Microbiology

Unit 2 · Bacteria, Fungi, Algae and Protozoa

Bacteria, fungi, algae, protozoa and the parasitic worms studied with them: what each looks like, how it is built, how it reproduces and where it helps or harms us. After this unit you can draw and explain a bacterial cell and its wall, tell a Gram-positive from a Gram-negative by four tests, identify common moulds from a slide, classify algae and protozoa, trace a tapeworm life cycle, and explain how extremophiles survive.

Taught at 8 of 10 | Usually Sem 3 | 7 chapters

About this unit

This unit meets the main kinds of cellular microbe one by one. It starts with bacteria: their shapes and the parts of the cell (Chapter 2.1), then the cell wall that splits them into Gram-positive and Gram-negative groups (2.2), then two groups that break the usual pattern, the wall-less mycoplasmas and the filamentous actinomycetes (2.3). Next come the eukaryotic microbes: fungi (2.4), algae (2.5), and protozoa together with the parasitic worms diagnosed alongside them (2.6). The last chapter (2.7) returns to prokaryotes that live at the limits of heat, cold, salt and acid. Viruses have their own unit (Unit 3).

Chapters

1. Bacterial morphology and cell structure
2. Bacterial cell wall: Gram-positive and Gram-negative
3. Mycoplasma and actinomycetes
4. Fungi: morphology, reproduction and diseases
5. Algae: morphology, reproduction and industrial uses
6. Protozoa and helminths
7. Extremophiles and their adaptations ADVANCED

How to use this book

- Read the lead paragraph and the figure of each chapter first; they carry the one idea.
- Cover the solution of the worked example and do it yourself with a pencil, then compare step by step.
- Answer each "Check yourself" question in your head or on paper before you open it.

2.1 Bacterial morphology and cell structure

Bacterial morphology is the shape, size and arrangement of bacterial cells; cell structure is the set of parts inside and outside the cell membrane. A bacterium is a prokaryote: it has no nucleus and no membrane-bound organelles, yet it grows, divides and senses its world with a small kit of structures. Each structure has a job that an examiner will ask about (the capsule resists engulfment, the flagellum moves the cell, the endospore survives boiling), and many of them are targets for antibiotics or tools in biotechnology, such as plasmids for cloning.

You need: prokaryotic and eukaryotic cells compared (Unit 1) and the Gram stain and special stains (Unit 1, Staining). The cell wall is long enough to have its own chapter: see 2.2.

Shape, size and arrangement

Most bacteria take one of three basic shapes. **Cocci** (singular coccus) are spheres. **Bacilli** (singular bacillus) are rods; short, oval rods are called coccobacilli. **Spiral** forms come in three kinds: the comma-shaped **vibrio** (*Vibrio cholerae*), the rigid, helical **spirillum**, and the flexible, corkscrew **spirochete** (*Treponema pallidum*, *Leptospira*). A few bacteria are **pleomorphic**: they change shape with age or conditions, and *Mycoplasma*, which has no wall at all, has no fixed shape (Chapter 2.3).

Arrangement depends on how a cell divides and whether the daughters stay together. Cocci dividing in one plane and staying in pairs are **diplococci** (*Neisseria*, *Streptococcus pneumoniae*); in chains, **streptococci**; in two planes, groups of four called **tetrads**; in three planes, cubes of eight called **sarcinae**; and in random planes, grape-like clusters called **staphylococci**. Rods can occur singly, in pairs (diplobacilli) or in chains (streptobacilli, as in *Bacillus anthracis*).

Size is measured in micrometres (1 μm = 10^{-6} m). A typical rod such as *Escherichia coli* is about 1–2 μm long and 0.5–1 μm wide; a typical coccus is about 1 μm across. This is why bacteria need the oil-immersion objective of a light microscope (Unit 1). Small size has a direct benefit, worked out in the example below: a high surface-area-to-volume ratio, so nutrients and wastes cross the membrane fast enough to support rapid growth.

Structures outside the cell wall

- **Glycocalyx.** A sugary coat outside the wall. When it is organised and firmly attached it is a **capsule** (seen with the negative stain of Unit 1); when loose and diffuse it is a **slime layer**. Capsules protect pathogens from phagocytosis (*S. pneumoniae*, *Klebsiella pneumoniae*), and

slime helps cells stick to surfaces and form biofilms (Unit 6). The capsule of *Bacillus anthracis* is unusual: it is a polypeptide (poly-D-glutamic acid), not a polysaccharide.

- **Flagella.** Long, helical filaments that rotate like propellers. Each has three parts: the **filament** made of the protein **flagellin**, a curved **hook**, and a **basal body** of rings anchored in the wall and membrane. The motor is driven by the flow of protons (or Na^+ ions in some marine bacteria) across the membrane, not by ATP directly. By number and position: **monotrichous** (one flagellum at one end, *V. cholerae*), **lophotrichous** (a tuft at one end), **amphitrichous** (one at each end) and **peritrichous** (all over the surface, *E. coli*, *Salmonella*). Bacteria without flagella are **atrichous**. Spirochetes move with **axial filaments** (endoflagella) that lie inside an outer sheath.
- **Fimbriae and pili.** Fimbriae are many short, thin protein hairs used for attachment, for example of *E. coli* to the urinary tract. A **sex pilus** (F pilus) is longer, usually one or two per cell, and joins a donor to a recipient in conjugation (Unit 4). Type IV pili let some bacteria crawl by "twitching" motility.

The envelope and the cytoplasm

Under the wall lies the **plasma membrane**, a phospholipid bilayer with proteins. Because a bacterium has no mitochondria, the membrane carries the electron transport chain and ATP synthase, and it does transport and senses the outside. Bacterial membranes contain hopanoids rather than sterols; *Mycoplasma* is the exception (Chapter 2.3). Folds called **mesosomes** seen in old electron micrographs are now regarded as artefacts of chemical fixation, a favourite trick question.

Inside, the **cytoplasm** holds:

- **Nucleoid**: the region where the chromosome sits, usually one circular, double-stranded DNA molecule, supercoiled and packed by proteins, with no nuclear membrane.
- **Plasmids**: small, circular, self-replicating DNA molecules that carry extra genes such as antibiotic resistance or toxin genes. They are not essential for normal growth, and they are the vectors of genetic engineering.
- **Ribosomes** of the **70S** type, made of a 50S and a 30S subunit (S is the Svedberg unit of sedimentation, so 50 + 30 does not add to 70). Streptomycin, tetracycline and erythromycin act on these ribosomes (Unit 5).
- **Inclusions**: stores and special bodies. Glycogen; **poly-β-hydroxybutyrate** (PHB), a lipid store and the raw material of a biodegradable plastic (Unit 6); **volutin** or metachromatic granules of polyphosphate, typical of *Corynebacterium diphtheriae*; sulphur granules; **gas vacuoles** that float aquatic cyanobacteria; **magnetosomes** of magnetite that align a cell with the Earth's field; and **carboxysomes** holding the CO_2-fixing enzyme RuBisCO.

Endospores

Some Gram-positive rods, mainly *Bacillus* and *Clostridium*, form an **endospore** when nutrients run out. The spore is a dormant, dehydrated cell with a thick cortex, protein coats and a core rich in **calcium dipicolinate** and small acid-soluble proteins that protect the DNA. Spores survive boiling, drying, radiation and many disinfectants, which is why autoclaving at 121 °C is the standard for sterilisation (Unit 5). When conditions improve, the spore **germinates** into one vegetative cell, so sporulation is survival, not reproduction. Spore position (central, subterminal, terminal) helps identification: *Clostridium tetani* has a terminal spore that gives a "drumstick" look.

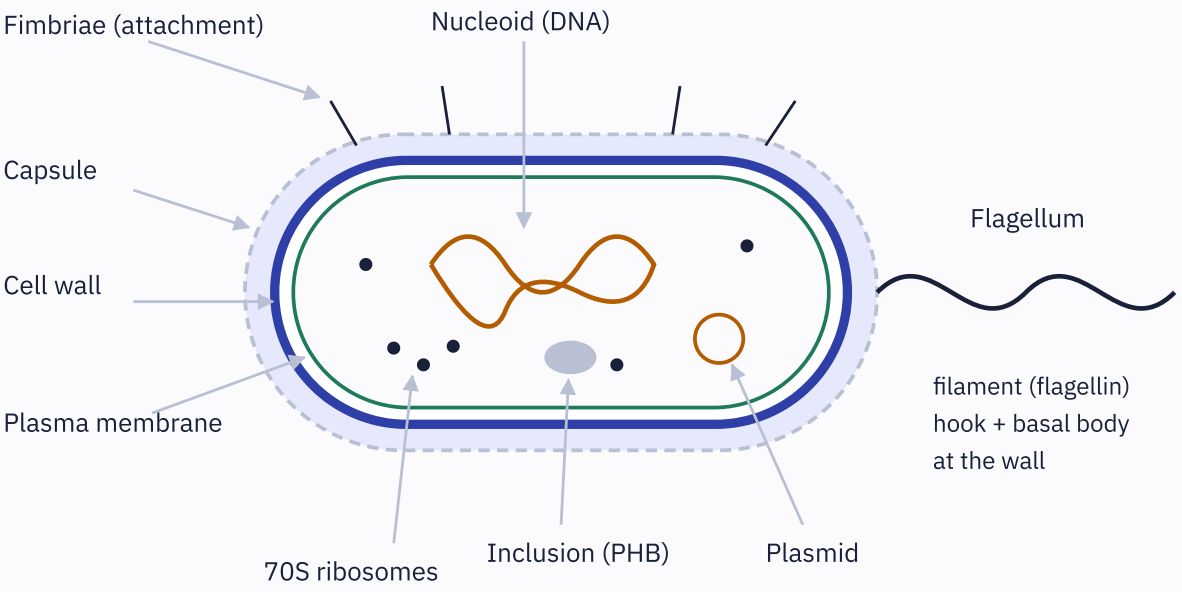


Figure 2.1.1 A rod-shaped bacterium in section. From outside in: capsule (dashed), thick cell wall, plasma membrane, then a cytoplasm with no nucleus. Notice that the DNA lies free in a nucleoid and the plasmid is a separate small circle.

WORKED EXAMPLE

Question (4 marks). A coccus has a diameter of 1 µm. A spherical eukaryotic cell has a diameter of 20 µm. Calculate the surface-area-to-volume (S/V) ratio of each and explain what the result means for growth.

1. For a sphere of radius r, surface area $S = 4\pi r^2$ and volume $V = (4/3)\pi r^3$. So $S/V = 4\pi r^2 \div (4/3)\pi r^3 = 3/r$. The ratio depends only on the radius.
2. Coccus: r = 0.5 µm, so S/V = 3 / 0.5 µm = **6 µm^{-1}**.
3. Eukaryotic cell: r = 10 µm, so S/V = 3 / 10 µm = **0.3 µm^{-1}**.
4. Compare: 6 ÷ 0.3 = 20. The coccus has 20 times more membrane per unit of cytoplasm.
5. Meaning: every nutrient enters and every waste leaves through the membrane. With 20 times more membrane per volume, the coccus can feed its cytoplasm far faster, which is one reason bacteria can divide every 20–30 minutes in good conditions while large cells need internal transport systems.

EXAM TRAPS

- Shape and arrangement are different words. "Coccus" is a shape; "staphylococcus" and "streptococcus" are arrangements (and genus names). Say both in an answer.
- 70S ribosomes have 50S and 30S subunits. Svedberg units do not add, so never write "50S + 30S = 80S" or correct 70 to 80.
- Fimbriae attach; the sex pilus transfers DNA; flagella move. Do not call fimbriae "short flagella".
- An endospore is not a reproductive spore: one cell makes one spore, which gives back one cell. Compare fungal spores in Chapter 2.4.
- Mesosomes are artefacts of fixation in current textbooks, though older Indian texts still list them as organelles. If asked, mention both views.

CHECK YOURSELF

– REMEMBER **Name the four arrangements of flagella with one example each.** 2 marks

Monotrichous (*Vibrio cholerae*), lophotrichous (*Pseudomonas* species with a polar tuft), amphitrichous (*Spirillum*), peritrichous (*E. coli*, *Salmonella*).

– REMEMBER **What is the chemical that makes endospores heat resistant, together with low water content?** 1 mark

Calcium dipicolinate in the spore core, along with small acid-soluble proteins that bind the DNA.

– UNDERSTAND **Distinguish a capsule from a slime layer.** 2 marks

Both are glycocalyx. A capsule is organised and firmly attached to the wall and resists phagocytosis; a slime layer is loose, diffuse and easily washed off, and mainly helps attachment and biofilm formation.

– UNDERSTAND **Why are plasmids useful in biotechnology but not essential to the bacterium?** 3 marks

They replicate on their own and carry extra genes (resistance, toxins, catabolic pathways) that help only in some environments, so a cell survives without them. Their small size, self-replication and selectable markers make them easy carriers of cloned genes.

– APPLY **A rod is 2 μm long and 1 μm wide. Treat it as a cylinder (ignore the ends) and find S/V.** 3 marks

For a cylinder, side area = $2\pi rL$ and $V = \pi r^2 L$, so $S/V = 2/r$. With $r = 0.5$ μm, $S/V =$ **$4\ \mu m^{-1}$**. Including the two end caps adds $2\pi r^2$ to S and raises the ratio a little, to $5\ \mu m^{-1}$.

– APPLY **A smear shows Gram-positive cocci in grape-like clusters. Name the arrangement and the likely genus.** 2 marks

Staphylococci, from division in random planes: genus *Staphylococcus* (for example *S. aureus*).

– ANALYZE **A food sample was boiled for 10 minutes and still spoiled when stored. Which structure explains this, and what treatment would you use?** 4 marks

Endospores of *Bacillus* or *Clostridium* survive boiling at 100 °C, then germinate. Use moist heat under pressure (autoclaving or retort processing at 121 °C), which kills spores; boiling only kills vegetative cells.

BEYOND THE SYLLABUS

Electron cryo-tomography now shows bacterial cells frozen intact, and it has revealed a real cytoskeleton: FtsZ (a tubulin relative that rings the dividing cell), MreB (an actin relative that keeps rods rod-shaped) and crescentin in curved *Caulobacter*. Bacterial microcompartments such as carboxysomes are being engineered into crops and factories to concentrate enzymes. The flagellar motor is a model of a molecular machine studied in biophysics. None of this is in the exam; search "bacterial cytoskeleton MreB FtsZ" or "bacterial microcompartments" to go further.

2.2 Bacterial cell wall: Gram-positive and Gram-negative

The bacterial cell wall is a rigid layer outside the plasma membrane that gives the cell its shape and stops it bursting. The cytoplasm is much more concentrated than most surroundings, so water rushes in by osmosis; without a wall the cell would swell and lyse. Almost all bacteria build this wall from **peptidoglycan**, a material found nowhere in human cells, which is why penicillin and lysozyme can attack bacteria and spare us. How the wall is built splits bacteria into two great groups, Gram-positive and Gram-negative, and that split decides how a bacterium stains, which antibiotics work, and whether it releases endotoxin.

You need: the Gram stain procedure (Unit 1, Staining) and the cell structures of 2.1. How antibiotics act is taught in Unit 5.

Peptidoglycan: the common building block

Peptidoglycan (also called **murein**) is a mesh made of sugar chains held together by short peptides.

1. **Glycan backbone.** Two amino sugars alternate along a chain: **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**, joined by **β-1,4 glycosidic bonds**.
2. **Tetrapeptide side chain.** Each NAM carries a short peptide, typically L-alanine, D-glutamic acid, a diamino acid (meso-diaminopimelic acid, DAP, in most Gram-negatives; L-lysine in many Gram-positives) and D-alanine. The D-amino acids are unusual; human proteins use L-amino acids.
3. **Cross-links.** Peptides on neighbouring chains are joined by **transpeptidase** enzymes, also called penicillin-binding proteins (PBPs). In *E. coli* the link is direct, from DAP of one

peptide to D-alanine of the next. In *Staphylococcus aureus* a bridge of five glycines (the **pentaglycine bridge**) joins lysine to D-alanine, giving more and longer cross-links.

The result is one giant bag-shaped molecule around the cell, the **sacculus**. Two classic agents attack it. **Lysozyme**, an enzyme of tears, saliva and egg white, cuts the β-1,4 bond between NAM and NAG. **Penicillin** and other β-lactams block the transpeptidases, so a growing cell makes a weak wall and bursts.

The Gram-positive wall

A Gram-positive wall is a **thick** peptidoglycan layer, many sheets deep, directly over the plasma membrane. Textbooks give its thickness as roughly 20–80 nm, and some up to 100 nm; peptidoglycan makes up most of its dry weight. Woven through it are **teichoic acids**, chains of glycerol phosphate or ribitol phosphate. **Wall teichoic acids** are attached to the peptidoglycan; **lipoteichoic acids** are anchored in the membrane lipid. Teichoic acids give the wall a negative charge, bind metal ions such as Mg^{2+}, help control the wall's own autolysins, and in pathogens like *Streptococcus* help the cell stick to host tissue. There is no outer membrane, so a Gram-positive cell has no true periplasm (some books describe a thin space between membrane and wall).

The Gram-negative wall

A Gram-negative envelope has more layers, and its peptidoglycan is only a few nanometres thick, often a single sheet. From outside in:

- **Outer membrane.** A second bilayer whose inner leaflet is phospholipid and whose outer leaflet is mostly **lipopolysaccharide (LPS)**. LPS has three parts: **lipid A**, which anchors it and is the toxic part called **endotoxin** (it triggers fever and septic shock when released from dying cells); a **core polysaccharide**; and the **O-antigen** (O side chain), a repeating sugar chain that varies between strains and is used to type them, as in *E. coli* O157.
- **Porins.** Channel proteins in the outer membrane that let small hydrophilic molecules through. They make the outer membrane a sieve: it keeps out bile salts, detergents, lysozyme and many antibiotics, which is why Gram-negatives are harder to treat.
- **Braun's lipoprotein.** The most abundant protein of the outer membrane; it ties the outer membrane to the peptidoglycan.
- **Periplasm.** The gel-filled space between the outer and plasma membranes. It holds the thin peptidoglycan layer, binding proteins for nutrient uptake, and enzymes such as β-lactamases that destroy penicillins before they reach their targets.
- **Plasma membrane**, as in all bacteria.

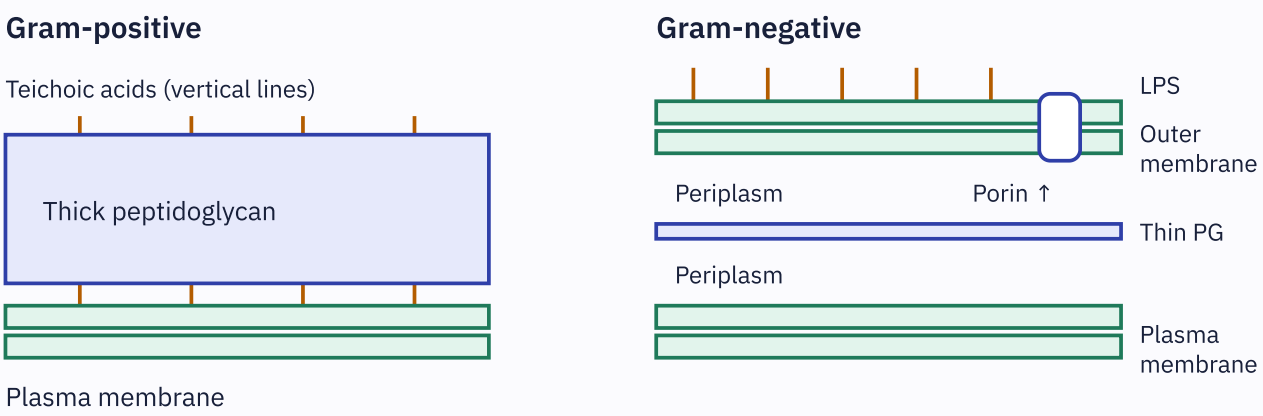


Figure 2.2.1 The two envelopes, outside at the top. The Gram-positive cell has one thick peptidoglycan layer threaded with teichoic acids; the Gram-negative cell has an outer membrane with LPS and porins, a periplasm, and only a thin peptidoglycan layer.

Why the Gram stain works, and when the wall is missing

In the Gram stain both kinds of cell take up crystal violet, which iodine (the mordant) turns into a large crystal violet–iodine complex. The decolouriser, alcohol or acetone, is the deciding step. In a Gram-positive cell it dehydrates and shrinks the thick peptidoglycan, closing its pores, so the complex is trapped and the cell stays purple. In a Gram-negative cell the alcohol dissolves lipid from the outer membrane, and the thin peptidoglycan cannot hold the complex, so the cell loses its colour and takes up the pink counterstain, safranin. Old Gram-positive cultures with damaged walls can stain Gram-variable.

Walls can also be removed. Lysozyme in a medium of high osmotic strength (for example sucrose) turns a Gram-positive cell into a **protoplast**, a round cell bounded only by its membrane. A Gram-negative cell treated the same way (with EDTA to open the outer membrane) keeps outer-membrane remnants and is called a **spheroplast**. Some bacteria lose their walls under penicillin and keep growing as **L-forms**. *Mycoplasma* never had one (Chapter 2.3). Two other walls are worth one line each: *Mycobacterium* has a waxy wall rich in **mycolic acids**, the reason it is acid-fast (Unit 1); and archaea have no true peptidoglycan, some having **pseudomurein** with N-acetyltalosaminuronic acid and β-1,3 bonds, so lysozyme and penicillin do not affect them.

WORKED EXAMPLE

Question (6 marks). Two unknown rods, X and Y, are tested. X: lysed by lysozyme alone; very sensitive to penicillin G; no LPS detected. Y: not lysed by lysozyme unless EDTA is added; less sensitive to penicillin G; gives a positive test for endotoxin; a loopful mixed with 3% KOH on a slide becomes stringy. Assign the Gram reaction of each and explain every result.

1. **Lysozyme.** It must reach the peptidoglycan. In X the peptidoglycan is exposed on the surface, so X lyses. In Y the outer membrane blocks lysozyme; EDTA removes the Mg^{2+} and Ca^{2+} that hold LPS molecules together, opening the membrane. So X is Gram-positive, Y Gram-negative.
2. **Penicillin G.** X has a thick, exposed wall made by transpeptidases, so blocking them is lethal. In Y penicillin G crosses the outer membrane poorly, and periplasmic β-lactamases may destroy it. Consistent.
3. **Endotoxin.** Only Gram-negatives have LPS, and lipid A is endotoxin. Y is positive. Consistent.
4. **KOH string test.** 3% KOH dissolves the thin Gram-negative wall and releases DNA, which makes the suspension viscous and stringy. A Gram-positive wall resists, so no string forms. Y is Gram-negative.
5. **Answer.** X is Gram-positive (purple in the Gram stain); Y is Gram-negative (pink). Four independent tests agree, which is stronger than one stain alone.

EXAM TRAPS

- "Gram-negative bacteria have no peptidoglycan" is wrong. They have a thin layer in the periplasm. Only *Mycoplasma* and its relatives lack a wall entirely.
- Lysozyme breaks the glycan backbone (the NAM–NAG β-1,4 bond); penicillin blocks cross-linking (transpeptidation). Do not swap them.
- Endotoxin is lipid A of LPS, released when cells die; exotoxins are proteins secreted by living cells, from both Gram groups.
- The decolouriser is the critical step of the Gram stain. Over-decolourising makes Gram-positives look negative.
- Protoplast (Gram-positive, wall fully gone) and spheroplast (Gram-negative, outer membrane bits remain) are a favourite 2-mark distinction.

CHECK YOURSELF

- REMEMBER **Name the two sugars of peptidoglycan and the bond between them.** 2 marks

  N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), alternating, joined by β-1,4 glycosidic bonds.

- REMEMBER **List the three parts of LPS and say which is endotoxin.** 2 marks

  Lipid A, core polysaccharide and O-antigen. Lipid A is the endotoxin.

- UNDERSTAND **Why does an alcohol rinse decolourise Gram-negative but not Gram-positive cells?** 4 marks

  Alcohol dehydrates the thick Gram-positive peptidoglycan and shrinks its pores, trapping the crystal violet–iodine complex. In Gram-negatives it extracts outer-membrane lipid and the thin peptidoglycan cannot retain the complex, which washes out; safranin then stains the cells pink.

- UNDERSTAND **Give two functions of teichoic acids.** 2 marks

  Any two: negative charge and binding of Mg^{2+}; rigidity of the wall; control of autolysins during growth; adhesion to host cells in pathogens; surface antigens.

- APPLY **A Gram-negative patient isolate is treated with an antibiotic that kills the bacteria quickly, and the patient's fever and blood pressure fall sharply soon after. Explain.** 3 marks

  Killing Gram-negative cells releases LPS from their outer membranes. Lipid A (endotoxin) triggers cytokine release, fever and a fall in blood pressure (endotoxic shock). The drug worked; the dying cells released the toxin.

- APPLY **You must remove the wall of *Bacillus subtilis* to fuse cells. What would you use and why must the medium contain sucrose?** 3 marks

  Lysozyme, which digests the exposed peptidoglycan to give protoplasts. A high concentration of sucrose (an osmotic stabiliser) balances the internal osmotic pressure so the wall-less protoplasts do not burst.

- ANALYZE **Why are Gram-negative bacteria generally more resistant to antibiotics and disinfectants than Gram-positives?** 5 marks

  The outer membrane is a permeability barrier: the LPS layer repels hydrophobic molecules and porins admit only small hydrophilic ones. Periplasmic β-lactamases destroy β-lactams on the way in. Efflux pumps across both membranes push drugs out. Together these lower the drug level at the target; Gram-positives have the target wall exposed.

BEYOND THE SYLLABUS

Wall synthesis is still the most successful antibiotic target, and new ones keep being found: teixobactin, reported in 2015 from a soil bacterium grown with the iChip method, binds the lipid-linked wall precursors. LPS is the reason injectable drugs and biotech proteins made in *E. coli* must be tested for endotoxin, using the *Limulus* amoebocyte lysate (LAL) test or the newer recombinant factor C test. Search "lipid II antibiotics" or "endotoxin testing recombinant factor C" to go deeper. This is not for the exam.

2.3 Mycoplasma and actinomycetes

Mycoplasma and actinomycetes are two groups of bacteria that break the textbook picture of a bacterium in opposite directions. **Mycoplasmas** are the smallest free-living cells known and have no cell wall at all, so they are shapeless, pass through bacteria-proof filters and ignore penicillin. **Actinomycetes** are Gram-positive bacteria that grow as branching filaments and make spores in chains, so they look like fungi, and they are the richest natural source of antibiotics. Both matter to a biotechnologist: mycoplasma is the most common hidden contaminant of animal cell cultures, and *Streptomyces* is a workhorse of the antibiotic industry.

You need: the cell wall of 2.2, because both groups are defined by their walls; and classification of microbes (Unit 1).

Mycoplasma: bacteria without a wall

Mycoplasmas belong to the class **Mollicutes** ("soft skin"). They evolved from Gram-positive ancestors by losing genes, including those for peptidoglycan. Their key features:

- **No cell wall.** Only a plasma membrane bounds the cell, so the cells are **pleomorphic**: spheres, pear shapes and branched filaments in the same culture. They stain poorly with the Gram stain and are not truly Gram-positive or negative.
- **Sterols in the membrane.** Most mycoplasmas take cholesterol from the medium or the host and put it in their membrane for strength, the only bacteria to do so. This is why their growth media contain serum. (*Acholeplasma* is a genus that does not need sterols.)
- **Very small.** Cells are about 0.2–0.3 µm across in their smallest forms, so many pass through the 0.45 µm filters used to sterilise solutions, and some through 0.22 µm filters.

- **Tiny genomes.** *Mycoplasma genitalium* has a genome of about 580 kilobase pairs, among the smallest of any cell that grows on its own. Many mycoplasmas read the codon UGA as tryptophan rather than "stop", a variant of the genetic code.
- **"Fried-egg" colonies.** On agar they form tiny colonies, often under 1 mm, that need a hand lens or low-power microscope. The centre grows down into the agar (dense, raised) and the edge spreads thin on the surface, giving a fried-egg look.
- **Drug pattern.** Resistant to penicillins, cephalosporins and lysozyme (no wall to attack); sensitive to drugs that act on the ribosome, such as tetracyclines and macrolides.

Importance. *M. pneumoniae* causes primary atypical pneumonia, often called "walking pneumonia", and is spread by droplets. *M. genitalium* and *Ureaplasma urealyticum* cause urogenital infections. In plants, wall-less relatives called **phytoplasmas** live in phloem and are spread by leafhoppers; in India they cause little leaf of brinjal and phyllody of sesame. In the laboratory, mycoplasma contamination of cell lines is common and invisible: the medium stays clear, yet the cells' growth, metabolism and experimental results change. Labs screen cultures by PCR, by DNA staining with DAPI or Hoechst (which shows tiny dots outside the nuclei), or by culture on special agar.

Actinomycetes: filamentous bacteria

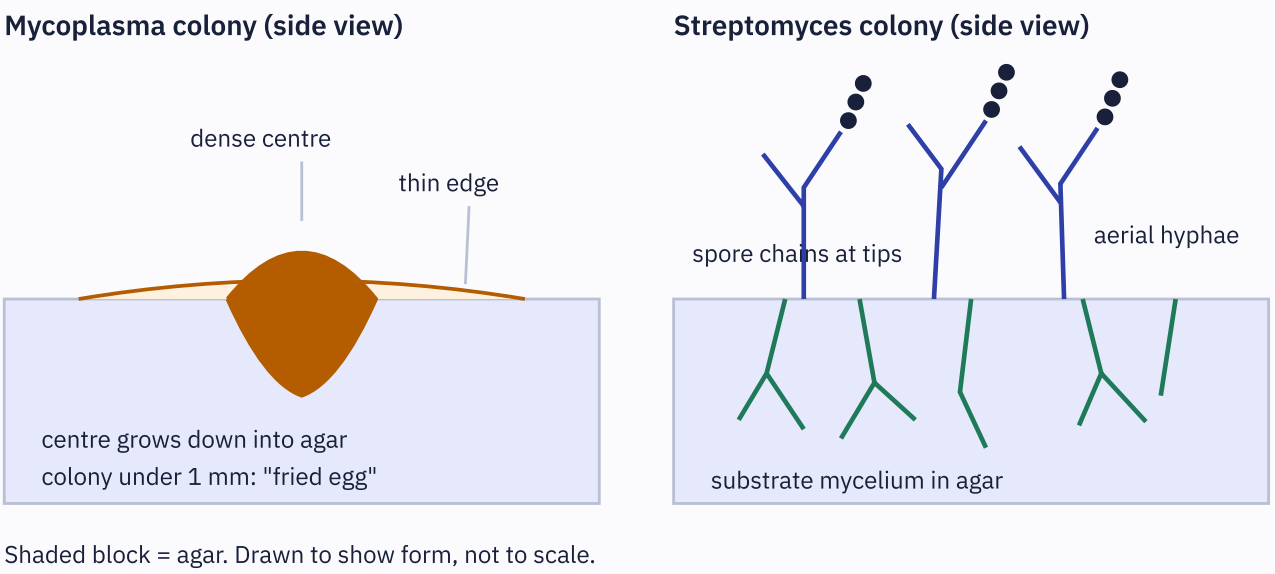
Actinomycetes (phylum Actinomycetota, formerly Actinobacteria) are Gram-positive bacteria with a high G+C content in their DNA. Most live in soil, where they break down tough plant material such as cellulose and chitin. The earthy smell of wet soil after the first monsoon rain comes partly from **geosmin**, a compound made by *Streptomyces*.

Structure. Their hyphae are about 0.5–1 µm wide, much thinner than fungal hyphae, and are true prokaryotic cells with peptidoglycan walls. A colony has two kinds of mycelium: the **substrate (vegetative) mycelium** that grows into the agar and absorbs food, and the **aerial mycelium** that rises above it and makes spores. In *Streptomyces* the tips of aerial hyphae break up into chains of **conidia**; other genera make spores inside sporangia (*Streptosporangium*, *Actinoplanes*, whose spores swim). Colonies are tough, chalky or leathery and stick firmly to the agar, unlike soft bacterial colonies or fluffy mould colonies. Actinomycete spores are for dispersal and resist drying, but unlike endospores they are not highly heat resistant.

Important genera.

- *Streptomyces*: aerobic soil saprophytes and the main antibiotic producers, including streptomycin (*S. griseus*, discovered in Selman Waksman's laboratory in 1943), chloramphenicol (*S. venezuelae*), tetracyclines (*S. aureofaciens* and *S. rimosus*), and the antifungals nystatin and amphotericin B. The antiparasitic avermectins come from *S. avermitilis*.
- *Saccharopolyspora erythraea* (erythromycin) and *Amycolatopsis* (vancomycin, rifamycin): actinomycetes outside *Streptomyces*.

- *Frankia*: fixes nitrogen in root nodules of non-legume trees such as *Casuarina* and alder.
- *Nocardia*: aerobic, partially acid-fast; causes lung nocardiosis and actinomycetoma (Madura foot) in people who walk barefoot on soil.
- *Actinomyces israelii*: anaerobic, part of normal mouth flora; causes actinomycosis, with "sulphur granules" (yellow clumps of filaments) in the pus.
- The group also includes *Mycobacterium* and *Corynebacterium*, which are not filamentous but are related; they are covered as pathogens in Unit 6.



Shaded block = agar. Drawn to show form, not to scale.

Figure 2.3.1 Two colonies seen from the side. The wall-less mycoplasma pushes its centre into the agar and spreads a thin edge, so from above it looks like a fried egg. *Streptomyces* grows a branching substrate mycelium into the agar and aerial hyphae that end in chains of conidia.

WORKED EXAMPLE

Question (8 marks). A student isolates an organism from garden soil. On starch–casein agar it forms small, hard, chalky white colonies that stick to the agar and smell of wet earth. A smear shows thin (under 1 µm) branching Gram-positive filaments with chains of spores. (a) Identify the group and likely genus. (b) Describe how you would check whether it makes an antibiotic against *Staphylococcus aureus* and *E. coli*.

1. **Group.** Gram-positive, filamentous, branching, hyphae thinner than 1 µm (too thin for a fungus), tough adherent colonies, earthy smell (geosmin): an **actinomycete**. Spores in chains on aerial hyphae point to *Streptomyces*.
2. **Primary screen: cross-streak method.** Streak the isolate as a single band across one side of a nutrient agar plate and incubate (often about 5–7 days at 28 °C) so it grows and releases any antibiotic into the agar.
3. Streak the test bacteria, *S. aureus* and *E. coli*, as lines at right angles to the band, starting near it and running outwards. Incubate overnight at 37 °C.
4. **Read.** Measure the length of each test streak that fails to grow next to the band. Inhibition of *S. aureus* only suggests an antibiotic active against Gram-positives; inhibition of both suggests a broad-spectrum compound.
5. **Secondary screen.** Grow the isolate in broth, filter off the cells, and put the culture filtrate in wells cut in agar seeded with each test organism (agar well diffusion). A clear zone around the well confirms a diffusible antibiotic, and its diameter allows comparison between isolates.

EXAM TRAPS

- Actinomycetes are bacteria, not fungi. Give the proof: prokaryotic cells, peptidoglycan walls, hyphae under 1 µm, sensitivity to antibacterial drugs.
- Mycoplasma has no cell wall; it is not a Gram-negative organism, even though it does not stain purple.
- Sterols in the membrane are a mycoplasma feature; most bacteria have hopanoids instead. Penicillin resistance follows from the missing wall, not from β-lactamase.
- *Actinomyces* (anaerobic, actinomycosis) and *Nocardia* (aerobic, partially acid-fast) are a common "distinguish between" pair.

CHECK YOURSELF

- REMEMBER **Name the class to which mycoplasmas belong and the disease caused by *M. pneumoniae*.** 2 marks

  Class Mollicutes; primary atypical ("walking") pneumonia.

- REMEMBER **Name the antibiotic made by *Streptomyces griseus* and one made by *S. venezuelae*.** 2 marks

  Streptomycin; chloramphenicol.

- UNDERSTAND **Why do mycoplasma colonies look like fried eggs?** 2 marks

  Without a wall, the cells can push into the gel: the centre grows down into the agar and stays dense and raised, while the edge spreads as a thin surface layer.

- UNDERSTAND **Distinguish substrate mycelium from aerial mycelium.** 2 marks

  Substrate mycelium grows into the medium and absorbs nutrients; aerial mycelium grows into the air and bears the spores (conidia), often with a different colour.

- APPLY **A cell line grows slowly but the medium is clear and nothing is seen under a normal microscope. What do you suspect and how do you test it?** 3 marks

  Mycoplasma contamination. Test by PCR for mycoplasma 16S rRNA genes, or stain with DAPI/Hoechst and look for small fluorescent dots outside the nuclei; culture on mycoplasma agar is slower.

- APPLY **A culture medium was filter-sterilised through a 0.45 µm membrane but later showed mycoplasma. Explain and suggest a fix.** 3 marks

  Wall-less mycoplasma cells are small and flexible and can pass 0.45 µm pores. Use 0.1 µm filters for media and serum, buy certified mycoplasma-free serum, and screen cultures regularly.

- ANALYZE **Compare mycoplasma and actinomycetes as "unusual bacteria" under wall, form, size and importance.** 6 marks

  Wall: none (sterol membrane) vs thick Gram-positive peptidoglycan. Form: pleomorphic single cells vs branching filaments with spore chains. Size: about 0.2–0.3 µm cells vs hyphae 0.5–1 µm wide forming macroscopic colonies. Drugs: penicillin-resistant vs usually penicillin-sensitive. Importance: pneumonia, phytoplasma plant diseases and cell-culture contamination vs antibiotics, soil decomposition, nitrogen fixation (*Frankia*) and some infections (*Nocardia*, *Actinomyces*).

BEYOND THE SYLLABUS

In 2016 the J. Craig Venter Institute reported JCVI-syn3.0, a *Mycoplasma mycoides* cell running on a designed, chemically synthesised genome cut down to under 500 genes, a step toward a "minimal cell". On the actinomycete side, genome sequencing shows each *Streptomyces* carries 20–30 or more biosynthetic gene clusters, most silent in the lab; genome mining tools such as antiSMASH find them, and synthetic biology wakes them up. Search "minimal cell JCVI-syn3" or "antiSMASH genome mining". Not for the exam.

2.4 Fungi: morphology, reproduction and diseases

Fungi are eukaryotic, heterotrophic organisms that feed by absorbing nutrients and have cell walls of **chitin**. The group includes single-celled **yeasts**, filamentous **moulds**, and mushrooms. Fungi rot dead matter and recycle carbon, make bread rise and give us penicillin and citric acid, but they also spoil grain, poison food with mycotoxins and cause infections from ringworm to the "black fungus" mucormycosis seen in India in 2021. A biotechnology student meets them on both sides: as production organisms and as contaminants.

You need: prokaryotic and eukaryotic cells compared (Unit 1). Antifungal drugs are in Unit 5; industrial fermentations by fungi are in Unit 6.

Morphology: yeasts, moulds and dimorphism

Every fungal cell has a nucleus, mitochondria and 80S ribosomes. The wall is made of chitin (a polymer of N-acetylglucosamine) and glucans, and the membrane contains **ergosterol** instead of cholesterol. Ergosterol is the target of the antifungals amphotericin B and the azoles, which is how they spare human cells.

- **Yeasts** are single oval cells, about 3–5 µm across in *Saccharomyces cerevisiae*, that usually reproduce by **budding**. When buds stay attached in a chain they form a **pseudohypha**, as in *Candida albicans*.
- **Moulds** grow as **hyphae**, tubular filaments about 2–10 µm wide that extend at their tips and branch. A mass of hyphae is a **mycelium**. Hyphae are either **septate** (divided by cross-walls with pores, as in *Aspergillus* and *Penicillium*) or **coenocytic** (aseptate: one long multinucleate tube, as in *Rhizopus* and *Mucor*). The vegetative mycelium absorbs food; the reproductive (aerial) mycelium carries spores.

- **Dimorphic fungi** switch form with temperature: a mould at 25 °C in soil and a yeast at 37 °C in the body. *Histoplasma capsulatum*, *Blastomyces* and *Talaromyces* (formerly *Penicillium*) *marneffei* are examples. The switch is part of how they cause disease.

Reproduction and classification

Fungi reproduce mainly by **spores**, which are true reproductive units: one mycelium releases millions. Asexual spores are made by mitosis, and sexual spores follow the fusion of two nuclei and meiosis.

- **Asexual spores. Sporangiospores** form inside a sac (sporangium) at the tip of a sporangiophore (*Rhizopus*). **Conidia** form free, not enclosed, at the tips or sides of a conidiophore (*Aspergillus*, *Penicillium*). **Arthroconidia** form when a hypha breaks into cells; **chlamydospores** are thick-walled resting cells; **blastoconidia** are buds.
- **Sexual spores** define the main groups. **Zygomycetes** (now Mucoromycota) form a thick-walled **zygospore** where two hyphae fuse; their hyphae are coenocytic (*Rhizopus*, *Mucor*). **Ascomycota** make **ascospores**, usually eight, in a sac called an **ascus** (*Saccharomyces*, *Neurospora*, and the sexual stages of many *Aspergillus* and *Penicillium*). **Basidiomycota** make **basidiospores** on a club-shaped **basidium**, usually four (mushrooms, rusts, smuts, *Cryptococcus*). **Chytrids** have flagellated spores and live in water.
- Fungi with no known sexual stage were placed in the **Deuteromycota** or **Fungi Imperfecti**. Many Indian syllabi still use this group; DNA sequencing now places most of them within the Ascomycota.

Water moulds (oomycetes) such as *Phytophthora infestans*, the cause of late blight of potato, look like fungi but have cellulose walls and belong to a different lineage. Name them as "fungus-like" in an answer.

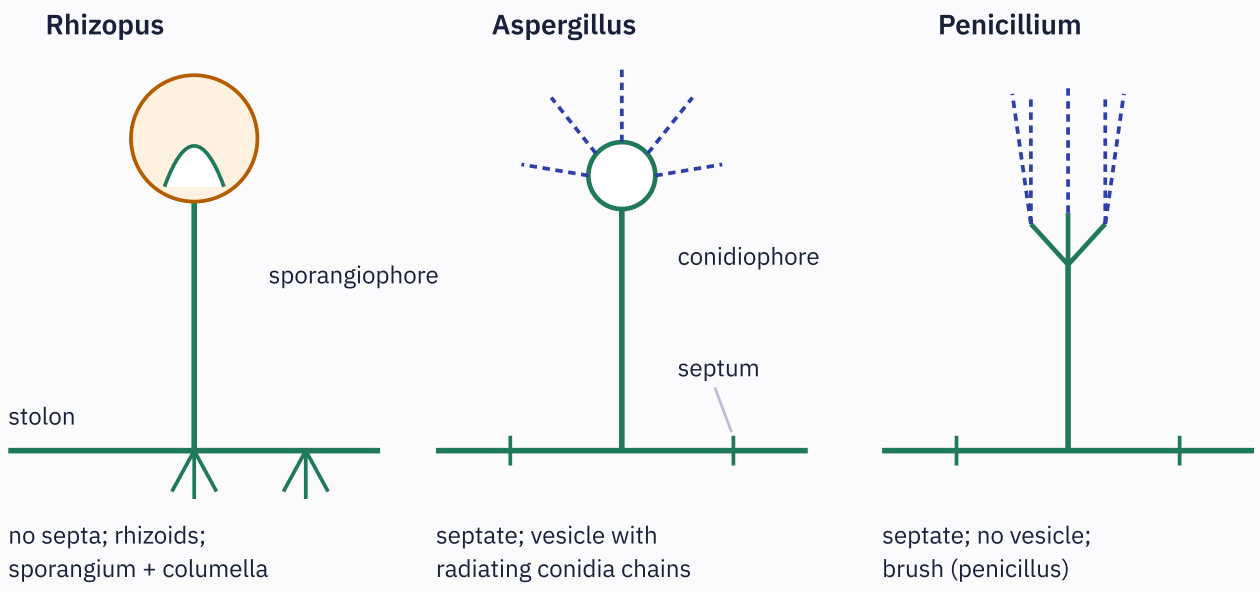


Figure 2.4.1 Three common moulds under the low-power microscope, as seen in a lactophenol cotton blue mount. Dashed lines are chains of conidia. Notice the two things to check first: are there septa, and are spores enclosed in a sac (*Rhizopus*) or free on a swollen vesicle (*Aspergillus*) or on brush-like branches (*Penicillium*)?

Fungal diseases and mycotoxins

Human fungal infections (**mycoses**) are grouped by how deep they go:

- **Superficial**: outer skin and hair only, for example pityriasis versicolor (*Malassezia*), with pale or dark patches.
- **Cutaneous**: skin, hair and nails, caused by **dermatophytes** (*Trichophyton*, *Microsporum*, *Epidermophyton*) that digest keratin. The diseases are called **tinea** or ringworm (tinea corporis, tinea pedis or athlete's foot, tinea capitis), common in hot, humid Indian summers.
- **Subcutaneous**: entering through a wound, for example sporotrichosis and eumycetoma (fungal Madura foot).
- **Systemic**: starting in the lungs after spores are inhaled and spreading, mainly by dimorphic fungi (histoplasmosis).
- **Opportunistic**: in people with weak immunity, uncontrolled diabetes or long steroid use. Candidiasis (oral thrush, vaginal infection), aspergillosis, cryptococcal meningitis in AIDS, and **mucormycosis** by *Rhizopus* and *Mucor*, which spread widely in India among COVID-19 patients in 2021.

Fungi also harm crops: rice blast (*Magnaporthe oryzae*), rusts and smuts of wheat, and red rot of sugarcane. **Aflatoxins** from *Aspergillus flavus* and *A. parasiticus* on groundnuts and maize damage the liver, and aflatoxin B1 is a potent liver carcinogen; **ochratoxin A** (*Aspergillus*, *Penicillium*) damages kidneys; **patulin** occurs in rotting apples; **ergot alkaloids** from *Claviceps*

purpurea on rye and millets cause ergotism. Mycotoxins are heat stable, so cooking does not make mouldy grain safe; the control is dry storage.

WORKED EXAMPLE

Question (6 marks). Mould growth on stored groundnuts is mounted in lactophenol cotton blue. You see septate hyphae; upright unbranched conidiophores ending in a swollen, round vesicle; flask-shaped cells covering the vesicle; and chains of greenish-yellow spores radiating from them. (a) Identify the mould. (b) Name the toxin risk. (c) Say how you would tell it from *Penicillium* and *Rhizopus*.

1. **Septa present**, so it is not a coenocytic zygomycete such as *Rhizopus* or *Mucor*.
2. **Spores free in chains** (conidia), not enclosed in a sporangium, from flask-shaped phialides on a **vesicle**: genus **Aspergillus**.
3. Yellow-green colour on groundnuts suggests ***A. flavus***, the main aflatoxin producer (species identification needs culture or DNA, so say "likely").
4. **Toxin risk**: aflatoxins, especially B1, which harm the liver and are carcinogenic; the lot should be tested before use.
5. **Distinguish**: *Penicillium* has septate hyphae and conidia chains too, but its conidiophore branches into a brush (penicillus) with no vesicle. *Rhizopus* has aseptate hyphae, rhizoids and a sporangium with a columella.

EXAM TRAPS

- Fungal walls are chitin, plant walls cellulose. Oomycetes have cellulose walls and are not true fungi.
- Conidia are free spores; sporangiospores are inside a sporangium. This single difference separates *Aspergillus*/*Penicillium* from *Rhizopus*/*Mucor*.
- Ringworm is a fungal (dermatophyte) infection, not a worm. Do not list it under helminths.
- Antibacterial drugs do not kill fungi: fungi have no peptidoglycan and have 80S ribosomes. Antifungals target ergosterol or the wall glucans.
- Ascus: usually 8 ascospores, inside. Basidium: usually 4 basidiospores, outside on stalks. Examiners love the count and the position.

CHECK YOURSELF

– REMEMBER **Name the sterol in fungal membranes and one drug that targets it.** 2 marks

Ergosterol; amphotericin B (binds it) or an azole such as fluconazole (blocks its synthesis).

– REMEMBER **Name the three genera of dermatophytes.** 2 marks

Trichophyton, *Microsporum* and *Epidermophyton*.

– UNDERSTAND **What is a dimorphic fungus and why does dimorphism matter in disease?** 3 marks

A fungus that grows as a mould at environmental temperature (about 25 °C) and as a yeast at body temperature (37 °C). Inhaled mould spores convert to the yeast form in the body, which can survive and spread inside host cells, as in histoplasmosis.

– UNDERSTAND **Distinguish septate and coenocytic hyphae with an example of each.** 2 marks

Septate hyphae have cross-walls dividing them into cells (*Aspergillus*, *Penicillium*); coenocytic hyphae are continuous multinucleate tubes without regular septa (*Rhizopus*, *Mucor*).

– APPLY **A diabetic patient after a long course of steroids develops black tissue in the nose and palate. Name the likely infection, the fungi and one feature you would see in tissue.** 3 marks

Mucormycosis, by *Rhizopus* or *Mucor* (order Mucorales). In tissue: broad, ribbon-like, mostly aseptate hyphae with wide-angle branching, invading blood vessels.

– APPLY **A rice mill asks how to prevent aflatoxin in its stored grain. Give two measures and the reason.** 3 marks

Dry grain quickly to low moisture and store it dry and cool; reject damaged grain, and keep stores clean and ventilated. Aflatoxin is heat stable, so it must be prevented by stopping *Aspergillus* growth, not removed by cooking.

– ANALYZE **Why is a yeast like *Saccharomyces* classified as a fungus although it has no hyphae?** 4 marks

Classification rests on cell type and life cycle, not on shape. Yeast is eukaryotic, heterotrophic and absorptive, has a chitin–glucan wall and ergosterol, and forms ascospores in an ascus on mating, placing it in the Ascomycota. Some yeasts also form pseudohyphae, showing the link to filamentous growth.

BEYOND THE SYLLABUS

The WHO published its first fungal priority pathogens list in 2022, naming *Candida auris*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans* among the critical group, partly because antifungal resistance is rising. In industry, *Aspergillus niger* makes most of the world's citric acid and many enzymes, and *Trichoderma reesei* makes cellulases for biofuel. Fungal mycelium is also being grown into packaging and leather-like materials. Search "WHO fungal priority pathogens list" or "mycelium materials". Not for the exam.

2.5 Algae: morphology, reproduction and industrial uses

Algae are photosynthetic eukaryotes, mostly aquatic, that have chlorophyll a but lack the roots, stems, leaves and vascular tissue of plants. They range from single cells a few micrometres across (**microalgae**, such as *Chlorella*) to seaweeds many metres long (**macroalgae**, such as kelps). Algae make a large share of the oxygen on Earth and stand at the base of aquatic food chains. Industry harvests them for agar, carrageenan and alginate, grows them for food supplements and pigments, and studies them as a source of biofuel; some also make deadly toxins that reach us through seafood.

You need: prokaryotic and eukaryotic cells compared (Unit 1). Photosynthesis in microbes is in Unit 4; algae in wastewater ponds are in Unit 6.

Thallus organisation and the main groups

The body of an alga is called a **thallus**. Its forms run from simple to complex: **unicellular** (*Chlamydomonas*, motile; *Chlorella*, non-motile), **colonial** (*Volvox*, a hollow ball of cells), **filamentous** (*Spirogyra*, *Ulothrix*), **siphonous** (*Caulerpa*, one large multinucleate cell) and **parenchymatous** sheets and blades (*Ulva*, the brown kelps). The study of algae is called **phycology**.

Algae are classified by four features: their pigments, their storage food, their cell wall and their flagella. The table gives the groups most asked in exams.

GROUP	PIGMENTS	STORAGE FOOD	CELL WALL	EXAMPLES
Green algae (Chlorophyta)	chl a, b; carotenoids	starch	cellulose	*Chlamydomonas*, *Chlorella*, *Ulva*, *Spirogyra*

GROUP	PIGMENTS	STORAGE FOOD	CELL WALL	EXAMPLES
Brown algae (Phaeophyta)	chl a, c; fucoxanthin	laminarin, mannitol	cellulose + alginate	*Sargassum*, *Laminaria*, *Fucus*
Red algae (Rhodophyta)	chl a; phycoerythrin, phycocyanin	floridean starch	cellulose + agar or carrageenan	*Gracilaria*, *Gelidium*, *Kappaphycus*
Diatoms (Bacillariophyta)	chl a, c; fucoxanthin	chrysolaminarin, oil	silica frustule in two halves	*Navicula*, *Pseudo-nitzschia*
Dinoflagellates (Dinophyta)	chl a, c; peridinin	starch, oil	cellulose plates (theca)	*Alexandrium*, *Gonyaulax*
Euglenoids (Euglenophyta)	chl a, b	paramylon	no wall; protein pellicle	*Euglena*

The "blue-green algae" are not algae: they are **cyanobacteria**, prokaryotes with chlorophyll a and phycobilins. *Spirulina* (correctly *Arthrospira*), *Nostoc* and *Anabaena* are cyanobacteria. Most syllabi still discuss them with algae because their uses overlap, but in an answer always say they are bacteria.

Reproduction and life cycles

Algae reproduce in three ways.

- **Vegetative**: by cell division in unicells and by **fragmentation** of filaments and thalli.
- **Asexual**: by spores made by mitosis. **Zoospores** are flagellated and swim (*Chlamydomonas*, *Ulothrix*); **aplanospores** are non-motile; **akinetes** are thick-walled resting cells.
- **Sexual**: by fusion of gametes. In **isogamy** the two gametes look alike (many *Chlamydomonas* species); in **anisogamy** they differ in size (*Chlamydomonas braunii*); in **oogamy** a large non-motile egg is fertilised by a small motile sperm (*Volvox*, *Fucus*). *Spirogyra* shows **conjugation**: a tube forms between two filaments and the content of one cell moves into the other.

Where meiosis falls in the life cycle gives three patterns (Figure 2.5.1). In a **haplontic** cycle the organism is haploid (n) and only the zygote is diploid (2n); the zygote undergoes meiosis at once. In a **diplontic** cycle the organism is diploid and only the gametes are haploid. In a **haplodiplontic** cycle (alternation of generations) a haploid gametophyte and a diploid sporophyte are both multicellular; in *Ulva* they even look the same (isomorphic).

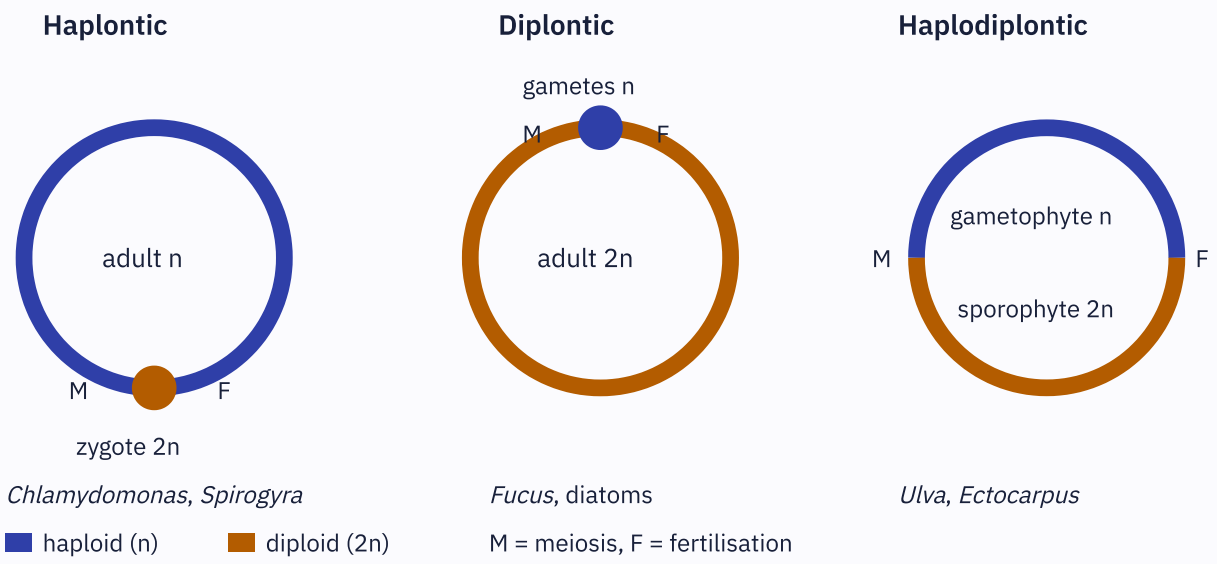


Figure 2.5.1 The three life cycles of algae as rings, read clockwise. Blue is haploid, orange is diploid. Notice where meiosis (M) falls: straight after the zygote in haplontic algae, at gamete formation in diplontic algae, and at spore formation on the sporophyte in haplodiplontic algae.

Industrial uses

- **Phycocolloids** (gels from seaweed walls). **Agar** from red algae such as *Gelidium* and *Gracilaria* solidifies microbiological media because almost no bacteria digest it and it melts near 85 °C but sets only around 32–40 °C (Unit 4). **Carrageenan** from *Kappaphycus alvarezii*, farmed along the Tamil Nadu coast near Ramanathapuram, thickens ice cream, toothpaste and chocolate milk. **Alginate** from brown algae (*Sargassum*, *Turbinaria*, *Laminaria*) is used in wound dressings, textile printing and to entrap cells and enzymes as beads in bioprocessing.
- **Food and feed**: *Chlorella* and the cyanobacterium *Arthrospira* (Spirulina) are sold as protein-rich supplements; microalgae feed fish and shrimp larvae in hatcheries.
- **Pigments and nutraceuticals**: β-carotene from *Dunaliella salina*, grown in salt ponds; astaxanthin from *Haematococcus pluvialis*; phycocyanin from Spirulina as a natural blue colour; DHA (an omega-3 fatty acid) from the dinoflagellate *Crypthecodinium cohnii* for infant formula.
- **Diatomaceous earth**: fossil diatom silica, used as a filter aid in breweries, as a mild abrasive and as an insecticide dust.
- **Environment and energy**: algae in oxidation ponds supply oxygen to bacteria treating sewage (Unit 6); oil-rich microalgae can be turned into biodiesel, though cost of growing and harvesting is still the main barrier.

During harmful algal blooms ("red tides") shellfish concentrate toxins: saxitoxins from *Alexandrium* cause **paralytic shellfish poisoning**; domoic acid from the diatom *Pseudo-nitzschia* causes **amnesic shellfish poisoning**; okadaic acid from *Dinophysis* causes **diarrhetic shellfish poisoning**; ciguatoxins from *Gambierdiscus* reach reef fish and cause **ciguatera**. In fresh water,

the cyanobacterium *Microcystis* makes microcystins that damage the liver. These toxins are heat stable, so cooking does not help.

WORKED EXAMPLE

Question (6 marks). A start-up in Gujarat runs an open raceway pond of 2,000 m² growing an oil-rich microalga. Assume an areal productivity of 15 g dry biomass per m² per day, a lipid content of 25% of dry weight, 300 operating days a year, 80% lipid extraction efficiency and an oil density of 0.9 kg/L. Find (a) the daily biomass, (b) the oil recovered per year in litres, and (c) the oil yield per hectare per year.

1. **Daily biomass** = area × productivity = 2,000 m² × 15 g m^{-2} day^{-1} = 30,000 g day^{-1} = **30 kg dry biomass per day**.
2. **Daily lipid in the biomass** = 30 kg × 0.25 = 7.5 kg day^{-1}.
3. **Lipid recovered per year** = 7.5 kg day^{-1} × 300 days × 0.80 = 1,800 kg $year^{-1}$.
4. **Volume** = mass ÷ density = 1,800 kg ÷ 0.9 kg L^{-1} = **2,000 L of oil per year**.
5. **Per hectare**: 1 ha = 10,000 m², which is 5 times the pond area, so 2,000 L × 5 = **10,000 L ha^{-1} $year^{-1}$**.
6. **Comment**: the yield per hectare is high compared with oil crops, which is the case for algal biofuel; the catch is the energy and cost of mixing, harvesting dilute cells and extracting the oil.

EXAM TRAPS

- Blue-green algae are cyanobacteria (prokaryotes). Spirulina is a cyanobacterium, not a green alga.
- Agar comes from red algae; alginate from brown algae. Students swap them every year.
- Storage foods are a classic one-mark question: starch (green), laminarin and mannitol (brown), floridean starch (red), paramylon (euglenoids).
- In a haplontic cycle the zygote is the only diploid cell; do not draw a diploid adult for *Chlamydomonas*.
- Algal toxins in shellfish are not destroyed by cooking. "Boil the prawns" is a wrong control measure.

CHECK YOURSELF

- REMEMBER **Name the pigment that gives brown algae their colour and the one that gives red algae theirs.** 2 marks

  Fucoxanthin in brown algae; phycoerythrin in red algae.

- REMEMBER **Name one alga used for each: agar, carrageenan, alginate, β-carotene.** 2 marks

  Gelidium or *Gracilaria*; *Kappaphycus*; *Sargassum*, *Laminaria* or *Turbinaria*; *Dunaliella salina*.

- UNDERSTAND **Distinguish isogamy, anisogamy and oogamy.** 3 marks

  Isogamy: fusing gametes alike in size and form. Anisogamy: both motile but of different sizes. Oogamy: a large non-motile egg and a small motile male gamete.

- UNDERSTAND **Why is agar preferred to gelatin for solid culture media?** 2 marks

  Very few microbes digest agar, so it stays solid; and it remains solid at 37 °C (and higher incubation temperatures) once set, while gelatin melts near body temperature and is digested by many bacteria.

- APPLY **Repeat the worked example with 20 g m^{-2} day^{-1} and 30% lipid, other values unchanged. Find the oil per year.** 3 marks

  Biomass 2,000 × 20 = 40 kg day^{-1}; lipid 12 kg day^{-1}; recovered 12 × 300 × 0.8 = 2,880 kg; volume 2,880 ÷ 0.9 = **3,200 L per year**.

- APPLY **Several people fall ill with tingling lips and numbness after eating mussels collected during a red tide. Name the syndrome and the likely toxin and organism.** 3 marks

  Paralytic shellfish poisoning, caused by saxitoxins from dinoflagellates such as *Alexandrium*, concentrated by the filter-feeding mussels.

- ANALYZE **Compare open raceway ponds with closed photobioreactors for growing microalgae.** 5 marks

  Ponds: cheap to build and run, easy to scale, but lower cell density, contamination by other algae and grazers, water loss by evaporation and poor control of temperature and CO_2. Photobioreactors: higher density and productivity, sterile and controlled, suited to high-value products (astaxanthin, DHA), but costly to build, cool and clean. Choice follows product value.

BEYOND THE SYLLABUS

Seaweed farming is growing in India's coastal states as a livelihood and a carbon-friendly crop, and seaweed-based biostimulants are sold to farmers. Microalgae are being engineered as "green cell factories" for proteins and vaccines, and diatom silica is studied as a template for nanomaterials. The economics of algal biofuel remain hard; life-cycle assessments are the tool used to judge it. Search "seaweed cultivation India Kappaphycus" or "microalgae techno-economic analysis". Not for the exam.

2.6 Protozoa and helminths

Protozoa are single-celled eukaryotes that feed like animals, by eating or absorbing organic matter, and most of them move. Helminths are parasitic worms: multicellular animals with organs, which are not microbes at all but are taught in microbiology because their eggs, larvae and cysts are found by the microscope in stool, blood and food. Both groups spread mainly through contaminated water and food, insect bites and poor sanitation, so they matter a great deal in India, where amoebic dysentery, giardiasis, malaria, kala-azar, worm infections and filariasis are all public health problems.

You need: eukaryotic cell structure (Unit 1). Malaria is treated in detail in Unit 6 (Common infections); here it appears only as an example of a sporozoan.

Protozoa: structure and life stages

A protozoan cell has a nucleus, mitochondria (absent or reduced in some, such as *Giardia*), and no cell wall; many have a firm, flexible outer layer called the **pellicle**. Freshwater forms use **contractile vacuoles** to pump out water. Food is taken in by phagocytosis into food vacuoles or, in ciliates, through a mouth-like **cytostome**.

Many parasitic protozoa have two stages. The **trophozoite** is the active, feeding, dividing form found in the host. The **cyst** is a dormant form with a tough wall that survives outside the body and is the infective stage when swallowed. This is why cysts, not trophozoites, spread amoebiasis and giardiasis through water: trophozoites die in the stomach acid, cysts pass through.

Reproduction. Asexual reproduction is by **binary fission** (longitudinal in flagellates, transverse in ciliates), **multiple fission** or **schizogony** (one cell divides into many, as in *Plasmodium* in red

blood cells) and budding. Sexual reproduction is by **conjugation** in ciliates (two cells exchange micronuclei) and by **gamete fusion** in sporozoa, as in the sexual cycle of *Plasmodium* inside the mosquito.

Classification by locomotion, with diseases

The traditional classification, still used in Indian syllabi, groups protozoa by how they move. Modern classification by DNA splits them across many lineages, but the four classic groups remain the best map for exams.

- **Sarcodina (amoebae)**: move and feed by **pseudopodia**. *Entamoeba histolytica* causes **amoebic dysentery** (stools with blood and mucus) and can reach the liver to form an **amoebic liver abscess**. Its cyst has four nuclei when mature; the harmless gut amoeba *Entamoeba coli* has up to eight.
- **Mastigophora (flagellates)**: move by **flagella**. *Giardia lamblia* (also called *G. intestinalis* or *G. duodenalis*) has a pear-shaped trophozoite with a ventral sucking disc and causes **giardiasis**, with greasy, foul-smelling diarrhoea. *Leishmania donovani*, spread by sandflies (*Phlebotomus*), causes **kala-azar** (visceral leishmaniasis), still found in Bihar, Jharkhand and nearby states. *Trypanosoma brucei* causes African sleeping sickness (tsetse fly) and *T. cruzi* causes Chagas disease in Latin America. *Trichomonas vaginalis* is sexually transmitted.
- **Ciliophora (ciliates)**: move by many **cilia** and have two kinds of nuclei (a macronucleus for daily function and a micronucleus for reproduction). *Paramecium* is the free-living model; *Balantidium coli* is the only ciliate that commonly infects humans, causing dysentery, often with pig contact.
- **Sporozoa (Apicomplexa)**: adults have no organs of locomotion and all are parasites with complex life cycles. *Plasmodium* causes **malaria** (female *Anopheles* mosquito; Unit 6). *Toxoplasma gondii* comes from cat faeces and undercooked meat and is dangerous in pregnancy. *Cryptosporidium* causes watery diarrhoea and its oocysts resist chlorine, so filtration is needed in water treatment.

Helminths: flukes, tapeworms and roundworms

Helminths are classified by body shape:

- **Platyhelminthes (flatworms)**, flat and without a body cavity. **Trematodes (flukes)** are leaf-shaped with suckers and usually use snails as intermediate hosts: *Schistosoma* (blood flukes; larvae penetrate skin in water), *Fasciola hepatica* (liver fluke). **Cestodes (tapeworms)** are ribbon-like, with a head (**scolex**) bearing suckers and sometimes hooks, and a chain of segments (**proglottids**), each with its own reproductive organs. *Taenia solium* (pork tapeworm), *T. saginata* (beef tapeworm) and *Echinococcus granulosus* (dog tapeworm, causes hydatid cysts in humans).

- **Nematodes (roundworms)**, round in section, with a complete gut and separate sexes. *Ascaris lumbricoides* (large roundworm; eggs in soil and on vegetables), *Enterobius vermicularis* (pinworm; night-time anal itching in children), the hookworms *Ancylostoma duodenale* and *Necator americanus* (larvae enter through bare feet and the adults suck blood, causing iron-deficiency anaemia), *Trichuris trichiura* (whipworm), *Trichinella spiralis* (larvae in undercooked pork muscle) and *Wuchereria bancrofti* (lymphatic filariasis or elephantiasis, spread by *Culex* mosquitoes).

The soil-transmitted worms (*Ascaris*, hookworm, *Trichuris*) affect school learning and growth in children, which is why India runs mass deworming of children with albendazole through its National Deworming Day programme.

Diagnosis. Stool microscopy is the base test: a **saline wet mount** shows motile trophozoites and helminth eggs, and an **iodine mount** stains cyst nuclei. Concentration methods (formalin–ether sedimentation, flotation) find scanty eggs. Blood films detect malaria and, taken at night, the microfilariae of *W. bancrofti*, which circulate mainly at night. Antigen tests and PCR are increasingly used.

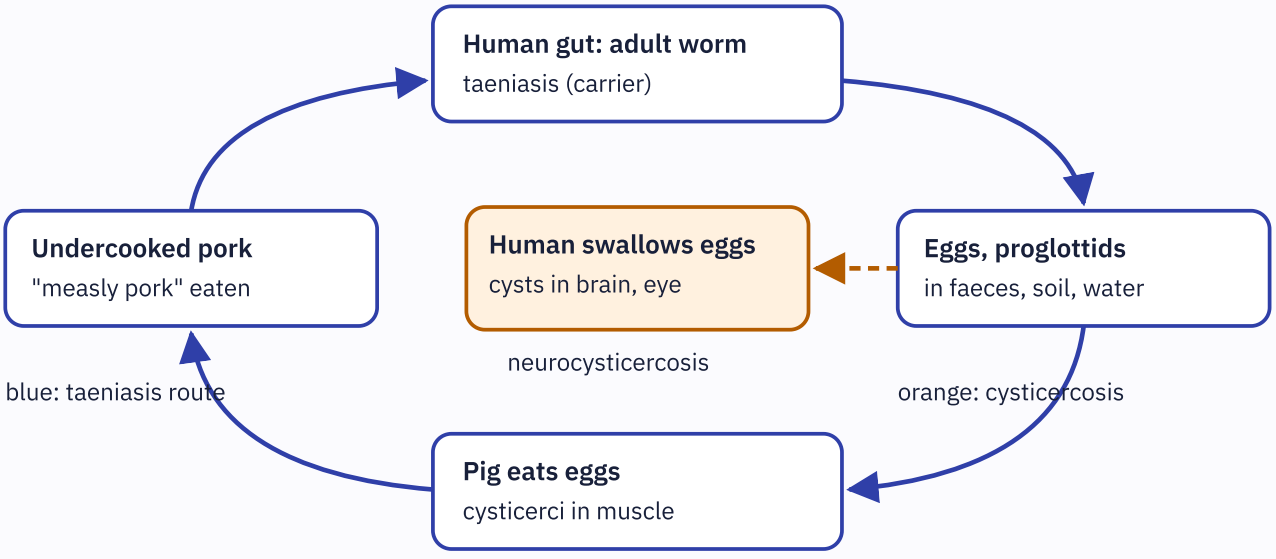


Figure 2.6.1 The two ways *Taenia solium* reaches people. Eating cysticerci in undercooked pork gives an adult tapeworm in the gut (taeniasis, blue ring). Swallowing eggs shed by a human carrier, through food, water or hands, makes the human the intermediate host, with cysts in tissues including the brain (cysticercosis, orange).

WORKED EXAMPLE

Question (6 marks). A 24-year-old vegetarian in Madurai who has never eaten pork has a seizure. A brain scan shows small cysts and a blood test is positive for *Taenia solium* antibodies. The family cook was recently treated for a "tapeworm". Explain how the patient was infected, and propose two control measures.

1. **Name the disease**: **neurocysticercosis**, cysticerci (larval cysts) of *T. solium* in the brain, a leading cause of acquired epilepsy in India.
2. **Which stage infects whom?** Pork with cysticerci gives the **adult worm** (taeniasis). **Eggs** give **cysticercosis**. So the patient must have swallowed eggs; eating pork is not needed, and pork cannot cause cysticercosis directly.
3. **Source**: the cook carried an adult worm and shed eggs in faeces. Without proper hand-washing after the toilet, eggs reached food the patient ate.
4. **Inside the patient**: eggs hatch in the gut, the larvae (oncospheres) enter the blood, lodge in brain and muscle and form cysts. The patient is acting as the intermediate host, a dead end for the worm.
5. **Control**: treat tapeworm carriers, especially food handlers (praziquantel or niclosamide); hand-washing with soap and safe sanitation; wash raw vegetables. For the taeniasis route, meat inspection and thorough cooking of pork.

EXAM TRAPS

- Cysts, not trophozoites, are the infective stage of *E. histolytica* and *Giardia*. Trophozoites are what you see in dysenteric stool.
- Taeniasis (adult worm, from undercooked pork) and cysticercosis (larval cysts, from eggs) are different diseases from the same worm. This is the examiner's favourite trap.
- Helminths are animals, not microbes; only their eggs and larvae are microscopic.
- *Anopheles* spreads malaria; *Culex* spreads bancroftian filariasis; sandflies spread kala-azar; tsetse flies spread sleeping sickness. Match vector to disease carefully.
- Ringworm is a fungus (Chapter 2.4) and pinworm is a nematode.

CHECK YOURSELF

- REMEMBER **Name the four classical groups of protozoa and their means of locomotion.** 2 marks

  Sarcodina, pseudopodia; Mastigophora, flagella; Ciliophora, cilia; Sporozoa, none in the adult (gliding in some stages).

- REMEMBER **Name the vector and the causative agent of kala-azar.** 2 marks

  Sandfly (*Phlebotomus*); *Leishmania donovani*.

- UNDERSTAND **Why do cysts spread protozoan diseases through water better than trophozoites?** 3 marks

  The cyst wall resists drying, cold and stomach acid, so cysts survive for weeks in water and pass through the stomach unharmed. Trophozoites are fragile and die outside the host and in gastric acid.

- UNDERSTAND **How do the bodies of cestodes and nematodes differ?** 3 marks

  Cestodes: flat, segmented into proglottids, with a scolex, no gut (absorb food through the body surface), mostly hermaphrodite. Nematodes: round, unsegmented, with a complete gut and separate sexes.

- APPLY **A child from a village who often walks barefoot has pale skin and low haemoglobin. Stool shows thin-shelled oval eggs. What is likely and why?** 3 marks

  Hookworm infection (*Ancylostoma* or *Necator*). Larvae in soil enter through bare feet; adults attach to the intestinal wall and suck blood, causing iron-deficiency anaemia. Treat with albendazole and advise footwear and toilets.

- APPLY **A city's water passed chlorination, yet an outbreak of watery diarrhoea is traced to *Cryptosporidium*. Explain and suggest a fix.** 3 marks

  Cryptosporidium oocysts resist chlorine at normal doses. Add effective filtration (coagulation plus sand or membrane filtration) or UV disinfection, and protect the source from faecal contamination.

– ANALYZE **Compare protozoa and helminths as parasites under cell organisation, multiplication in the host and diagnosis.** 5 marks

Protozoa are single cells and most multiply inside the host, so a few cysts can cause heavy infection; diagnosed by trophozoites or cysts in stool or parasites in blood. Most helminths are multicellular and do not multiply in the human host (worm burden depends on how many eggs or larvae were taken in), with exceptions such as *Strongyloides*; diagnosed by eggs, larvae or proglottids in stool, or microfilariae in night blood.

BEYOND THE SYLLABUS

India has run a kala-azar elimination programme for years, and it uses single-dose liposomal amphotericin B and indoor residual spraying against sandflies. Malaria control now faces artemisinin partial resistance first seen in Southeast Asia and an invasive urban mosquito, *Anopheles stephensi*. Helminths have another side too: the "hygiene hypothesis" studies whether their absence raises allergy and autoimmune disease. Search "kala-azar elimination India" or "One Health parasitology". Not for the exam.

2.7 Extremophiles and their adaptations

Extremophiles are microbes that grow best in conditions that would kill most life: near boiling water, below freezing, in saturated brine, in acid or alkali, under crushing pressure or heavy radiation. Most are archaea or bacteria. Their enzymes, called **extremozymes**, keep working where ordinary enzymes fall apart; the best known is *Taq* DNA polymerase, which made PCR practical.

You need: the cell wall and membrane of 2.2 (archaeal walls and lipids) and the effect of temperature on growth (Unit 4, growth kinetics).

The kinds of extremophile

Every organism has three **cardinal temperatures**: a minimum, an optimum and a maximum for growth. Groups are named by the optimum (Brock's definitions; some books shift the limits slightly).

- **Psychrophiles**: optimum 15 °C or lower, maximum about 20 °C; polar seas and glaciers.

- **Thermophiles**: optimum 45–80 °C, for example *Thermus aquaticus*, first isolated from a hot spring in Yellowstone.
- **Hyperthermophiles**: optimum above 80 °C, mostly archaea such as *Pyrococcus furiosus* and *Methanopyrus kandleri*. *M. kandleri* strain 116 was reported in 2008 to grow at 122 °C under 20 MPa pressure.
- **Halophiles**: need salt. Extreme halophiles such as *Halobacterium salinarum* need very high NaCl and colour solar salt pans red.
- **Acidophiles** (optimum below about pH 5; *Acidithiobacillus*, and *Picrophilus*, which grows near pH 0) and **alkaliphiles** (optimum above about pH 9; soda lakes, alkaliphilic *Bacillus* species).
- **Piezophiles** grow best under deep-sea pressure; *Deinococcus radiodurans* survives huge radiation doses by repairing its broken DNA.

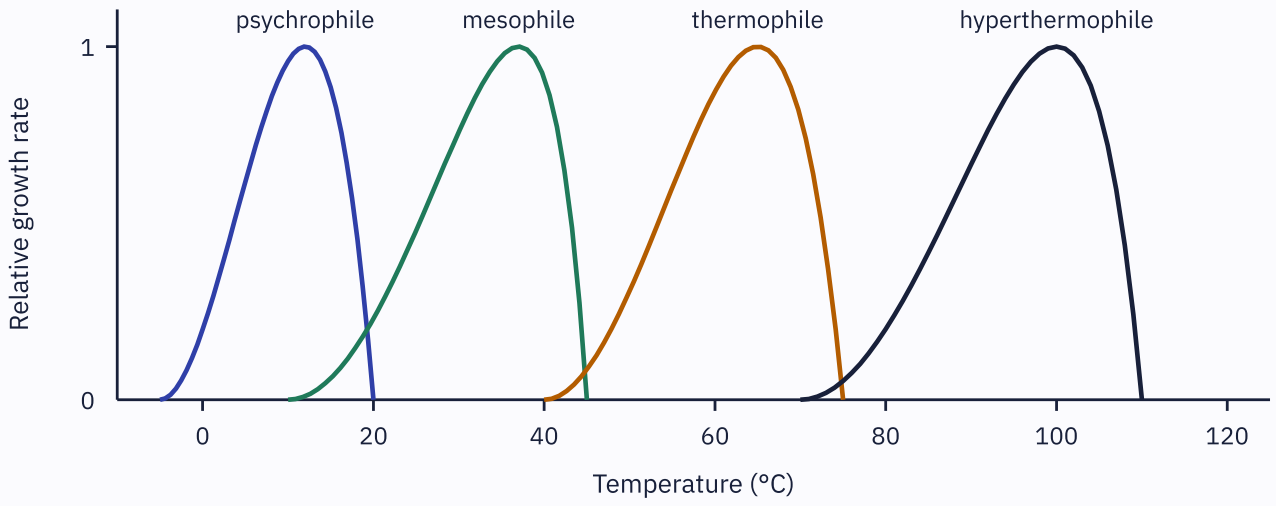


Figure 2.7.1 Growth rate against temperature for four groups, computed from a standard cardinal-temperature model with illustrative minimum, optimum and maximum values (for example 10, 37 and 45 °C for the mesophile). Each curve rises slowly to its optimum, then crashes within a few degrees.

How they survive

- **Heat.** Proteins have more salt bridges and tighter hydrophobic cores. Archaea have **ether-linked lipids**, more stable than the ester links of bacteria, and many hyperthermophiles have **monolayer membranes** of tetraether lipids that cannot peel apart. **Reverse gyrase** adds positive supercoils that stabilise DNA; it is found in hyperthermophiles and not in mesophiles.
- **Cold.** More unsaturated fatty acids keep membranes fluid; flexible enzymes work in the cold; cold-shock and antifreeze proteins help.
- **Salt.** Brine pulls water out of the cell. The **salt-in** strategy (*Halobacterium*) piles up K^+ inside to match the outside, so its proteins are acidic and need salt. The **compatible-solute** strategy

(most halotolerant bacteria) makes small organic molecules such as glycine betaine and ectoine that balance water without harming enzymes.

- **Acid and alkali.** The cytoplasm is kept close to neutral in both. Acidophiles keep protons out; alkaliphiles often run transport on a sodium gradient.

WORKED EXAMPLE

Question (5 marks). An isolate from the Manikaran hot spring in Himachal Pradesh gives these specific growth rates, μ (h^{-1}): 45 °C, 0.05; 55 °C, 0.45; 65 °C, 0.90; 70 °C, 1.10; 75 °C, 0.95; 80 °C, 0.30; 85 °C, 0. (a) Estimate the cardinal temperatures and classify the organism. (b) Find the doubling time at the optimum. (c) Suggest one industrial use of its enzymes.

1. **Optimum**: highest μ in the data, 1.10 h^{-1} at **about 70 °C**.
2. **Minimum**: growth is barely detectable at 45 °C, so the minimum is a little below that, about 40–45 °C. **Maximum**: between 80 °C (still growing) and 85 °C (none), about 82 °C.
3. **Class**: optimum between 45 and 80 °C, so a **thermophile** (not a hyperthermophile, whose optimum is above 80 °C).
4. **Doubling time** in exponential growth: $t_d = \ln 2 / \mu = 0.693 / 1.10\ h^{-1} = 0.63$ h ≈ **38 minutes**.
5. **Use**: heat-stable enzymes, such as a PCR polymerase or amylases for hot starch processing.

EXAM TRAPS

- Thermophile versus hyperthermophile is set by the optimum (80 °C), not by the maximum.
- Psychrotolerant is not psychrophile: *Listeria* grows in the fridge but has a mesophilic optimum.
- Archaeal lipids are ether-linked and built from isoprenoid chains; bacterial lipids are ester-linked fatty acids.
- *Taq* polymerase is from the bacterium *Thermus aquaticus*; *Pfu* polymerase is from the archaeon *Pyrococcus furiosus* and has proofreading.

CHECK YOURSELF

– REMEMBER **Define a hyperthermophile and name one.** 2 marks

A microbe with an optimum growth temperature above 80 °C, for example *Pyrococcus furiosus* or *Methanopyrus kandleri*.

– REMEMBER **Name two compatible solutes.** 1 mark

Glycine betaine and ectoine (also proline, trehalose).

– UNDERSTAND **Why do growth curves fall steeply above the optimum temperature?** 3 marks

Above the optimum, key proteins denature and membranes become too fluid and leaky; these failures are cooperative and fast, so growth stops within a few degrees. Below the optimum, reaction rates fall gradually.

– APPLY **A psychrophile grows with μ = 0.10 h^{-1} at its optimum. Find its doubling time.** 2 marks

$t_d = 0.693 / 0.10 =$ **6.9 h**.

– ANALYZE **Explain why *Taq* polymerase made automated PCR possible.** 4 marks

Each PCR cycle heats DNA to about 94–95 °C to separate the strands. The first enzyme used, from *E. coli*, was destroyed each cycle and had to be re-added. *Taq* survives repeated heating, so one addition lasts the run, and its 72 °C working temperature improves specificity.

BEYOND THE SYLLABUS

Extremophiles shape the search for life on Mars and the icy moons Europa and Enceladus. Ectoine is sold in skin creams. Search "astrobiology extremophiles" or "extremozymes industrial applications". Not for the exam.

Unit summary

KEY TERMS AND FORMULAS

TERM	MEANING OR FORMULA	CHAPTER
S/V of a sphere	S/V = 3/r; smaller cells exchange faster	2.1
70S ribosome	50S + 30S subunits (Svedberg units do not add)	2.1
Endospore	Dormant, heat-resistant cell with calcium dipicolinate; *Bacillus*, *Clostridium*	2.1
Peptidoglycan	NAG–NAM chains (β-1,4) cross-linked by peptides; cut by lysozyme, built by penicillin-sensitive transpeptidases	2.2
Gram-positive wall	Thick peptidoglycan + teichoic acids; no outer membrane	2.2
Gram-negative wall	Thin peptidoglycan in periplasm + outer membrane with LPS and porins	2.2
LPS	Lipid A (endotoxin) + core polysaccharide + O-antigen	2.2
Mycoplasma	Wall-less, sterol-containing, pleomorphic; penicillin-resistant; fried-egg colonies	2.3
Actinomycetes	Gram-positive filamentous bacteria; *Streptomyces* makes most natural antibiotics	2.3
Fungal cell	Chitin–glucan wall, ergosterol membrane, 80S ribosomes	2.4
Sexual spores of fungi	Zygospore, ascospore (8 in an ascus), basidiospore (4 on a basidium)	2.4
Algal groups	Classified by pigment, storage food, wall and flagella	2.5
Microalgal oil yield	area × productivity × lipid fraction × days × extraction ÷ density	2.5
Trophozoite / cyst	Active feeding form / resistant infective form	2.6
Taeniasis / cysticercosis	Adult worm from pork / larval cysts from swallowed eggs	2.6
Doubling time	$t_d = \ln 2 / \mu = 0.693 / \mu$	2.7
Thermophile / hyperthermophile	Optimum 45–80 °C / above 80 °C	2.7

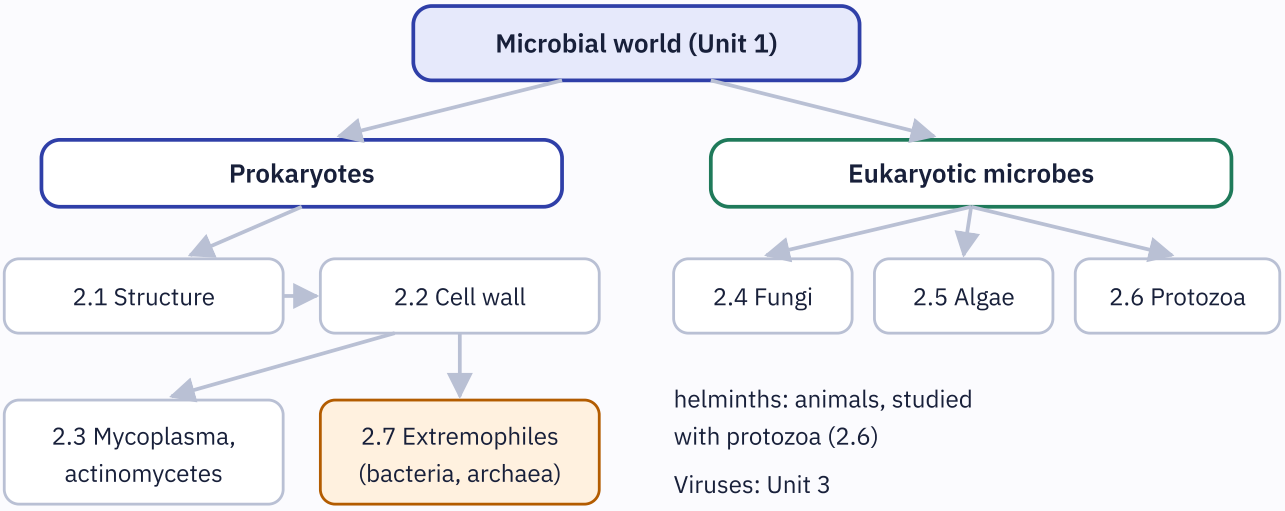


Figure 2.S.1 How the chapters connect. The prokaryote branch builds from the general cell (2.1) to its wall (2.2), then to bacteria that break the pattern (2.3) and life at the limits (2.7). The eukaryote branch covers the three groups of eukaryotic microbes, with helminths added because they are diagnosed the same way.

1. Bacteria are named by shape (coccus, bacillus, spiral) and arrangement (diplo-, strepto-, staphylo-, tetrad, sarcina); small size gives a high surface-to-volume ratio and fast growth.
2. Outside the wall: capsule, flagella (filament, hook, basal body) and pili; inside: nucleoid, plasmids, 70S ribosomes and inclusions; endospores are for survival, not reproduction.
3. Peptidoglycan is the target of lysozyme (glycan bond) and penicillin (cross-links). Gram-positives have a thick layer with teichoic acids; Gram-negatives a thin layer plus an outer membrane with LPS.
4. The Gram stain works because the decolouriser shrinks thick peptidoglycan but strips the Gram-negative outer membrane.
5. Mycoplasmas have no wall, contain sterols, pass 0.45 µm filters and resist penicillin; actinomycetes are filamentous Gram-positive bacteria and the main source of natural antibiotics.
6. Fungi have chitin walls and ergosterol; identify moulds by septa and spore type; mycoses range from superficial to opportunistic, and mycotoxins such as aflatoxin are heat stable.
7. Algae are grouped by pigment, storage food and wall; they supply agar, carrageenan, alginate, food and pigments, and some make shellfish toxins.
8. Protozoa are grouped by locomotion; cysts spread infection. Helminths are flukes, tapeworms and roundworms; the stage swallowed decides the disease.
9. Extremophiles survive heat, cold, salt, acid and pressure by special lipids, proteins and solutes, and their enzymes power PCR and industry.

Exam question bank

Remember and understand

– UNDERSTAND **With a labelled diagram, describe the structure of a bacterial cell and the function of each part.** 12 marks

Diagram with capsule, wall, membrane, nucleoid, plasmid, ribosomes, inclusions, flagellum, fimbriae. Functions: capsule (anti-phagocytic, adhesion); wall (shape, osmotic protection); membrane (transport, respiration); nucleoid and plasmids (genes; extra traits); 70S ribosomes (protein synthesis); inclusions (stores); flagella (motility with filament, hook, basal body; arrangements); pili (attachment, conjugation); endospore (survival).

– UNDERSTAND **Compare the cell walls of Gram-positive and Gram-negative bacteria and explain the mechanism of the Gram stain.** 16 marks

Peptidoglycan chemistry (NAG–NAM, peptides, cross-links). Table: thickness, teichoic acids, outer membrane, LPS, porins, periplasm, lysozyme and penicillin sensitivity, endotoxin. Two side-by-side diagrams. Stain mechanism: crystal violet–iodine complex, decolouriser dehydrates thick PG vs extracts outer membrane lipid, safranin counterstain.

– REMEMBER **Write short notes on (a) mycoplasma and (b) actinomycetes.** 8 marks

(a) Mollicutes, no wall, sterols, size, pleomorphism, fried-egg colonies, penicillin resistance, *M. pneumoniae*, phytoplasmas, cell-culture contamination. (b) Gram-positive, high G+C, substrate and aerial mycelium, conidia chains, geosmin, *Streptomyces* antibiotics with examples, *Frankia*, *Nocardia*, *Actinomyces*.

– UNDERSTAND **Describe the general characteristics, reproduction and classification of fungi.** 12 marks

Eukaryotic, heterotrophic, absorptive; chitin, ergosterol; yeasts, moulds, dimorphism; septate vs coenocytic hyphae; asexual spores (sporangiospores, conidia, arthro-, chlamydo-, blastoconidia); sexual spores and the groups (Zygo-, Asco-, Basidiomycota, Deuteromycota); examples.

– UNDERSTAND **Classify algae with the basis of classification and describe their modes of reproduction.** 12 marks

Basis: pigments, storage, wall, flagella; table of green, brown, red, diatoms, dinoflagellates, euglenoids; thallus forms; vegetative, asexual (zoospores, aplanospores, akinetes) and sexual (iso-, aniso-, oogamy, conjugation); three life cycles with diagram; note that cyanobacteria are prokaryotes.

Apply

– APPLY **Discuss the industrial uses of algae, and calculate the annual oil output of a 5,000 m² pond at 12 g m^{-2} day^{-1}, 30% lipid, 320 days and 85% extraction (oil density 0.9 kg/L).** 12 marks

Uses: agar, carrageenan, alginate; Spirulina, *Chlorella*; β-carotene, astaxanthin, phycocyanin, DHA; diatomaceous earth; wastewater ponds; biofuel. Calculation: 5,000 × 12 = 60 kg day^{-1}; lipid 18 kg day^{-1}; × 320 × 0.85 = 4,896 kg; ÷ 0.9 = **5,440 L per year**.

– APPLY **Describe the classification of protozoa with one human disease from each group, giving the mode of transmission.** 12 marks

Sarcodina: *E. histolytica*, amoebic dysentery, cysts in water/food. Mastigophora: *Giardia* (cysts in water), *Leishmania* (sandfly), *Trypanosoma* (tsetse). Ciliophora: *Balantidium* (cysts, pig contact). Sporozoa: *Plasmodium* (*Anopheles*), *Toxoplasma*, *Cryptosporidium*. Life stages and reproduction.

– APPLY **Outline a programme to screen soil actinomycetes for new antibiotics, from sample to confirmed activity.** 10 marks

Sample collection; pretreatment (drying, heat) to reduce other bacteria; selective media (starch–casein, with antifungals); recognising colonies; pure culture; primary screen by cross-streak; secondary screen by agar-well diffusion of culture filtrate; activity spectrum against Gram-positive, Gram-negative and fungal test organisms; preservation of the isolate.

– APPLY **Explain how food can transmit algal toxins, mycotoxins and parasites, with one example and one control measure for each.** 12 marks

Algal toxins: saxitoxin in shellfish (paralytic shellfish poisoning); monitor blooms and close harvest areas. Mycotoxins: aflatoxin in groundnuts and maize; dry storage and testing. Protozoa: *Giardia*, *Cryptosporidium* in water and produce; filtration, safe water. Helminths: *T. solium* and *Trichinella* in pork; meat inspection, thorough cooking, hygiene of food handlers. Note heat stability of toxins.

Analyze and evaluate

– ANALYZE **"The cell wall decides the fate of a bacterium." Evaluate this statement using Gram-positive bacteria, Gram-negative bacteria, mycoplasma and archaea.** 16 marks

Wall gives shape and osmotic protection; decides Gram reaction, antibiotic and lysozyme sensitivity, endotoxin, surface antigens. Mycoplasma: no wall, so pleomorphic, sterols, penicillin resistance, need for osmotically stable habitat. Archaea: pseudomurein or protein walls, no penicillin target, ether lipids (link to 2.7). Counterpoint: other traits (plasmids, capsule, metabolism) also decide fate. Spans 2.1, 2.2, 2.3, 2.7.

– ANALYZE **Compare bacteria, fungi, algae and protozoa in a table under cell type, wall, nutrition, reproduction, size and one useful and one harmful example each.** 16 marks

Bacteria: prokaryotic, peptidoglycan, varied nutrition, binary fission, 1–2 µm, *Streptomyces*/*V. cholerae*. Fungi: eukaryotic, chitin, absorptive heterotrophs, spores, yeasts 3–5 µm to large mycelia, *Penicillium*/*A. flavus*. Algae: eukaryotic, cellulose (+ agar, alginate, silica), photosynthetic, spores and gametes, µm to metres, *Kappaphycus*/*Alexandrium*. Protozoa: eukaryotic, no wall, ingestive heterotrophs, fission and conjugation, *Paramecium* or soil protozoa in wastewater/*E. histolytica*. Spans 2.1–2.6.

– ANALYZE **A vegetarian student develops seizures from cysticercosis, while their pork-eating roommate has only intestinal taeniasis. Analyse the life cycle of *Taenia solium* to explain both, and evaluate the control strategies.** 10 marks

Life cycle with definitive host (human, adult worm) and intermediate host (pig, cysticerci). Roommate: cysticerci in undercooked pork, adult worm. Student: swallowed eggs from a carrier, became an accidental intermediate host. Control: treat carriers, hygiene, sanitation, meat inspection, cooking, pig management; which breaks which route.

– ANALYZE **Discuss the adaptations of thermophiles, psychrophiles and halophiles, and evaluate the industrial value of extremophiles.** 12 marks

Definitions by optimum; heat: salt bridges, ether and tetraether lipids, reverse gyrase; cold: unsaturated lipids, flexible enzymes, antifreeze proteins; salt: salt-in vs compatible solutes. Value: *Taq* and *Pfu* in PCR, alkaline proteases in detergents, cold-active enzymes, ectoine; limits: slow growth, special reactors. Growth-curve sketch.

Glossary

Actinomycetes	Gram-positive, high G+C bacteria that grow as branching filaments; *Streptomyces* is the main genus.
Aflatoxin	Heat-stable, liver-damaging mycotoxin from *Aspergillus flavus* and *A. parasiticus*.
Agar	Gel from red algae, used to solidify culture media.
Alginate	Gel-forming polysaccharide from brown algae; used to entrap cells and enzymes.
Ascus	Sac in which ascomycete fungi form their sexual spores, usually eight.
Basidium	Club-shaped cell bearing basidiospores, usually four, on its outside.
Capsule	Organised, attached layer of the glycocalyx that resists phagocytosis.
Cestode	Tapeworm: flat, segmented helminth with a scolex and proglottids.
Compatible solute	Small organic molecule (glycine betaine, ectoine) that balances osmotic stress without harming enzymes.
Conidia	Asexual fungal or actinomycete spores formed free, not inside a sac.
Cyst	Dormant, thick-walled stage of a protozoan; usually the infective stage.
Cysticercosis	Infection of tissues with larval cysts of *Taenia solium*, acquired by swallowing eggs.
Dimorphic fungus	Fungus that grows as a mould at about 25 °C and a yeast at 37 °C.
Endospore	Dormant, highly resistant cell formed inside some Gram-positive rods.
Endotoxin	Lipid A of Gram-negative LPS, released when cells die; causes fever and shock.
Ergosterol	The main sterol of fungal membranes; target of many antifungals.
Extremophile	Microbe that grows best under extreme temperature, salt, pH, pressure or radiation.

Lipopolysaccharide (LPS)	Outer-leaflet molecule of the Gram-negative outer membrane: lipid A, core, O-antigen.
Lysozyme	Enzyme that cuts the β-1,4 bond between NAM and NAG in peptidoglycan.
Mycelium	Mass of branching hyphae.
Mycoplasma	Wall-less bacterium of the class Mollicutes with sterols in its membrane.
Peptidoglycan	Wall polymer of NAG and NAM chains cross-linked by short peptides.
Periplasm	Space between the outer and plasma membranes of Gram-negative bacteria.
Plasmid	Small, circular, self-replicating DNA outside the chromosome.
Protoplast	Cell with its wall completely removed, bounded only by the membrane.
Teichoic acid	Polymer of glycerol or ribitol phosphate in Gram-positive walls.
Thallus	Body of an alga (or fungus) not divided into root, stem and leaf.
Trophozoite	Active, feeding, dividing stage of a protozoan.

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Syllabus: the University free n easy universal syllabus, built from top universities' syllabi.

Built for the University free n easy YouTube channel from the universal syllabus of Microbiology (Oct 2026). Written with top universities' syllabi and standard textbooks; check a formula against your prescribed book before an exam, and report mistakes on the channel.