) colonies. Capsules are often lost during in vitro culture. They are not essential to cell viability and some strains within a species will produce a capsule, while others do not. Capsules are sometimes referred as K antigens (in Enterobacteriaceae) or as Vi antigen (in *Salmonella typhi*). Capsular antigens may either be specific to a species or may be shared by few different bacteria. For example, K2 antigen of Klebsiella cross-reacts with Pneumococcal type2 antigen.

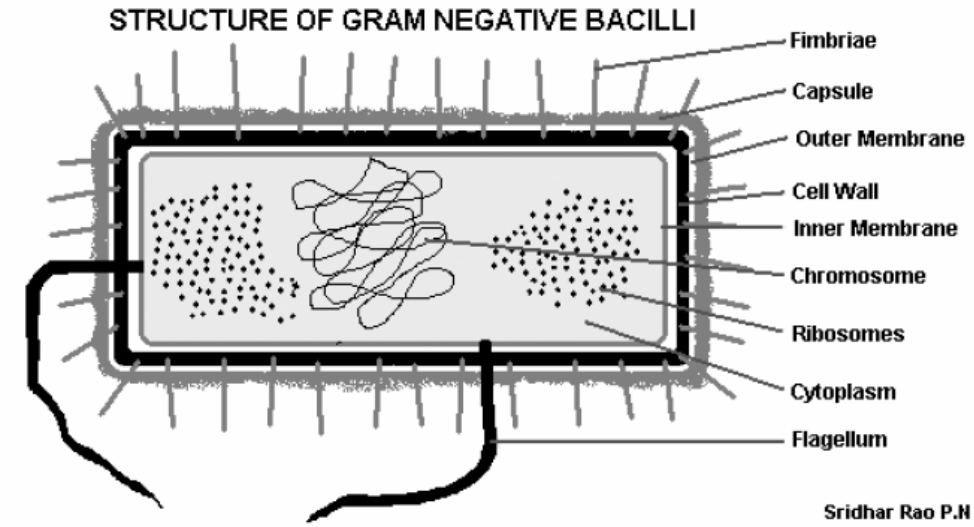
Significance:
- Virulence factor. Capsules of pathogenic bacteria inhibit ingestion and killing by phagocytes. It can also prevent complement-mediated bacterial cell lysis. Capsules protect the cells from lysozyme. Mutant strains lacking capsule are avirulent.
- Permit bacteria to adhere to cell surfaces and structures such as medical implants and catheters. This is a first step in colonization and sometimes leads to disease.
- Capsules can be a source of nutrients and energy to microbes. *Streptococcus mutans*, which colonizes teeth, ferments the sugar in the capsule and acid byproducts contribute to tooth decay.
- Prevent cell from drying out (desiccation)
- Toxicity to the host cell; capsule of *Bacteroides fragilis* induces abscess formation.
- Capsules may protect cells from bacteriophages.
- Capsules play a role in antigenic mosaic.
- Capsules may trap ions.

Examples:
Streptococcus pneumoniae, *Streptococcus mutans, Klebsiella pneumoniae, Bacillus anthracis*, *Neisseria meningitidis*

Demonstration:
Since capsules don't take up any stain, they can be demonstrated by negative staining techniques. In Gram stain, they may appear as a clear halo around the bacteria where they represent capsule. They are best demonstrated by Negative staining using India ink, Nigrosine, Maneval's method (using Congo Red, acetic acid and acid fuchsin) or Welch method (involving crystal violet and copper sulfate solution). They can also be demonstrated immunologically by Quellung reaction.

Application:

- Since the polysaccharides are good antigens, their presence on bacteria may be used to identify or type them by serological methods. Detection of capsular antigen in clinical specimens such as CSF, blood provides rapid method of diagnosis. Rapid identification of *Streptococcus pneumoniae* in CSF can be made by serological tests such as coagglutination. At least 13 serogroups of *Neisseria meningitidis* are identified on the basis of antigenicity of capsular polysaccharides.
- Since polysaccharides from certain capsules can be good antigen, they have been used in vaccines. Purified polysaccharide vaccines are available against *Neisseria meningitidis* and *Streptococcus pneumoniae*. They can also be conjugated with other vaccine as in *Hemophilus influenzae* vaccine.

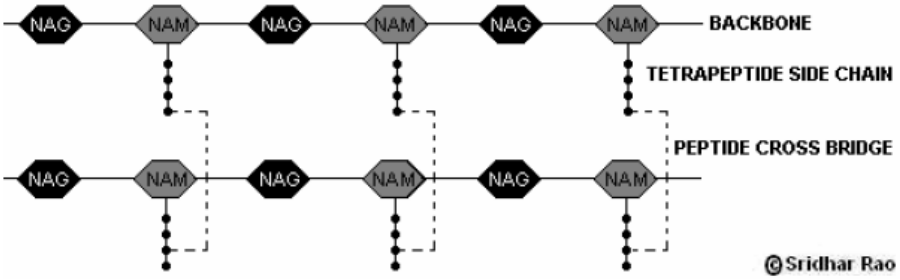


CELL WALL:

The layers of cell envelope lying between the cytoplasmic membrane and the capsule are referred to collectively as cell wall. In gram positive bacteria, the cell wall mainly consists of peptidoglycan and teichoic acid while the cell wall in gram negative bacteria includes peptidoglycan, lipoprotein, outer membrane and lipopolysaccharide layers. Cell wall does not take up any stain and hence are not seen by light microscope.

Most bacteria have a complex cell wall consisting of peptidoglycan (also called murein, mucopeptide). This complex polymer consists of three parts,

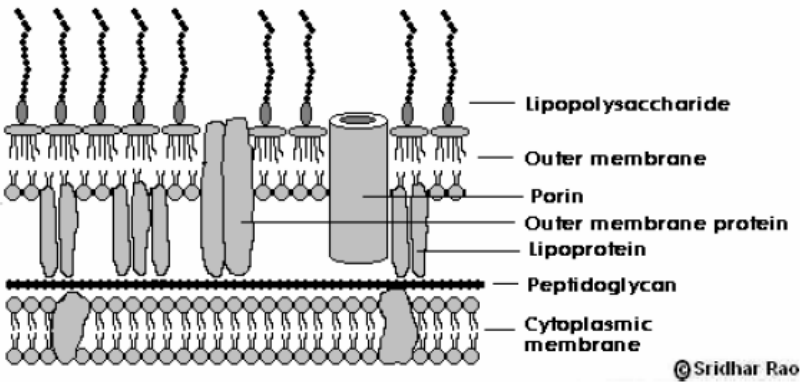
- A backbone consisting of alternating units of NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid).
- Tetrapeptide side chain attached to NAM
- Peptide cross-bridges, which are short chains of amino acids that crosslink the backbone.



Gram positive bacterial cell wall: In gram positive bacteria, there may be as many as 40 sheets of peptidoglycan, comprising up to 50% of cell wall material. Electron micrographs show the peptidoglycan of Gram positive cells to be 20-80 nm thick. Most gram positive cell walls contain additional substances such as teichoic acid and teichuronic acid. These are water soluble polymers of ribitol or glycerol. There are two types of teichoic acid, wall teichoic acid (linked to peptidoglycan) and lipoteichoic acid (linked to membrane). Some gram positive bacteria may lack wall teichoic acid but all contain lipoteichoic acid. The teichoic acid constitutes major antigens of cells that possess them. Teichoic acid binds to Magnesium ions and plays a role in supply of this ion to the cell. Teichuronic acids are

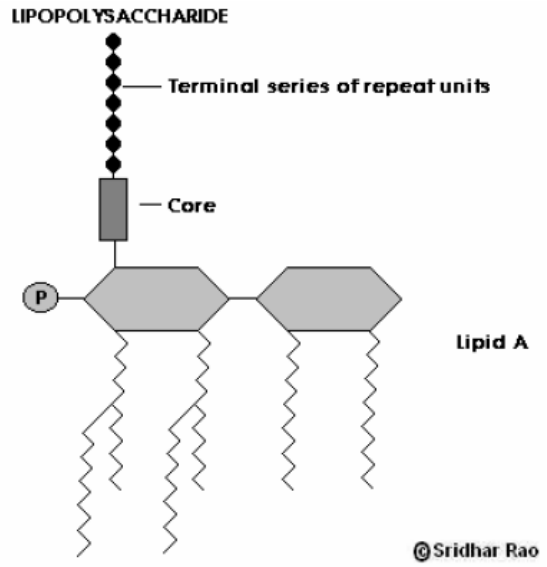
produced in place of teichoic acid when phosphate is limiting. Teichoic acid in *Streptococcus pneumoniae* bears the Forssman antigen. Gram positive cells stain purple due to retention of the crystal violet dye during the Gram stain procedure. If peptidoglycan is digested away from the cell, gram positive cells lose their cell walls and become protoplasts while the gram negative cells become spheroplasts. In some cases the cell wall of Gram-positive bacteria may contain proteins of special significance such as M, T and R proteins of the group A streptococci and Protein A of *Staphylococcus aureus*.

Gram negative bacterial cell wall: Gram negative cells consist of a relatively thin layer of peptidoglycan (approximately 10 nm). There appears to be only one or two sheets of peptidoglycan, comprising 5-10% of cell wall material. Gram negative bacteria do not retain the primary dye in Gram stain and hence appear pink. Gram negative bacteria have an additional outer membrane. The outer membrane is the major permeability barrier in Gram negative bacteria. The space between the inner and outer membranes is known as the periplasmic space, which contains digestive enzymes and other transport proteins.



Gram negative cell walls contain three components that lie outside the peptidoglycan layer: lipoprotein, outer membrane and lipopolysaccharide. Lipoprotein stabilizes the outer membrane by anchoring it to peptidoglycan. Outer membrane is phospholipid bilayer in which the outer phospholipids are replaced by lipopolysaccharides. It is structurally similar to cytoplasmic membrane and serves to prevent leakage of periplasmic proteins and protects the cell from bile salts and protelolytic enzymes. The outer membranes contain several important porins, which specifically allow transport of solutes.

Lipopolysaccharide consists of a polysaccharide core, a complex lipid called Lipid A and a terminal series of repeat units. The polysaccharide core is similar in all gram negative bacteria. Each species contains unique terminal repeat units. Lipopolysaccharide (LPS) is toxic in nature and is called endotoxin because it is firmly bound to the cell wall and released only when cell is lysed. Endotoxin can trigger fever and septic shock in gram negative infections. Endotoxin can be detected in IV fluids by Limulus lysate reaction. The lysate of amebocytes (circulating cells) from the horseshoe crab (*Limulus polyphemus*) are highly sensitive to endotoxin and gels (clots) immediately on exposure to it. LPS also protects the cell from phagocytosis and lysozyme.



LPS confers a negative charge and also repels hydrophobic molecules such as bile in the intestine. If LPS is split into Lipid A and polysaccharide, all the toxicity is associated with Lipid A and polysaccharide represents the major surface antigen of bacterial cell. This antigen is designated as somatic "O" antigen and is used in serological typing of species. It was formerly known as Boivin antigen. Antigenic specificity is conferred by the terminal repeat units

The cell envelopes of Mycobacteria are more complex than other bacteria. Long chained branched fatty acids (Mycolic acid) are covalently bound via a polysaccharide to peptidoglycan. Other mycolic acid containing compounds and complex lipids form a thick waxy membranous layer outside the peptidoglycan layer.

Significance of cell wall:

- Maintains cell shape, any cell that loses its cell wall, loses its shape as well.
- Protects bacteria from osmotic lysis
- Acts as a barrier, protects cell contents from external environment
- Determines reactivity to Gram stain, cells become gram negative if they lose cell wall
- Attachment site for flagella
- Site of action of certain antimicrobial agents (E.g. Penicillins, Cephalosporins)
- Bacteria may attach to surface, produce slime, divide and produce microcolonies within the slime layer and construct a biofilm. E.g. formation of dental plaque mediated by the bacterium *Streptococcus mutans*.
- Confer specific antigenicity to a strain/species that can be exploited to detect and identify an isolate.

Substances acting against cell wall:

- ✓ Lysozyme, an enzyme found in tears and saliva breaks down a component of cell walls
- ✓ Antibiotics that inhibits cell wall synthesis such as Penicillins and cephalosporins
- ✓ Autolytic enzymes produced by some bacteria such as *Streptococcus pneumoniae*

Demonstration of cell wall:

Since cell wall does not take up stain, they can't be demonstrated by light microscopy. Their presence can be demonstrated by placing a cell in hypertonic solution, where they undergo plasmolysis. The cytoplasm shrinks as the water is lost by osmosis but the cell wall retains its original shape (due to its rigidity). This is described as "bacterial ghost". The cell wall may also be demonstrated by micro-dissection, electron microscopy and immunological reactivity.

CELL MEMBRANE

Cell membrane or cytoplasmic membrane is a typical unit membrane composed of phospholipids (40%) and proteins (60%). It measures approximately 5-10 nm in thickness. It lies below the peptidoglycan layer of the cell wall and encloses the cytoplasm. The arrangement of proteins and lipids to form a membrane is called the fluid mosaic model. The membranes of bacteria (except Mycoplasma) do not contain sterols. It is a phospholipid bilayer with polar heads on either side of the membrane. Hydrophobic tails are oriented to the interior of the membrane. Specialized structures called mesosomes or chondroids are formed from the convoluted invaginations of cytoplasmic membrane. There are two types of mesosomes, septal mesosome and lateral mesosome. The bacterial chromosome is attached to the septal mesosome. During cell division, the septal mesosome participates in the formation of cross-walls. Mesosomes are more prominant in gram positive bacteria. They are believed to be analogous to eukaryotic mitochondria since they are rich in respiratory enzymes.

Functions of cell membrane:

- A selectively permeable barrier: substances that pass through the membrane are limited by pore sizes and the hydrophobic nature of the membrane
- Integral (transmembrane) proteins form channels and act as carriers
- Peripheral proteins can act as receptors and as enzymes for metabolic reactions
- Electron transport and oxidative phosphorylation: cytochromes and dehydrogenases of respiratory chain are located in the cell membrane
- Excretion of hydrolytic enzymes
- Site of initiation of cell wall synthesis
- Site of attachment of the chromosome
- Site of synthesis of phospholipids
- Bear receptors and proteins of sensory transduction system

Substances acting on cell membrane:

- ❑ Detergents that contain lipophilic and hydrophilic groups disrupt cytoplasmic membranes
- ❑ Antibiotics such as Polymyxin B and Gramicidin selectively damages membrane
- ❑ Ionophores (E.g. Valinomycin) are compounds that permit rapid diffusion of cations through the membrane.
- ❑ Chemical agents such as alcohols and quaternary ammonium compounds

CYTOPLASM

The cytoplasm or protoplasm is the portion of the cell that lies within the cytoplasmic membrane. It is gel-like in consistency and includes the procaryotic chromosome and ribosomes. Constituents of cytoplasm include proteins (including enzymes), vitamins, ions, nucleic acids and their precursors, amino acids and their precursors, carbohydrates and their derivatives, fatty acids and their derivatives. The cytoplasm does not exhibit any internal

mobility (cytoplasmic streaming). The cytoplasm also lacks organelles such as mitochondria, golgi apparatus or endoplasmic reticulum. Cytoplasm stains uniformly in young cultures. Recent studies suggest that some bacteria (*Bacillus subtilis*) possess cytoskeleton.

Chromosome:
The chromosome in bacteria is typically a single, closed circle DNA that is concentrated in a nucleoid region. It is not membrane bound as in eukaryotes. Some bacteria possess smaller extrachromosomal pieces of DNA called plasmids. Plasmids replicate independently of the chromosome and carry genes that are not essential for cell survival but may give some advantage to an organism. The chromosome is attached to an invagination of the cytoplasmic membrane called mesosome. Mitotic apparatus and nuclear membrane are completely lacking. The length of *E.coli* chromosome is approximately 1.4 mm but is condensed inside the cell by supercoiling. DNA is mainly negatively charged hence bind readily to basic dyes. It can be demonstrated by Feulgen stain or by electron microscopy.

Ribosomes:
Bacterial cells can contain thousands of ribosomes, which are the sites of protein synthesis. The distinct granular appearance of procaryotic cytoplasm is due to the presence and distribution of ribosomes. Often they aggregate to form structures known as polysomes. Bacterial ribosomes are termed 70 S (Svedberg units) and eukaryotic ribosomes are termed 80S. The difference between bacterial and eukaryotic ribosomes is often exploited during antibiotic therapy.

Inclusion bodies:
Intracytoplasmic inclusions can be vacuoles, crystals or storage bodies. Bacteria often store reserve material in the form of insoluble cytoplasmic granules. Inclusions accumulate when a cell is grown in the presence of excess nutrients and they are often observed under laboratory conditions. Various examples of these bodies are:

- Starch/Glycogen granules - blue-greens and enteric bacteria
- Poly-ß-hydroxybutyrate granules - Azotobacter and Rhizobium
- Nitrogen-reserve granules - blue-greens
- Sulphur inclusions – Thiotrix
- Lipid inclusions
- Volutin granules – *Corynebacterium diphtheriae*

The inclusion bodies can be appreciated using phase contrast microscope or using special stains such as Albert's stain (volutin granules) or Sudan black (lipid inclusion).

FLAGELLA
Some bacteria are motile and some are not. Almost all motile bacteria possess flagella as the organ of locomotion. Such bacteria tend to move towards or away from the source of stimulus. These stimuli can be chemicals (chemotaxis), light (phototaxis), air (aerotaxis) or magnetism (magnetotaxis).

Structure:
Procaryotic flagella are much thinner than eukaryotic flagella and they lack the typical 9 + 2 arrangement of microtubules. Over 40 genes are involved in its assembly and function. They are approximately 3-20µm long and end in a square tip. Since flagella are very thin (20-30 nm in diameter), they are below the resolution limits of a normal light microscope and cannot be seen. The bacterial flagellum is a non contractile, composed of single kind of protein subunit called flagellin. It is anchored to the bacterial cytoplasmic membrane and cell well by means of disk-like structures. A flagellum comprises of three parts, filament, hook and basal body. The flagellum is attached to the cell body by hook and basal body. While the hook and basal body are embedded in the cell envelope, the filament is free. If a flagellum is cut off it will regenerate until reaches a maximum length. This is so because the growth is not from base, but from tip. The basal body bears a set of rings, one pair in gram positive bacteria and two pairs in gram negative bacteria. While the rings named S and M are common to both, the rings names P and L are found only in gram negative bacteria. Rings in the basal body rotate relative to each other causing the flagella to turn like a propeller. The energy to drive the basal body is obtained from the proton motive force. Bacteria move at average speed of 50µm/sec, the fastest being *Vibrio cholerae* that moves 200µm/sec.

The numbers of flagella, as well as their location on the cell surface are characteristic of a species.

Flagella arrangements are:
1. Monotrichous - a single flagellum at one pole (also called polar flagellum) E.g. *Vibrio cholerae*
2. Amphitrichous - single flagellum at both poles. Eg. Spirilla
3. Lophotrichous - two or more flagella at one or both poles of the cell E.g. *Spirillum undula*
4. Peritrichous - completely surrounded by flagella E.g. *E.coli*

Other mechanisms of bacterial locomotion include gliding and motion by axial filament contraction. Gliding is movement of bacteria along solid surfaces by an unknown mechanism. Spirochetes have internally-located axial filaments or endoflagella. Axial filaments wrap around the spirochete towards the middle from both ends. They are located above the peptidoglycan cell wall but below the outer membrane.

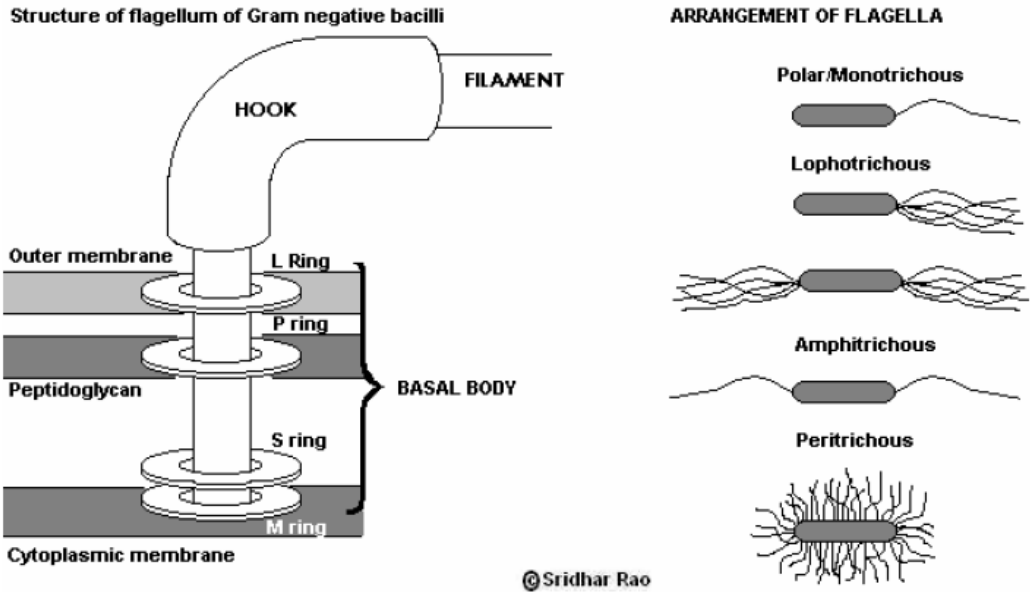
Detection bacterial motility:

1) Direct observation by means of hanging drop preparation
2) Special-purpose microscopes (phase-contrast and dark-field)
3) Motility media (semi solid agar)
4) Indirectly, by demonstration of flagella
   - Flagella staining (Silver impregnation, Leifson's method)
   - Electron microscopy
   - Immunological detection of flagellar "H" antigen

Types of bacterial motility:

- Stately motility: *Bacillus sps*
- Active motility: *Pseudomonas sps*
- Darting motility: *Vibrio cholerae*
- Tumbling motility: *Listeria monocytogens*
- Corkscrew, extension-flexion motility: Spirochetes

Examples of non-motile bacteria: Most cocci, Shigella, Klebsiella

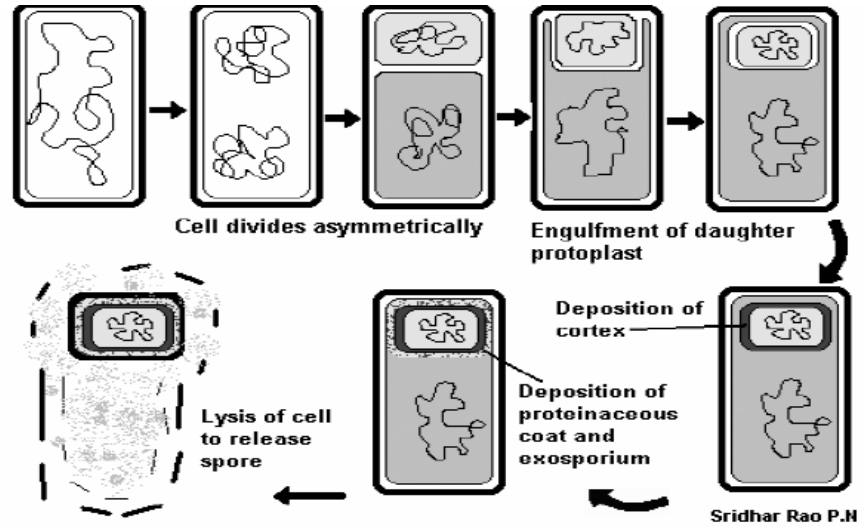


Significance of flagella:

- Primarily function is motility (chemotaxis, aerotaxis, phototaxis etc). Positive taxis is movement toward a favorable environment whereas negative taxis is movement away from a repellent.
- Flagella can help in identifying certain types of bacteria. For example, Proteus species show 'swarming' type of growth on solid media.
- Flagellar antigens are used to distinguish different species and strains of bacteria (serovars). Variations in the flagellar H antigen are used in serotyping.

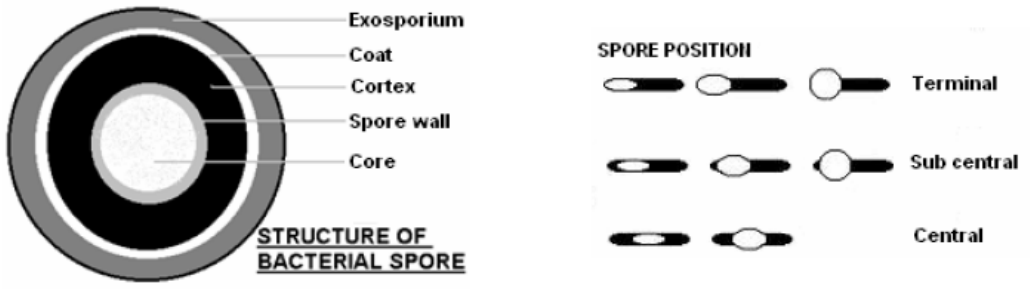
FIMBRIAE AND PILI

Fimbriae are short, hair-like structures made up of protein pilin and are present in many gram negative bacteria. Even though pili arise from plasma membrane they are not considered part of the plasma membrane. They are anchored in the membrane and protrude through the cell wall to the outside of the cell. Fimbriae are shorter and straighter than flagella and are more numerous. They are 0.5µm long and 10 nm thick. Since they are made up of protein, they are antigenic. Bacteria from different genera may possess common fimbrial antigens. Fimbriae are



The impermeability of the spore coat is thought to be responsible for the endospore's resistance to chemicals. The resistance of endospores is due to a variety of factors:

- Calcium-dipicolinate, abundant within the endospore, may stabilize and protect the endospore's DNA.
- Specialized DNA-binding proteins saturate the endospore's DNA and protect it from heat, drying, chemicals, and radiation.
- The cortex may osmotically remove water from the interior of the endospore and the dehydration that results is thought to be very important in the endospore's resistance to heat and radiation.
- DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.



The size of the endospore and its position within the vegetative cell is characteristic for a given species. The position of spore in a bacterium can be central, sub terminal or terminal. The shape of the spore can be spherical or oval. Sometimes the width of the spore is slightly more than the width of the bacillus such that spore appears to be bulging from the cell, as seen in Clostridium. In *Bacillus sps*, the spore does not cause the bacillus to bulge.
Bacteria with central or sub terminal spores: *Clostricium welchii, Clostridium sporogenes*
Bacteria with oval and terminal spores: *Clostridium tertium*
Bacteria with spherical and terminal spores: *Clostridium tetani*

Demonstration of spores:
The dense endospore is impenetrable by basic dyes. Strong dyes and vigorous staining conditions such as heat are needed. Once stained, however, endospores are equally hard to decolorize.

- In the Gram stain the spore is not stained and may appear as a clear space
- Appear as refractile bodies when seen through phase contrast microscope
- Spores can be stained by Malachite Green
- Modified acid fast stain

Significance of spores:

- Since spores survive ordinary disinfection, they may contaminate surgical wounds.
- Since spores are everywhere, they may contaminate bacterial culture media.
- Since they are highly heat resistant, they can be used to monitor the efficacy of sterilization process in autoclave (*Bacillus stearothermophilus*) and hot air oven (*Clostridium tetani* var *niger*).
- They have also been used in biological warfare.

L-FORMS, PROTOPLAST AND SPHEROPLASTS:

When bacteria are treated with enzymes that hydrolyze the cell wall (e.g. lysozyme) or antibiotics that interfere with biosynthesis of peptidoglycan (penicillin), wall-less bacteria are often produced. Such a treatment of bacteria in osmotically protective medium liberates protoplasts from gram positive bacteria and spheroplasts from gram negative bacteria. Spheroplasts retain the outer membrane. Usually these treatments generate wall-less non-viable organisms that do not multiply. However, if such cells can grow and divide, they are called L forms. L forms were first reported by Klieneberger Nobel in cultures of *Streptobacillus monoliformis*. They are named L forms after Lister Institute, where they were discovered. They are produced more readily with penicillin than with lysozyme, suggesting that need for residual peptidoglycan. Some L forms are stable and some are unstable. Unstable forms are those which revert back to cell wall containing state when inducing stimulus (penicillin) is removed. Such forms usually have small amounts of residual peptidoglycan that serves as primer for building cell wall. Stable forms do not revert back to normal form since they completely lack peptidoglycan. While some L forms form spontaneously (e.g *Streptobacillus monoliformis*) others are inducible. Since they lack cell wall, they don't have a definite shape. L forms are difficult to cultivate and require medium that has right osmotic strength and low concentration of agar, inactivated serum and sucrose. L forms resemble mycoplasma in morphology, type of growth on agar "fried-egg colony". While mycoplasma lack cell wall and have sterols in their membrane, the L forms may have reminiscent of cell wall but do not have sterols in their membrane.

Significance of L forms: L forms may produce chronic infections in the host. They may persist in protective regions of the body. Since L forms are relatively resistant to antibiotics, they are difficult to treat. Their reversion to normal form can result in relapse of infection.

INVOLUTION FORMS AND PLEOMORPHISM:

Certain species of bacteria are known to exhibit variation in shape and size of individual cells. This variation is known as pleomorphism. Swollen and aberrant forms may be seen in ageing cultures of *Nesseria gonorrhoeae* and *Yersinia pestis* or in the presence of high salt concentration. Such forms are known as involution forms. Pleomorhism as well as involution forms are believed to be the result of defective cell wall synthesis or due to the action of autolytic enzymes that digest their own cell wall.

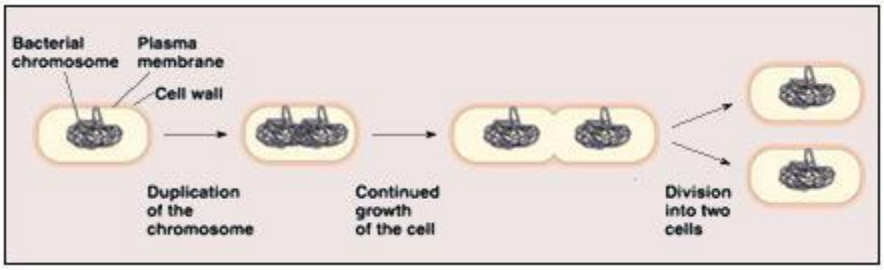
Reproduction in Bacteria

Asexual reproduction

Binary Fission in Bacteria

It is the most common mode of asexual reproduction. The cytoplasm and nucleoid of a bacterial cell divide equally into two, following replication of DNA. The cell wall and cytoplasm also split resulting in the formation of two daughter cells.

Under favourable conditions, a bacterial cell divides by fission once in every 20 minutes.

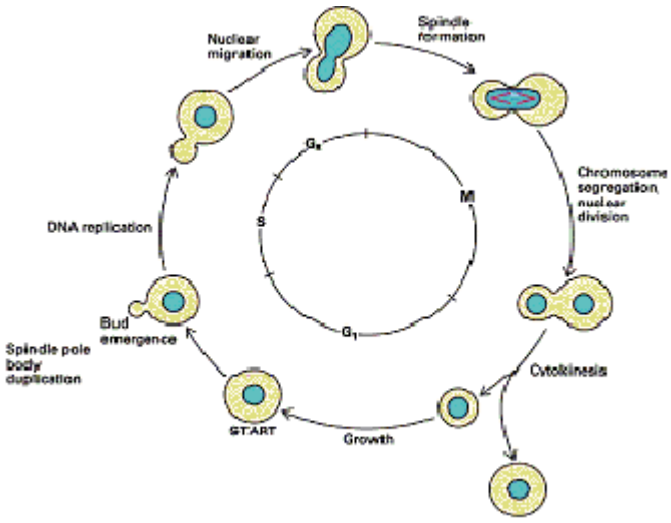


Fission in Bacteria

Budding

Budding is a form of asexual reproduction in which a new organism develops from an outgrowth or bud due to cell division at one particular site. The new organism remains attached as it grows, separating from the parent organism only when it is mature, leaving behind scar tissue. Since the reproduction is asexual, the newly created organism is a clone and is genetically identical to the parent organism.

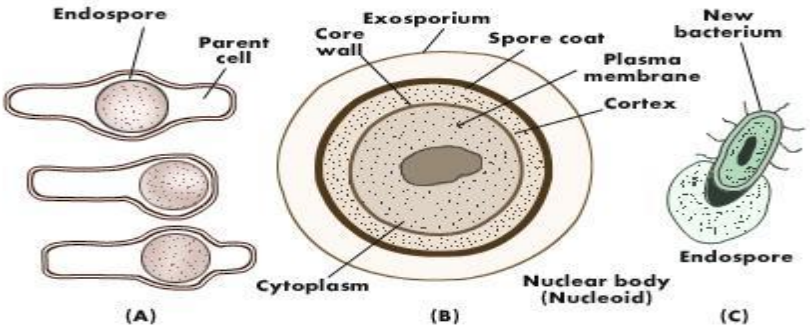
Budding bacterium, plural Budding Bacteria, any of a group of bacteria that reproduce by budding. Each bacterium divides following unequal cell growth; the mother cell is retained, and a new daughter cell is formed. (Binary fission, in which two equal daughter cells are produced from the unilateral growth and division of the mother cell, is typical of most bacteria.) In budding, the cell wall grows from one point on the cell (polar growth), rather than throughout the cell; this permits the development of more complex structures and processes. Most budding bacteria develop cytoplasmic extrusions, such as stalks (*Caulobacter*), hyphae (*Hyphomicrobium*), and appendages (*Stella*). Budding bacteria are most often aquatic and can attach to surfaces by their stalks; others are free-floating.



Sporulation - Endospore Formation

In certain bacteria like Clostridium and Bacillus, the cells tide over unfavourable conditions by forming **endospores**. During this process, a portion of the cytoplasm and a copy of the bacterial chromosome undergo dehydration and get surrounded by a three-layered covering. The remaining part of cytoplasm and cell wall degenerate. The resulting structure, called **endospore** can tolerate extreme environmental conditions and can remain viable for several years. When the environmental conditions are suitable, the endospore absorbs water, swells and the wall splits, releasing the cell inside. It develops a new cell wall and starts functioning as a typical bacterial cell.

Endospore formation is not a method of reproduction. It is only a method meant for tiding over unfavourable conditions and allowing dispersal of the bacterial cells into new habitats.



Endospore formation. A, Endospores according to their position in parent cells. B, An edospore in cross-section. C, Germination of endospore

Endospore of a Bacterium

Sexual Reproduction

In bacterial sexual reproduction there is no meiosis, formation of gametes and zygote. Instead, it involves transfer of a portion of genetic material (DNA) from a donor cell to a recipient cell. This process is called as **genetic recombination** or **parasexuality**. It is known to occur in the following three ways:

Transformation

In this process, one kind of bacterium is transformed into another kind. It takes place by a transfer of DNA from a capsulated bacterium into a non-capsulated bacterium. It has been observed in Diplococcus bacteria.

Transduction

In this process, DNA of a bacterial cell (donor) is transferred into another bacterial cell with the help of a bacteriophage. This process is known to occur in several bacterial species such as Salmonella, Escherichia, Micrococcus and Stigella.

Conjugation

It is a process in which the genetic material of a bacterial cell of a particular strain is transferred into that of another bacterial cell of a different strain. Of the two strains of bacteria involved, one acts as donor (or male) and the other as a recipient (or female). The donor cells are known to possess a sex factor or **fertility factor** (F factor) as a component of its circular DNA (F+ strain). The recipient cell does not have this factor and hence it is described as F- strain. A conjugation between cells of F+ and F- strains always results in the formation of F+ bacterial cells in the progeny.

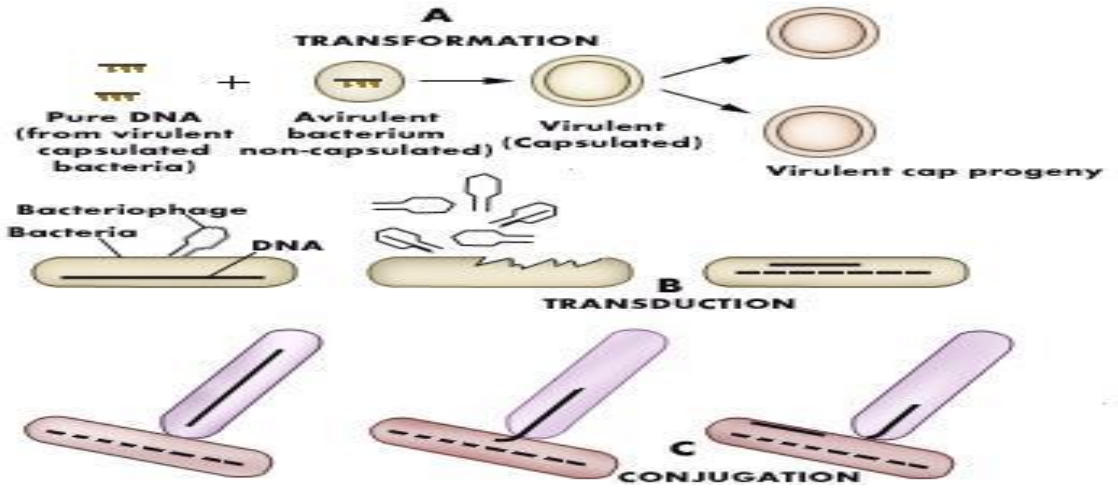


fig. 8.5 - Genetic Recombinations in Bacteria

Virus

A **virus** is a small infectious agent that replicates only inside the living cells of other organisms. Viruses can infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea.[1]

Since Dmitri Ivanovsky's 1892 article describing a non-bacterial pathogen infecting tobacco plants, and the discovery of the tobacco mosaic virus by Martinus Beijerinck in 1898,[2] about 5,000 virus species The study of viruses is known as virology, a sub-speciality of microbiology.

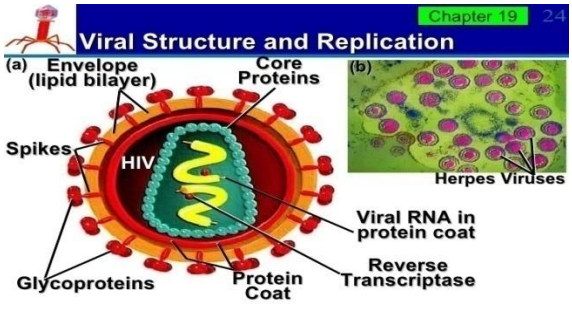
While not inside an infected cell or in the process of infecting a cell, viruses exist in the form of independent particles. These **viral particles**, also known as **virions**, consist of two or three parts: (i) the genetic material made from either DNA or RNA, long molecules that carry genetic information; (ii) a protein coat, called the capsid, which surrounds and protects the genetic material; and in some cases (iii) an envelope of lipids that surrounds the protein coat when they are outside a cell. The shapes of these virus particles range from simple helical and icosahedral forms for some virus species to more complex structures for others. Most virus species have virions that are too small to be seen with an optical microscope. The average virion is about one one-hundredth the size of the average bacterium.

Viral infections in animals provoke an immune response that usually eliminates the infecting virus. Immune responses can also be produced by vaccines, which confer an artificially acquired immunity to the specific viral infection. However, some viruses including those that cause AIDS and viral hepatitis evade these immune responses and result in chronic infections. Antibiotics have no effect on viruses, but several antiviral drugs have been developed.

Etymology

The word is from the Latin neuter *vīrus* referring to poison.

Structure



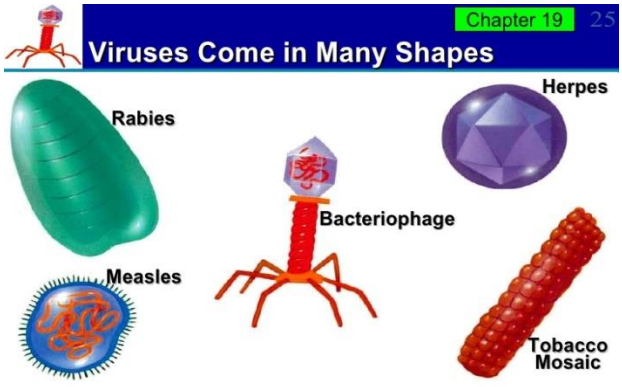
Viruses display a wide diversity of shapes and sizes, called *morphologies*. In general, viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and 300 nanometres. Some filoviruses have a total length of up to 1400 nm; their diameters are only

about 80 nm.[68] Most viruses cannot be seen with an optical microscope so scanning and transmission electron microscopes are used to visualise virions.[69] To increase the contrast between viruses and the background, electron-dense "stains" are used. These are solutions of salts of heavy metals, such as tungsten, that scatter the electrons from regions covered with the stain. When virions are coated with stain (positive staining), fine detail is obscured. Negative staining overcomes this problem by staining the background only.[70]

A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a capsid. These are formed from identical protein subunits called capsomeres.[71] Viruses can have a lipid "envelope" derived from the host cell membrane. The capsid is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction.[72][73] Virally coded protein subunits will self-assemble to form a capsid, in general requiring the presence of the virus genome. Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as nucleoproteins, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid. The capsid and entire virus structure can be mechanically (physically) probed through atomic force microscopy.[74][75] In general, there are four main morphological virus types:

Helical

> These viruses are composed of a single type of capsomer stacked around a central axis to form a helical structure, which may have a central cavity, or tube. This arrangement results in rod-shaped or filamentous virions: These can be short and highly rigid, or long and very flexible. The genetic material, in general, single-stranded RNA, but ssDNA in some cases, is bound into the protein helix by interactions between the negatively charged nucleic acid and positive charges on the protein. Overall, the length of a helical capsid is related to the length of the nucleic acid contained within it and the diameter is dependent on the size and arrangement of capsomers. The well-studied tobacco mosaic virus is an example of a helical virus.[76]



Icosahedral

Most animal viruses are icosahedral or near-spherical with chiral icosahedral symmetry. A regular icosahedron is the optimum way of forming a closed shell from identical sub-units. The minimum number of identical capsomers required is twelve, each composed of five identical sub-units. Many viruses, such as rotavirus, have more than twelve capsomers and appear spherical but they retain this symmetry. Capsomers at the apices are surrounded by five other capsomers and are called pentons. Capsomers on the triangular faces are surrounded by six others and are called hexons.[77] Hexons are in essence flat and pentons, which form the 12 vertices, are curved. The same protein may act as the subunit of both the pentamers and hexamers or they may be composed of different proteins.

Prolate

This is an icosahedron elongated along the fivefold axis and is a common arrangement of the heads of bacteriophages. This structure is composed of a cylinder with a cap at either end.[78]

Envelope

Some species of virus envelop themselves in a modified form of one of the cell membranes, either the outer membrane surrounding an infected host cell or internal membranes such as nuclear membrane or endoplasmic reticulum, thus gaining an outer lipid bilayer known as a viral envelope. This membrane is studded with proteins coded for by the viral genome and host genome; the lipid membrane itself and any carbohydrates present originate entirely from the host. The influenza virus and HIV use this strategy. Most enveloped viruses are dependent on the envelope for their infectivity.[79]

Complex

These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall. Some bacteriophages, such as Enterobacteria phage T4, have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibres. This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell.[80]

The poxviruses are large, complex viruses that have an unusual morphology. The viral genome is associated with proteins within a central disk structure known as a nucleoid. The nucleoid is surrounded by a membrane and two lateral bodies of unknown function. The virus has an outer envelope with a thick layer of protein studded over its surface. The whole virion is slightly pleiomorphic, ranging from ovoid to brick shape.[81] Mimivirus is one of the largest characterised

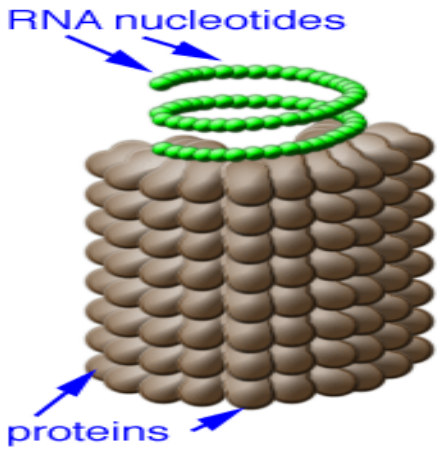
viruses, with a capsid diameter of 400 nm. Protein filaments measuring 100 nm project from the surface. The capsid appears hexagonal under an electron microscope, therefore the capsid is probably icosahedral.[82] In 2011, researchers discovered the largest then known virus in samples of water collected from the ocean floor off the coast of Las Cruces, Chile. Provisionally named *Megavirus chilensis*, it can be seen with a basic optical microscope.[83] In 2013, the Pandoravirus genus was discovered in Chile and Australia, and has genomes about twice as large as Megavirus and Mimivirus.[84]

Some viruses that infect Archaea have complex structures that are unrelated to any other form of virus, with a wide variety of unusual shapes, ranging from spindle-shaped structures, to viruses that resemble hooked rods, teardrops or even bottles. Other archaeal viruses resemble the tailed bacteriophages, and can have multiple tail structures.

TMV - Tobacco mosaic virus

(TMV) is a positive-sense single stranded RNA virus that infects a wide range of plants, especially tobacco and other members of the family Solanaceae. The infection causes characteristic patterns, such as "mosaic"-like mottling and discoloration on the leaves (hence the name). TMV was the first virus ever to be discovered. Although it was known from the late 19th century that an infectious disease was damaging tobacco crops, it was not until 1930 that the infectious agent was determined to be a virus.

Structure



Tobacco mosaic virus has a rod-like appearance. Its capsid is made from 2130 molecules of coat protein (see image to the left) and one molecule of genomic single strand RNA, 6400 bases long. The coat protein self-assembles into the rod like helical structure (16.3 proteins per helix turn) around the RNA which forms a hairpin loop structure (see the electron micrograph above). The protein monomer consists of 158 amino acids which are assembled into four main alpha-helices, which are joined by a prominent loop proximal to the axis of the virion. Virions are ~300 nm in length and ~18 nm in diameter.[12] Negatively stained electron microphotographs show a distinct

inner channel of ~4 nm. The RNA is located at a radius of ~6 nm and is protected from the action of cellular enzymes by the coat protein. There are three RNA nucleotides per protein monomer.[13] X-ray fiber diffraction structure of the intact virus was studied based on an electron density map at 3.6 Å resolution.[11]

Genome

The TMV genome consists of a 6.3-6.5 kb single-stranded (ss) RNA. The 3'-terminus has a tRNA-like structure. The 5' terminus has a methylated nucleotide cap (m7G5'pppG).[14] The genome encodes 4 open reading frames (ORFs), two of which produce a single protein due to ribosomal readthrough of a leaky UAG stop codon. The 4 genes encode a **replicase** (with methyltransferase [MT] and RNA helicase [Hel] domains), an RNA-dependent **RNA polymerase**, a so-called **movement protein** (MP) and a **capsid protein** (CP).[15]

Physicochemical properties

TMV is a thermostable virus. On a dried leaf, it can withstand up to 120 degrees Fahrenheit (50 °C) for 30 minutes.

TMV has an index of refraction of about 1.57.[16]

Disease cycle

TMV does not have a distinct overwintering structure. Rather, it will over-winter in infected tobacco stalks and leaves in the soil, on the surface of contaminated seed (TMV can even survive in contaminated tobacco products for many years). With the direct contact with host plants through its vectors (normally insects such as aphids and leaf hoppers), TMV will go through the infection process and then the replication process.

Infection

After its multiplication, it enters the neighboring cells through plasmodesmata. For its smooth entry, TMV produces a 30 kDa movement protein called P30 which enlarge the plasmodesmata. TMV most likely moves from cell-to-cell as a complex of the RNA, P30, and replicase proteins.

It can also spread through phloem for longer distance movement within the plant. Moreover, TMV can be transmitted from one plant to another by direct contact. Although TMV does not have defined transmission vectors, the virus can be easily transmitted from the infected hosts to the healthy plants, by human handling.

Replication

Following entry into its host via mechanical inoculation, TMV uncoats itself to release its viral [+]RNA strand. As uncoating occurs, the MetHel:Pol gene is translated to make the capping enzyme MetHel and the RNA Polymerase. Then the viral genome will further replicate to

produce multiple mRNAs via a [-]RNA intermediate primed by the $tRNA_{HIS}$ at the [+]RNA 3' end. The resulting mRNAs encode several proteins, including the coat protein and an RNA-dependent RNA polymerase (RdRp), as well as the movement protein. Thus TMV can replicate its own genome. After the coat protein and RNA genome of TMV have been synthesized, they spontaneously assemble into complete TMV virions in a highly organized process. The protomers co

me together to form disks or 'lockwashers' composed of two layers of protomers arranged in a helix. The helical capsid grows by the addition of protomers to the end of the rod. As the rod lengthens, the RNA passes through a channel in its center and forms a loop at the growing end. In this way the RNA can easily fit as a spiral into the interior of the helical capsid.[17]

HIV

The **human immunodeficiency virus** (**HIV**) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS).[1][2] AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.[3] Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.[4] HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including pyroptosis of abortively infected T cells,[5] apoptosis of uninfected bystander cells,[6] direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells.[7] When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections

Structure and genome

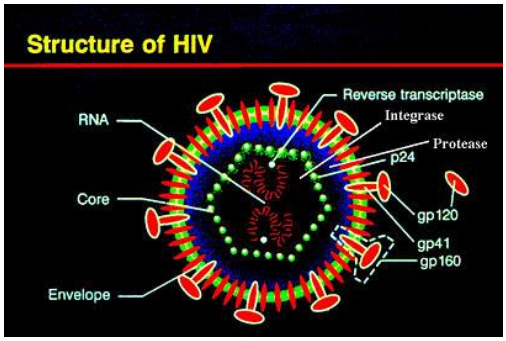


Diagram of HIV virion

HIV is different in structure from other retroviruses. It is roughly spherical[14] with a diameter of about 120 nm, around 60 times smaller than a red blood cell, yet large for a virus.[15] It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24.[16] The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle.[16]

This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the HIV Envelope protein,[16] which consists of a cap made of three molecules known as glycoprotein (gp) 120, and a stem consisting of three gp41 molecules which anchor the structure into the viral envelope.[17][18] The Envelope protein, encoded by the HIV *env* gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell membrane releasing the viral contents into the cell and initiating the infectious cycle.[17] As the sole viral protein on the surface of the virus, the Envelope protein is a major target for HIV vaccine efforts.[19]

The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of tat env and rev), encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles.[16] For example, *env* codes for a protein called gp160 that is broken down by a cellular protease to form gp120 and gp41. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease.[16]

The two Tat proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element. The TAR may also be processed into microRNAs that regulate the apoptosis genes ERCC1 and IER3.[20][21] The Rev protein (p19) is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the RRE RNA element. The Vif protein (p23) prevents the action of APOBEC3G (a cellular protein that deaminates Cytidine to Uridine in the single stranded viral DNA and/or interferes with reverse transcription[22]). The Vpr protein (p14) arrests cell division at G2/M. The Nef protein (p27) down-regulates CD4 (the major viral receptor), as well as the MHC class I and class II molecules.[23][24][25]

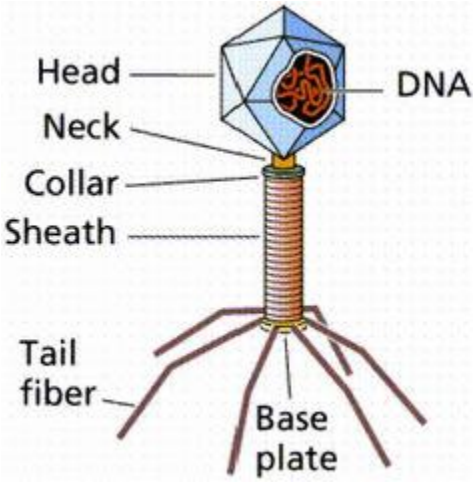
Nef also interacts with SH3 domains. The Vpu protein (p16) influences the release of new virus particles from infected cells.[16] The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral

genome packaging and recognized by Gag and Rev proteins. The SLIP element (TTTTTT) is involved in the frameshift in the Gag-Pol reading frame required to make functional Pol.[16]

T4 Bacteriophage

Enterobacteria phage T4 is a bacteriophage that infects *Escherichia coli* bacteria. The T4 phage is a member of the T-even phages, a group including enterobacteriophages T2 and T6. T4 is capable of undergoing only a lytic lifecycle and not the lysogenic lifecycle.

Genome and structure



The T4 phage's double-stranded DNA genome is about 169 kbp long[1] and encodes 289 proteins. The T4 genome is terminally redundant and is first replicated as a unit, then several genomic units are recombined end-to-end to form a concatemer. When packaged, the concatemer is cut at unspecific positions of the same length, leading to several genomes that represent circular permutations of the original.[2] The T4 genome bears eukaryote-like intron sequences.

Translation

The Shine-Dalgarno sequence GAGG dominates in bacteriophage T4 early genes, whereas the sequence GGAG is a target for the T4 endonuclease RegB that initiates the early mRNA degradation.[3]

Virus particle structure

T4 is a relatively large phage, at approximately 90 nm wide and 200 nm long (most phages range from 25 to 200 nm in length). The DNA genome is held in an icosahedral head, also known as a capsid. The T4's tail is hollow so that it can pass its nucleic acid into the cell it is infecting after attachment. The tail attaches to a host cell with the help of tail fibres. The tail fibres are also important in recognizing host cell surface receptors, so they determine if a bacterium is within the phage's host range.[*citation needed*]

Infection process

The T4 phage initiates an *E. coli* infection by binding OmpC porin proteins and Lipopolysaccharide (LPS) on the surface of *E. coli* cells with its long tail fibers (LTF).[4][5] A recognition signal is sent through the LTFs to the baseplate. This unravels the short tail fibers (STF) that bind irreversibly to the *E. coli* cell surface. The baseplate changes conformation and the tail sheath contracts, causing GP5 at the end of the tail tube to puncture the outer membrane of the cell. The lysozyme domain of GP5 is activated and degrades the periplasmic peptidoglycan layer. The remaining part of the membrane is degraded and then DNA from the head of the phage can travel through the tail tube and enter the *E. coli* cell.

Life cycle

The lytic lifecycle (from entering a bacterium to its destruction) takes approximately 30 minutes (at 37 °C) and consists of

- Adsorption and penetration (starting immediately)
- Arrest of host gene expression (starting immediately)
- Enzyme synthesis (starting after 5 minutes)
- DNA replication (starting after 10 minutes)
- Formation of new virus particles (starting after 12 minutes)

After the life cycle is complete, the host cell bursts open and ejects the newly built viruses into the environment, destroying the host cell. T4 has a burst size of approximately 100-150 viral particles per infected host. Complementation, deletion, and recombination tests can be used to map out the rII gene locus by using T4.

These bacteriophage infect a host cell with their information and then blow up the host cell, thereby propagating themselves.

he **lytic cycle** is one of the two cycles of viral reproduction, the other being the lysogenic cycle. The lytic cycle results in the destruction of the infected cell and its membrane. A key difference between the lytic and lysogenic phage cycles is that in the lytic phage, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterial DNA. The location of viral DNA in the lysogenic phage cycle is within the host DNA, therefore in both cases the virus/phage replicates using the host DNA machinery, but in the lytic phage cycle, the phage is a free floating separate molecule to the host DNA.

Description

Viruses that only use lytic cycle are called virulent viruses (in contrast to temperate viruses). The lytic cycle is a six-stage cycle. In the first stage, called "penetration," the virus injects its own nucleic acid into a host cell. In some viruses this genetic material is circular and mimics a bacterial plasmid. The virus hijacks the cell's replication and translation mechanisms, using them to make more viruses. Once enough virions have accumulated, specialized viral proteins are allowed to dissolve the bacterial cell wall. The cell bursts due to high internal osmotic pressure

(water pressure) that can no longer be constrained by the cell wall. This releases progeny virions into the surrounding environment, where they can go on to infect other cells.

Penetration

To infect a cell, a virus must first enter the cell through the plasma membrane and (if present) the cell wall. Viruses do so by either attaching to a receptor on the cell's surface or by simple mechanical force. The virus then releases its genetic material (either single- or double-stranded RNA or DNA) into the cell. In doing this, the cell becomes infected and can also be targeted by the immune system.

Biosynthesis

The virus' nucleic acid uses the host cell's metabolic machinery to make large amounts of viral components. In the case of DNA viruses, the DNA transcribes itself into messenger RNA (mRNA) molecules that are then used to direct the cell's ribosomes. One of the first polypeptides to be translated destroys the host's DNA. In retroviruses (which inject an RNA strand), a unique enzyme called reverse transcriptase transcribes the viral RNA into DNA, which is then transcribed again into RNA. Once the viral DNA has taken control it induces the host cell's machinery to synthesize viral DNA, protein and starts multiplying. About 25 minutes after initial infection, approximately 200 new bacteriophages are formed and the bacterial cell bursts i.e. it has undergone lysis. Newly formed phages are released to infect other bacteria and another lytic cycle begins. The phage which causes lysis of the host is called a lytic or virulent phage.[1] The biosynthesis is (e.g. T4) regulated in three phases of mRNA production followed by a phase of protein production.[2]

Early phase

> Enzymes modify the host's DNA replication by RNA polymerase. Amongst other modifications, virus T4 changes the sigma factor of the host by producing an anti-sigma factor so that the host promotors are not recognized any more but now recognize T4 middle proteins. For protein synthesis Shine-Dalgarno subsequence GAGG dominates an early genes translation.[3]

Middle phase

> Virus nucleic acid (DNA or RNA depending on virus type).

Late phase

> Structural proteins including those for the head and the tail.

Gene regulation biochemistry

There are three classes of genes in the phage genome that regulate whether the lytic or lysogenic cycles will emerge. The first are the immediate early genes, the second is the delayed early genes and the third is the late genes.

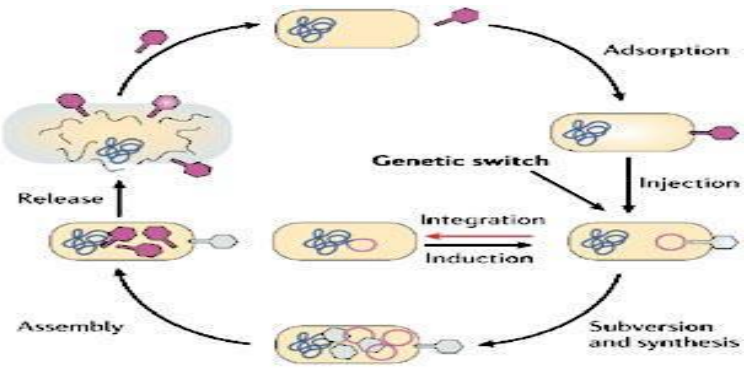
1. Immediate early genes: These genes code for two transcription factors: *N* and *cro*. *N* is an anti-termination factor that is needed for the transcription of the delayed early genes. *cro* has two functions. The first function is to repress the activity of the repressor that is needed to go into lysogeny. Note that a repressor coded by the *CI* gene is needed to repress the lytic cycle from taking place. The second function of cro is to initiate the transcription of the late genes needed for the lytic cycle to go to completion.
2. Delayed early genes: The immediate early gene N is required to express the delayed early genes. In lytic cells, the delayed early gene which is most important is *Q*. These genes are also used to express late genes.
3. The repressor: The repressor is needed to repress the lytic cycle for lysogeny to proceed. It has 2 *N* domains that bind the DNA via a helix turn helix motif and 2 C domains that dimerize to stabilize the protein.
4. Lysis inhibition: T4-like phages contain a gene called rI which can delay completed phage progeny from exiting an impregnated cell by suppressing the expression of holin gene products usually up to four hours in exponential phase growing cultures in rich media. Deletion of rI cancels the inhibition effect. This is only observed when higher concentrations of extracellular T4 phage particles are present.

Maturation and lysis

After many copies of viral components are made, they are assembled into complete viruses. The phage then directs production of lysin, an enzyme that breaks down the bacterial cell wall, which allows extracellular fluid to enter the cell. The cell eventually becomes filled with viruses (typically 100-200) and liquid, and bursts, or lyses; thus giving the lytic cycle its name. The new viruses are then free to infect other cells.

Lytic cycle without lysis

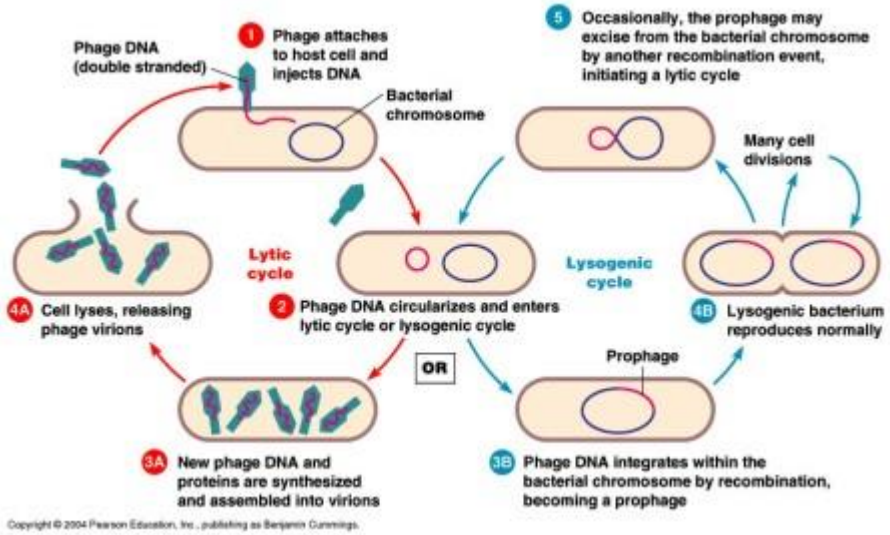
Some viruses escape the host cell without bursting the cell membrane, but rather bud/extrude off from it by taking a portion of the membrane with them. Because it otherwise is characteristic of the lytic cycle in other steps, it still belongs to this category, although it is sometimes named the Productive Cycle. HIV, influenza and other viruses that infect eukaryotic organisms generally use this method. These group includes all viruses that have a lipid membrane.



Lytic cycle

Lysogenic cycle

Lysogenic cycle



Lysogeny, or the **lysogenic cycle**, is one of two cycles of viral reproduction (the lytic cycle is the other). Lysogeny is characterized by integration of the bacteriophage nucleic acid into the host bacterium's genome or formations of a circular replicon in the bacterium's cytoplasm. In this condition the bacterium continues to live and reproduce normally. The genetic material of the bacteriophage, called a prophage, can be transmitted to daughter cells at each subsequent cell division, and a later event (such as UV radiation or the presence of certain chemicals) can release it, causing proliferation of new phages via the lytic cycle. [1] Lysogenic cycles can also occur in eukaryotes, although the method of DNA incorporation is not fully understood.

The distinction between lysogenic and lytic cycles is that the spread of the viral DNA occurs through the usual prokaryotic reproduction, while the lytic phage is spread through the production of thousands of individual phages capable of surviving and infecting other cells. The

key difference between the lytic cycle and the lysogenic cycle is that the lysogenic cycle does not lyse the host cell.[2] Phages that replicate only via the lytic cycle are known as virulent phages while phages that replicate using both lytic and lysogenic cycles are known as temperate phages.[1]

In the lysogenic cycle, the phage DNA first integrates into the bacterial chromosome to produce the prophage. When the bacterium reproduces, the prophage is also copied and is present in each of the daughter cells. The daughter cells can continue to replicate with the prophage present or the prophage can exit the bacterial chromosome to initiate the lytic cycle.[1]

Bacteriophages

Bacteriophages are viruses that infect and replicate within a bacteria. Temperate phages (such as lambda phage) can reproduce using both the lytic and the lysogenic cycle. Via the lysogenic cycle, the bacteriophage's genome is not expressed and is instead integrated into the bacteria's genome to form the prophage.[3] Since the bacteriophage's genetic information is incorporated into the bacteria's genetic information as a prophage, the bacteriophage replicates passively as the bacterium divides to form daughter bacteria cells.[3] In this scenario, the daughter bacteria cells contain prophage and are known as lysogens. Lysogens can remain in the lysogenic cycle for many generations but can switch to the lytic cycle at any time via a process known as induction.[3] During induction, prophage DNA is excised from the bacterial genome and is transcribed and translated to make coat proteins for the virus and regulate lytic growth.[3]

The model organism for studying lysogeny is lambda phage. Prophage integration, maintenance of lysogeny, induction, and control of phage genome excision in induction is described in detail in the lambda phage article.

Fitness tradeoffs for bacteria

Bacteriophages are parasitic because they infect their hosts, use bacterial machinery to replicate, and ultimately lyse the bacteria. Temperate phages can lead to both advantages and disadvantages for their hosts via the lysogenic cycle. During the lysogenic cycle, the virus genome is incorporated as prophage and a repressor prevents viral replication. Nonetheless, a temperate phage can escape repression to replicate, produce viral particles, and lyse the bacteria.[4] The temperate phage escaping repression would be a disadvantage for the bacteria. On the other hand, the prophage may transfer genes that enhance host virulence and resistance to the immune system. Also, the repressor produced by the prophage that prevents prophage genes

from being expressed confers an immunity for the host bacteria from lytic infection by related viruses.[4]

Lysogenic conversion

In some interactions between lysogenic phages and bacteria, lysogenic conversion may occur. It is when a temperate phage induces a change in the phenotype of the infected bacteria that is not part of a usual phage cycle. Changes can often involve the external membrane of the cell by making it impervious to other phages or even by increasing the pathogenic capability of the bacteria for a host. In this way, temperate bacteriophages also play a role in the spread of virulence factors, such as exotoxins and exoenzymes, amongst bacteria.

Bacterial survival

Lysogenic conversion has shown to enable biofilm formation in *Bacillus anthracis*[5] Strains of *B. anthracis* cured of all phage were unable to form biofilms, which are surface-adhered bacterial communities that enable bacteria to better access nutrients and survive environmental stresses.[6] In addition to biofilm formation in *B. anthracis*, lysogenic conversion of *Bacillus subtilis*, *Bacillus thuringiensis*, and *Bacillus cereus* has shown an enhanced rate or extent of sporulation.[5] Sporulation produces endospores, which are metabolically dormant forms of the bacteria that are highly resistant to temperature, ionizing radiation, desiccation, antibiotics, and disinfectants.[5]

Bacterial virulence

Non-virulent bacteria have also been shown to transform into highly virulent pathogens through lysogenic conversion with the virulence factors carried on the lysogenic prophage.[7] Virulence genes carried within prophages as discrete autonomous genetic elements, known as morons, confer an advantage to the bacteria that indirectly benefits the virus through enhanced lysogen survival.[5]

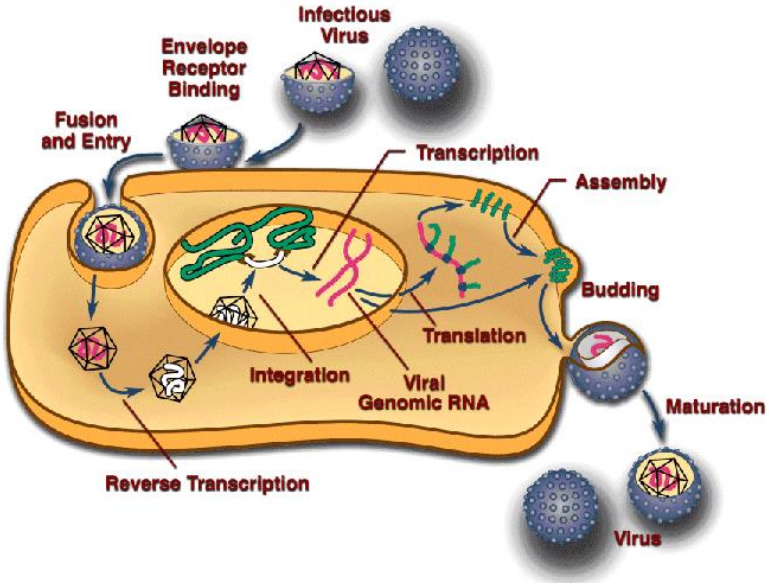
Examples:

- *Corynebacterium diphtheriae* produces the toxin of diphtheria only when it is infected by the phage β. In this case, the gene that codes for the toxin is carried by the phage, not the bacteria.[8]
- *Vibrio cholerae* is a non-toxic strain that can become toxic, producing cholera toxin, when it is infected with the phage CTXφ.
- *Shigella dysenteriae*, which produces dysentery has toxins that fall into two major groups, Stx1 and Stx2, whose genes are considered to be part of the genome of lambdoid prophages.
- *Streptococcus pyogenes*, produce a pyrogenic exotoxin, obtained by lysogenic conversion, which causes fever and a scarlet-red rash, Scarlet Fever.
- Certain strains of *Clostridium botulinum*, which causes botulism, express botulinum toxin from phage-tranduced genes

Genome

Genomic diversity among viruses	
Property	**Parameters**
Nucleic acid	• DNA • RNA • Both DNA and RNA (at different stages in the life cycle)
Shape	• Linear • Circular • Segmented
Strandedness	• Single-stranded • Double-stranded • Double-stranded with regions of single-strandedness
Sense	• Positive sense (+) • Negative sense (−) • Ambisense (+/−)

Replication cycle – Animal virus



Viral populations do not grow through cell division, because they are acellular. Instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they *assemble* in the cell.

The life cycle of viruses differs greatly between species but there are six *basic* stages in the life cycle of viruses:[102]

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV infects a limited range of human leucocytes. This is because its surface protein, gp120, specifically interacts with the CD4 molecule – a chemokine receptor – which is most commonly found on the surface of CD4+ T-Cells. This mechanism has evolved to favour those viruses that infect only cells in which they are capable of replication. Attachment to the receptor can induce the viral envelope protein to undergo changes that results in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

Penetration follows attachment: Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called viral entry. The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall.[103] However, nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called plasmodesmata.[104] Bacteria, like plants, have strong cell walls that a virus must breach to infect

the cell. However, given that bacterial cell walls are much less thick than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.[105]

Uncoating is a process in which the viral capsid is removed: This may be by degradation by viral enzymes or host enzymes or by simple dissociation; the end-result is the releasing of the viral genomic nucleic acid.

Replication of viruses involves primarily multiplication of the genome. Replication involves synthesis of viral messenger RNA (mRNA) from "early" genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: "late" gene expression is, in general, of structural or virion proteins.

Assembly – Following the structure-mediated self-*assembly* of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes called maturation) occurs *after* the virus has been released from the host cell.[106]

Release – Viruses can be *released* from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present: This is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a "provirus" or, in the case of bacteriophages a "prophage".[107] Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host. However, at some point, the provirus or prophage may give rise to active virus, which may lyse the host cells.[108] Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other, internal membrane.[109]

The genetic material within virus particles, and the method by which the material is replicated, varies considerably between different types of viruses.

DNA viruses

> The genome replication of most DNA viruses takes place in the cell's nucleus. If the cell has the appropriate receptor on its surface, these viruses enter the cell sometimes by direct fusion with the cell membrane (e.g., herpesviruses) or – more usually – by receptor-mediated endocytosis. Most DNA viruses are entirely dependent on the host cell's DNA and RNA synthesising machinery, and RNA processing machinery. However, viruses with larger genomes may encode much of this machinery themselves. In eukaryotes the viral genome must cross the cell's nuclear membrane to access this machinery, while in bacteria it need only enter the cell.[110]

RNA viruses

Replication usually takes place in the cytoplasm. RNA viruses can be placed into four different groups depending on their modes of replication. The polarity (whether or not it can be used directly by ribosomes to make proteins) of single-stranded RNA viruses largely determines the replicative mechanism; the other major criterion is whether the genetic material is single-stranded or double-stranded. All RNA viruses use their own RNA replicase enzymes to create copies of their genomes.[111]

Reverse transcribing viruses

These have ssRNA (*Retroviridae*, *Metaviridae*, *Pseudoviridae*) or dsDNA (*Caulimoviridae*, and *Hepadnaviridae*) in their particles. Reverse transcribing viruses with RNA genomes (retroviruses), use a DNA intermediate to replicate, whereas those with DNA genomes (pararetroviruses) use an RNA intermediate during genome replication. Both types use a reverse transcriptase, or RNA-dependent DNA polymerase enzyme, to carry out the nucleic acid conversion. Retroviruses integrate the DNA produced by reverse transcription into the host genome as a provirus as a part of the replication process; pararetroviruses do not, although integrated genome copies of especially plant pararetroviruses can give rise to infectious virus.[112] They are susceptible to antiviral drugs that inhibit the reverse transcriptase enzyme, e.g. zidovudine and lamivudine. An example of the first type is HIV, which is a retrovirus. Examples of the second type are the *Hepadnaviridae*, which includes Hepatitis B virus.[113]

Effects on the host cell

The range of structural and biochemical effects that viruses have on the host cell is extensive.[114] These are called *cytopathic effects*.[115] Most virus infections eventually result in the death of the host cell. The causes of death include cell lysis, alterations to the cell's surface membrane and apoptosis.[116] Often cell death is caused by cessation of its normal activities because of suppression by virus-specific proteins, not all of which are components of the virus particle.[117]

Host range

Viruses are by far the most abundant biological entities on Earth and they outnumber all the others put together.[123] They infect all types of cellular life including animals, plants, bacteria and fungi.[3] However, different types of viruses can infect only a limited range of hosts and many are species-specific. Some, such as smallpox virus for example, can infect only one species – in this case humans,[124] and are said to have a narrow host range. Other viruses, such as rabies virus, can infect different species of mammals and are said to have a broad range.[125] The viruses that infect plants are harmless to animals, and most viruses that infect other animals are harmless to humans.[126] The host range of some bacteriophages is limited to a single strain of bacteria and they can be used to trace the source of outbreaks of infections by a method called phage typing.[127]

Fungus

A **fungus** (/ˈfʌŋɡəs/; plural: **fungi**[3] or **funguses**[4]) is any member of the group of eukaryotic organisms that includes unicellular microorganisms such as yeasts and molds, as well as multicellular fungi that produce familiar fruiting forms known as mushrooms. These organisms are classified as a kingdom, **Fungi**, which is separate from the other life kingdoms of plants, animals, protists, and bacteria.

One difference that places fungi in a different kingdom is that its cell walls contain chitin, unlike the cell walls of plants, bacteria and some protists. Similar to animals, fungi are heterotrophs, that is, they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Growth is their means of mobility, except for spores, which may travel through the air or water (a few of which are flagellated). Fungi are the principal decomposers in ecological systems.

Etymology

The use of the word *mycology*, which is derived from the Greek *mykes* (μύκης "mushroom") and *logos* (λόγος "discourse"),[8] to denote the scientific study of fungi is thought to have originated in 1836 with English naturalist Miles Joseph Berkeley's publication *The English Flora of Sir James Edward Smith, Vol. 5*.[6] A group of all the fungi present in a particular area or geographic region is known as mycobiota (plural noun, no singular), e.g., "the mycobiota of Ireland".[9]

Characteristics

Fungal hyphae cells

1- Hyphal wall 2- Septum 3- Mitochondrion 4- Vacuole 5- Ergosterol crystal 6- Ribosome 7- Nucleus 8- Endoplasmic reticulum 9- Lipid body 10- Plasma membrane 11- Spitzenkörper 12- Golgi apparatus

Shared features:

- With other eukaryotes: Fungal cells contain membrane-bound nuclei with chromosomes that contain DNA with noncoding regions called introns and coding regions called exons. Fungi have membrane-bound cytoplasmic organelles such as mitochondria, sterol-containing membranes, and ribosomes of the 80S type.[12] They have a characteristic range of soluble carbohydrates and storage compounds, including sugar alcohols (e.g., mannitol), disaccharides, (e.g., trehalose), and polysaccharides (e.g., glycogen, which is also found in animals[13]).
- With animals: Fungi lack chloroplasts and are heterotrophic organisms and so require preformed organic compounds as energy sources.[14]
- With plants: Fungi have a cell wall[15] and vacuoles.[16] They reproduce by both sexual and asexual means, and like basal plant groups (such as ferns and mosses) produce spores. Similar to mosses and algae, fungi typically have haploid nuclei.[17]

- With euglenoids and bacteria: Higher fungi, euglenoids, and some bacteria produce the amino acid L-lysine in specific biosynthesis steps, called the α-aminoadipate pathway.[18][19]
- The cells of most fungi grow as tubular, elongated, and thread-like (filamentous) structures called hyphae, which may contain multiple nuclei and extend by growing at their tips. Each tip contains a set of aggregated vesicles—cellular structures consisting of proteins, lipids, and other organic molecules—called the Spitzenkörper.[20] Both fungi and oomycetes grow as filamentous hyphal cells.[21] In contrast, similar-looking organisms, such as filamentous green algae, grow by repeated cell division within a chain of cells.[13]
- In common with some plant and animal species, more than 70 fungal species display bioluminescence.[22]

Unique features:

- Some species grow as unicellular yeasts that reproduce by budding or binary fission. Dimorphic fungi can switch between a yeast phase and a hyphal phase in response to environmental conditions.[23]
- The fungal cell wall is composed of glucans and chitin; while glucans are also found in plants and chitin in the exoskeleton of arthropods,[24][25] fungi are the only organisms that combine these two structural molecules in their cell wall. Unlike those of plants and oomycetes, fungal cell walls do not contain cellulose.[26]

Diversity

Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments such as deserts or areas with high salt concentrations[31] or ionizing radiation,[32] as well as in deep sea sediments.[33] Some can survive the intense UV and cosmic radiation encountered during space travel.[34]

Mycology

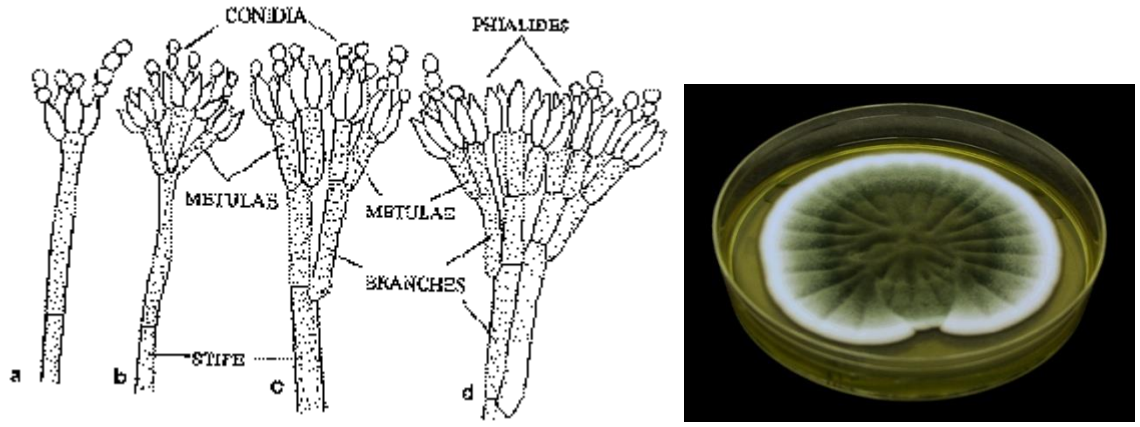
Mycology is the branch of biology concerned with the systematic study of fungi, including their genetic and biochemical properties, their taxonomy, and their use to humans as a source of medicine, food, and psychotropic substances consumed for religious purposes, as well as their dangers, such as poisoning or infection. The field of phytopathology, the study of plant diseases, is closely related because many plant pathogens are fungi.[43]

The use of fungi by humans dates back to prehistory; Ötzi the Iceman, a well-preserved mummy of a 5,300-year-old Neolithic man found frozen in the Austrian Alps, carried two species of

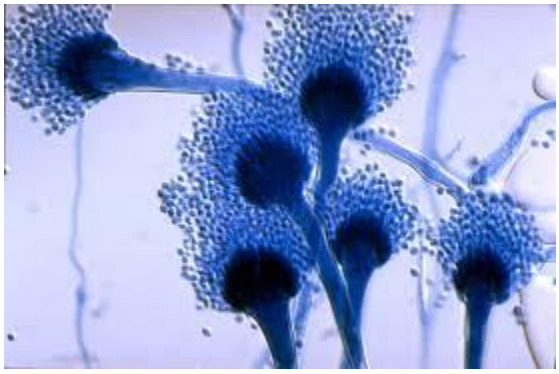
polypore mushrooms that may have been used as tinder (*Fomes fomentarius*), or for medicinal purposes (*Piptoporus betulinus*).[44] Ancient peoples have used fungi as food sources–often unknowingly–for millennia, in the preparation of leavened bread and fermented juices. Some of the oldest written records contain references to the destruction of crops that were probably caused by pathogenic fungi.[45]

Morphology

Microscopic structures



Penicillium
1. hypha 2. conidiophore 3. phialide 4. conidia 5. septa



Aspergillus niger

Most fungi grow as hyphae, which are cylindrical, thread-like structures 2–10 µm in diameter and up to several centimeters in length. Hyphae grow at their tips (apices); new hyphae are typically formed by emergence of new tips along existing hyphae by a process called *branching*, or occasionally growing hyphal tips fork, giving rise to two parallel-growing hyphae.[49] The combination of apical growth and branching/forking leads to the development of a mycelium, an interconnected network of hyphae.[23] Hyphae can be either septate or coenocytic. Septate hyphae are divided into compartments separated by cross walls (internal cell walls, called septa, that are formed at right angles to the cell wall giving the hypha its shape), with each compartment containing one or more nuclei; coenocytic hyphae are not compartmentalized.[50] Septa have

pores that allow cytoplasm, organelles, and sometimes nuclei to pass through; an example is the dolipore septum in fungi of the phylum Basidiomycota.[51] Coenocytic hyphae are in essence multinucleate supercells.[52]

Many species have developed specialized hyphal structures for nutrient uptake from living hosts; examples include haustoria in plant-parasitic species of most fungal phyla, and arbuscules of several mycorrhizal fungi, which penetrate into the host cells to consume nutrients.[53]

Although fungi are opisthokonts—a grouping of evolutionarily related organisms broadly characterized by a single posterior flagellum—all phyla except for the chytrids have lost their posterior flagella.[54] Fungi are unusual among the eukaryotes in having a cell wall that, in addition to glucans (e.g., β-1,3-glucan) and other typical components, also contains the biopolymer chitin.[55]

Macroscopic structures

Fungal mycelia can become visible to the naked eye, for example, on various surfaces and substrates, such as damp walls and spoiled food, where they are commonly called molds. Mycelia grown on solid agar media in laboratory petri dishes are usually referred to as colonies. These colonies can exhibit growth shapes and colors (due to spores or pigmentation) that can be used as diagnostic features in the identification of species or groups.[56] Some individual fungal colonies can reach extraordinary dimensions and ages as in the case of a clonal colony of *Armillaria solidipes*, which extends over an area of more than 900 ha (3.5 square miles), with an estimated age of nearly 9,000 years.[57]

The apothecium—a specialized structure important in sexual reproduction in the ascomycetes—is a cup-shaped fruit body that holds the hymenium, a layer of tissue containing the spore-bearing cells.[58] The fruit bodies of the basidiomycetes (basidiocarps) and some ascomycetes can sometimes grow very large, and many are well known as mushrooms.

Growth and physiology

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

The growth of fungi as hyphae on or in solid substrates or as single cells in aquatic environments is adapted for the efficient extraction of nutrients, because these growth forms have high surface area to volume ratios.[59] Hyphae are specifically adapted for growth on solid surfaces, and to invade substrates and tissues.[60] They can exert large penetrative mechanical forces; for example, the plant pathogen *Magnaporthe grisea* forms a structure called an appressorium that evolved to puncture plant tissues.[61] The pressure generated by the appressorium, directed against the plant epidermis, can exceed 8 megapascals (1,200 psi).[61] The filamentous fungus *Paecilomyces lilacinus* uses a similar structure to penetrate the eggs of nematodes.[62]

Reproduction

Fungal reproduction is complex, reflecting the differences in lifestyles and genetic makeup within this diverse kingdom of organisms.[75] It is estimated that a third of all fungi reproduce using more than one method of propagation; for example, reproduction may occur in two well-differentiated stages within the life cycle of a species, the teleomorph and the anamorph.[76] Environmental conditions trigger genetically determined developmental states that lead to the creation of specialized structures for sexual or asexual reproduction. These structures aid reproduction by efficiently dispersing spores or spore-containing propagules.

Asexual reproduction

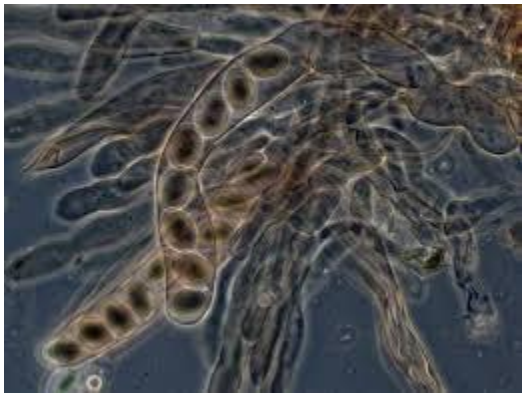
Asexual reproduction occurs via vegetative spores (conidia) or through mycelial fragmentation. Mycelial fragmentation occurs when a fungal mycelium separates into pieces, and each component grows into a separate mycelium. Mycelial fragmentation and vegatative spores maintain clonal populations adapted to a specific niche, and allow more rapid dispersal than sexual reproduction.[77] The "Fungi imperfecti" (fungi lacking the perfect or sexual stage) or Deuteromycota comprise all the species that lack an observable sexual cycle.[78]

Sexual reproduction

Sexual reproduction with meiosis exists in all fungal phyla except Glomeromycota.[79] It differs in many aspects from sexual reproduction in animals or plants. Differences also exist between fungal groups and can be used to discriminate species by morphological differences in sexual structures and reproductive strategies.[80][81] Mating experiments between fungal isolates may identify species on the basis of biological species concepts.[81] The major fungal groupings have initially been delineated based on the morphology of their sexual structures and spores; for example, the spore-containing structures, asci and basidia, can be used in the identification of ascomycetes and basidiomycetes, respectively. Some species may allow mating only between individuals of opposite mating type, whereas others can mate and sexually reproduce with any other individual or itself. Species of the former mating system are called heterothallic, and of the latter homothallic.[82]

Most fungi have both a haploid and a diploid stage in their life cycles. In sexually reproducing fungi, compatible individuals may combine by fusing their hyphae together into an interconnected network; this process, anastomosis, is required for the initiation of the sexual cycle. Ascomycetes and basidiomycetes go through a dikaryotic stage, in which the nuclei

inherited from the two parents do not combine immediately after cell fusion, but remain separate in the hyphal cells (see heterokaryosis).[83]



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

In ascomycetes, dikaryotic hyphae of the hymenium (the spore-bearing tissue layer) form a characteristic *hook* at the hyphal septum. During cell division, formation of the hook ensures proper distribution of the newly divided nuclei into the apical and basal hyphal compartments. An ascus (plural *asci*) is then formed, in which karyogamy (nuclear fusion) occurs. Asci are embedded in an ascocarp, or fruiting body. Karyogamy in the asci is followed immediately by meiosis and the production of ascospores. After dispersal, the ascospores may germinate and form a new haploid mycelium.[84]

Sexual reproduction in basidiomycetes is similar to that of the ascomycetes. Compatible haploid hyphae fuse to produce a dikaryotic mycelium. However, the dikaryotic phase is more extensive in the basidiomycetes, often also present in the vegetatively growing mycelium. A specialized anatomical structure, called a clamp connection, is formed at each hyphal septum. As with the structurally similar hook in the ascomycetes, the clamp connection in the basidiomycetes is required for controlled transfer of nuclei during cell division, to maintain the dikaryotic stage with two genetically different nuclei in each hyphal compartment.[85] A basidiocarp is formed in which club-like structures known as basidia generate haploid basidiospores after karyogamy and meiosis.[86] The most commonly known basidiocarps are mushrooms, but they may also take other forms (see Morphology section).

In glomeromycetes (formerly zygomycetes), haploid hyphae of two individuals fuse, forming a gametangium, a specialized cell structure that becomes a fertile gamete-producing cell. The gametangium develops into a zygospore, a thick-walled spore formed by the union of gametes. When the zygospore germinates, it undergoes meiosis, generating new haploid hyphae, which may then form asexual sporangiospores. These sporangiospores allow the fungus to rapidly disperse and germinate into new genetically identical haploid fungal mycelia.[87]

Spore dispersal

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

Specialized mechanical and physiological mechanisms, as well as spore surface structures (such as hydrophobins), enable efficient spore ejection.[88] For example, the structure of the spore-bearing cells in some ascomycete species is such that the buildup of substances affecting cell volume and fluid balance enables the explosive discharge of spores into the air.[89] The forcible discharge of single spores termed *ballistospores* involves formation of a small drop of water (Buller's drop), which upon contact with the spore leads to its projectile release with an initial acceleration of more than 10,000 g;[90] the net result is that the spore is ejected 0.01–0.02 cm, sufficient distance for it to fall through the gills or pores into the air below.[91] Other fungi, like the puffballs, rely on alternative mechanisms for spore release, such as external mechanical forces. The bird's nest fungi use the force of falling water drops to liberate the spores from cup-shaped fruiting bodies.[92] Another strategy is seen in the stinkhorns, a group of fungi with lively colors and putrid odor that attract insects to disperse their spores.[93]

Other sexual processes

Besides regular sexual reproduction with meiosis, certain fungi, such as those in the genera *Penicillium* and *Aspergillus*, may exchange genetic material via parasexual processes, initiated by anastomosis between hyphae and plasmogamy of fungal cells.[94] The frequency and relative importance of parasexual events is unclear and may be lower than other sexual processes. It is known to play a role in intraspecific hybridization[95] and is likely required for hybridization between species, which has been associated with major events in fungal evolution.[96]

Yeast

Yeasts are eukaryotic microorganisms classified as members of the fungus kingdom with 1,500 species currently identified[1] and are estimated to constitute 1% of all described fungal species.[2] Yeasts are unicellular, although some species may also develop multicellular characteristics by forming strings of connected budding cells known as pseudohyphae or false hyphae.[3] Yeast sizes vary greatly, depending on species and environment, typically measuring 3–4 µm in diameter, although some yeasts can grow to 40 µm in size.[4] Most yeasts reproduce asexually by mitosis, and many do so by the asymmetric division process known as budding.

By fermentation, the yeast species *Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohols – for thousands of years the carbon dioxide has been used in baking and the alcohol in alcoholic beverages.[5] It is also a centrally important 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.[6] Other species of yeasts, 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,[7] 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*,[8] 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.[9]

Nutrition and growth

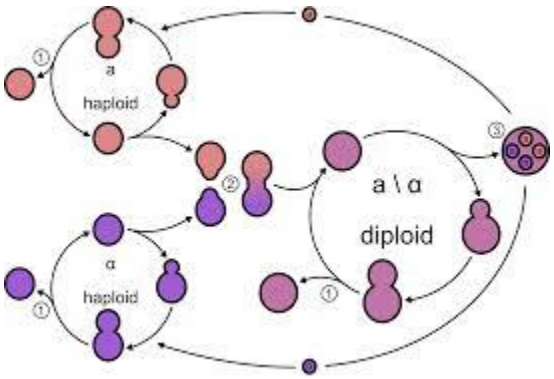
Yeasts are chemoorganotrophs, as they use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from hexose sugars, such as glucose and fructose, or disaccharides such as sucrose and maltose. Some species can metabolize pentose sugars such as ribose,[17] 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, no known yeast species 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).[18] The cells can survive freezing under certain conditions, with viability decreasing over time.

In general, yeasts are grown in the laboratory on solid growth media or in liquid broths. Common media used for the cultivation of yeasts include potato dextrose agar or potato dextrose broth, Wallerstein Laboratories nutrient agar, yeast peptone dextrose agar, and yeast mould agar or broth. Home brewers who cultivate yeast frequently use dried malt extract 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 flavor and must be removed regularly during fermentation.[19]

Reproduction



The yeast cell's life cycle:
1. Budding

2. Conjugation
3. Spore
See also: Mating of yeast

Yeasts, like all fungi, may have asexual and sexual reproductive cycles. The most common mode of vegetative growth in yeast is asexual reproduction by budding.[31] Here, a small bud (also known as a bleb), 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.[32] The daughter cell produced during the budding process is generally smaller than the mother cell. Some yeasts, including *Schizosaccharomyces pombe*, reproduce by fission instead of budding,[31] thereby creating two identically sized daughter cells.

In general, under high-stress conditions such as nutrient starvation, haploid cells will 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.[33]

The haploid fission yeast *Schizosaccharomyces pombe* is a facultative sexual microorganism that can undergo mating when nutrients are limiting.[34] Exposure of *S. pombe* to hydrogen peroxide, an agent that causes oxidative stress leading to oxidative DNA damage, strongly induces mating and the formation of meiotic spores.[35] The budding yeast *Saccharomyces cerevisiae* reproduces by mitosis as diploid cells when nutrients are abundant, but when starved, this yeast undergoes meiosis to form haploid spores.[36] Haploid cells may then reproduce asexually by mitosis. Katz Ezov et al.[37] presented evidence that in natural *S. cerevisiae* populations clonal reproduction and selfing (in the form of intratetrad mating) predominate. In nature, mating of haploid cells to form diploid cells is most often between members of the same clonal population and out-crossing is uncommon.[38] Analysis of the ancestry of natural *S. cerevisiae* strains led to the conclusion that out-crossing occurs only about once every 50,000 cell divisions.[38] These observations suggest that the possible long term benefits of outcrossing (e.g. generation of diversity) are likely to be insufficient for generally maintaining sex from one generation to the next.[39] Rather, a short term benefit, such as recombinational repair during meiosis,[40][41] may be the key to the maintenance of sex in *S. cerevisiae*.

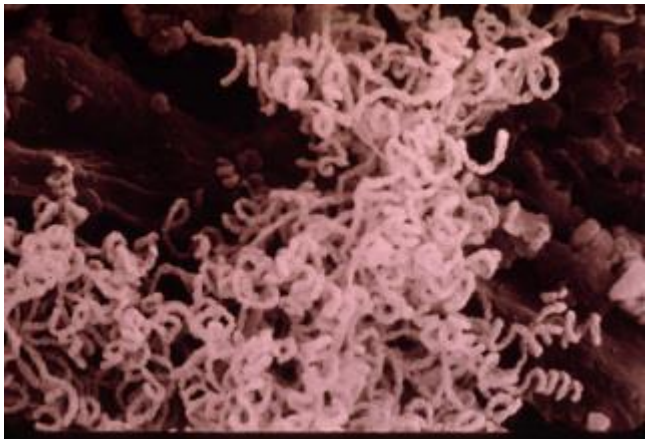
Some pucciniomycete yeasts, in particular species of *Sporidiobolus* and *Sporobolomyces*, produce aerially dispersed, asexual ballistoconidia.[42]

Actinomycetes

These are the organisms with characteristics common to both bacteria and fungi but yet possessing distinctive features to delimit them into a distinct category. In the strict taxonomic sense, actinomycetes are clubbed with bacteria the same class of Schizomycetes and confined to the order Actinomycetales. They are unicellular like bacteria, but produce a mycelium which is non-septate (coenocytic) and more slender, tike true bacteria they do not have distinct cell-wall and their cell wall is without chitin and cellulose (commonly found in the cell wall of fungi). On culture media unlike slimy distinct colonies of true bacteria which grow quickly, actinomycetes colonies grow slowly, show powdery consistency and stick firmly to agar surface. They produce hyphae and conidia / sporangia like fungi. Certain actinomycetes whose hyphae undergo segmentation resemble bacteria, both morphologically and physiologically.

Actinomycetes are numerous and widely distributed in soil and are next to bacteria in abundance. They are widely distributed in the soil, compost etc. Plate count estimates give values ranging from 10^4 to 10^8 per gram of soil. They are sensitive to acidity / low PH (optimum PH range 6.5 to 8.0) and waterlogged soil conditions. The population of actinomycetes increases with depth of soil even up to horizon 'C' of a soil profiler They are heterotrophic, aerobic and mesophilic (25-30 ^c) organisms and some species are commonly present in compost and manures are thermophilic growing at 55-65° c temperature (eg. Thermoatinomycetes, Streptomyces).

Actinomycetes belonging to the order of Actinomycetales are grouped under four families viz Mycobacteriaceae, Actinomycetaceae, Streptomycetaceae and Actinoplanaceae. Actinomycetous genera which are agriculturally and industrially important are present in only two families of Actinomycetaceae and Strepotmycetaceae. In the order of abundance in soils, the common genera of actinomycetes are Streptomyces (nearly 70%), Nocardia and Micromonospora although Actinomycetes, Actinoplanes, Micromonospora and Streptosporangium are also generally encountered.

- 

Actinomycetes

The actinomycetes comprise a group of procaryotes that have the ability to form Gram-positive, branching filaments of less than 1μm in diameter. The main animal pathogens in the actinomycetes are the genera *Actinomyces*, *Nocardia* and *Dermatophilus*.

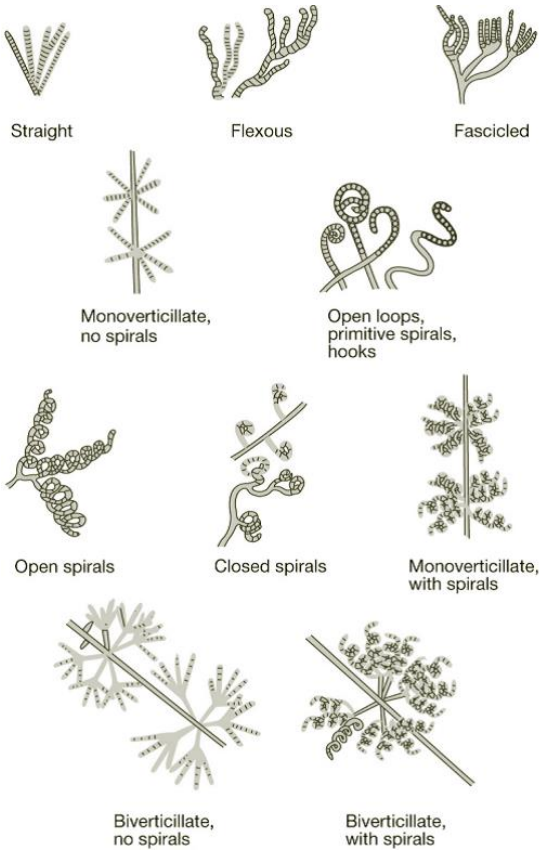
Streptomyces species are also included in this group, and they are prolific producers of antimicrobial substances.

Actinomyces species

Morphology and Staining

These are gram-positive diphtheroid or filamentous rods, about 0.5μm in width. Branching filaments are usually found in pathological specimens. In culture, diphtheroid forms predominate.

Spores



Structure and Composition

Actinomyces species have distinctive cell wall constituents. Surface fibrils in *A. viscosus* may be adhesins for host cells or other bacteria. Surface antigens are related to chemotactic and mitogenic activities.

Growth Characteristics

Animal *Actinomyces* species are capnophilic or facultative anaerobes. All require rich media, preferably containing serum or blood. No growth occurs on Sabouraud's agar. Development of macroscopic colonies may require several days at 37°C. Colonical morphology varies between and within species.

Biochemical Activities

Actinomyces are catalase-negative, with the exceptions of *A. viscosus* and *A. canis*.

Reservoir

Actinomyces species live in the nasopharyngeal and oral mucosa, and secondarily in the gastrointestinal tract.

Disease

Actinomyces species evoke pyogranulomatous reactions by unknown mechanisms. Bacterial colonies trigger the suppurative response, and there is also granulation, mononuclear infiltration and fibrosis.

Sinus tracts carry the exudate to the outside. This often contains 'sulfur granules' which are bacterial colonies surrounded by clubs.

Diseases caused by *Actinomyces* species include:

> Ruminants: *A. bovis* causes lumpy jaw.
> Horses: *Actinomyces* species have been isolated from cases of Poll Evil and Fistulous Withers as well as in cases of cervical lymphadenitis.
> Dogs and cats: *Actinomyces* are common causes of pyonecrotic processes, usually associated with a foreign body such as a migrating grass awn. Commonly isolated species include: *Actinomyces viscosus*, *Actinomyces hordeovulneris*, *A. canis*, A. catuli.
> Pigs: mastitis, pneumonia and abortion have all be associated with *Actinomyces* infections.

Nocardia species

Morphology and Staining

Gram-stained *Nocardia* species are indistinguishable from *Actinomyces* species. They are partially acid-fast so a Ziehl-Neelsen stain would differentiate them from *Actinomyces*. They alternate between the coccobacillary resting phase and the actively-growing filamentous forms.

Structure and Composition

The cell wall is typical of gram-positive bacteria. It contains a high concentration of lipids. A superoxide dismutase acts as a virulence factor.

Growth Characteristics

Nocardia species are obligate aerobes growing on simple media (Sabouraud's) over a wide temperature range. Colonies are opaque and variously pigmented. The colony surface, which is waxy to powdery to velvety depending on the abundance of growth, wrinkles with age. The diameter of colonies may reach several centimetres.

Biochemical activities include catalase production and acidification of various carbohydrates.

Reservoir

Pathogenic *Nocardia* species are saprophytic and found in many climates in soil and water, either as indigenous flora or as contaminants.

Disease

Nocardia species survive within phagocytic vacuoles and lead to a predominantly suppurative process. Lymph nodes are consistently involved. Exudates are serosanguinous and sometimes contain 'sulfur-granule-like' debris, which lack the microstructure of *Actinomyces* sulfur granules.

Diseases in different species include:

Ruminants: mastitis, bovine farcy, pneumonia, abortion and lymphadenitis
Horses: local or general infections due to underlying immunosuppression (EPPID), placentitis which causes abortion
Dogs and cats: pneumonia, suppurative pleuritis with empyema, dissemination to kidneys, CNS, bones, joints
Pigs: pneumonia, abortion, lymphadenitis

Dermatophilus species

Dermatophilus congolensis is the only species, and is a gram-positive filamentous bacterium.

Morphology and Composition

The reproductive unit of the bacterium is the motile coccoid zoospore. Upon germinating, the zoospores sprout a germ tube which elongates and thickens, dividing transversely and longitudinally, forming a strand several layers thick. Multiflagellated zoospores are liberated as the strand disintegrates.

Growth Characteristics

D. congolensis grows on blood media, but not on Sabouraud's agar. It is aerobic and capnophilic. Haemolytic colonies develop within 48 hours and vary from mucoid to viscous and waxy and whitish-grey to yellow. Catalase, urease and proteases are produced. The bacterium survives well in the soil and on fomites.

Reservoir

D. congolensis does not multiply saprophytically. Its reservoir is infected animals. Cattle, sheep, goats and horses are common hosts but it has been diagnosed in many other animals.

Disease

It causes an exudative dermatitis, with activity confined to the living epidermis. There is a scab consisting of layers of neutrophilic exudate and keratinising epidermis. Wetting favours expansion of the lesions.

It causes lesions on the back (rain scald in horses, lumpy wool in sheep), feet and legs (strawberry footrot in sheep and grease heel in horses).

Streptomyces species

These are not pathogenic but are similar to *Nocardia* species in cultures. Some species produce antibiotics, and they are common laboratory contaminants.