Microbiology

Unit 2 · Lesson 2 · Odd bacteria and fungi

The notes for Lesson 2 of Unit 2: chapters 2.3 to 2.4 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 3 | 2 chapters | Chapters 2.3 to 2.4

About these notes

These are the notes for **Lesson 2 · Odd bacteria and fungi** of the University free n easy video lessons on Microbiology Unit 2, Bacteria, Fungi, Algae and Protozoa. They are chapters 2.3 to 2.4 of the unit book, unchanged, so a page here is the same page in the full book.

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.

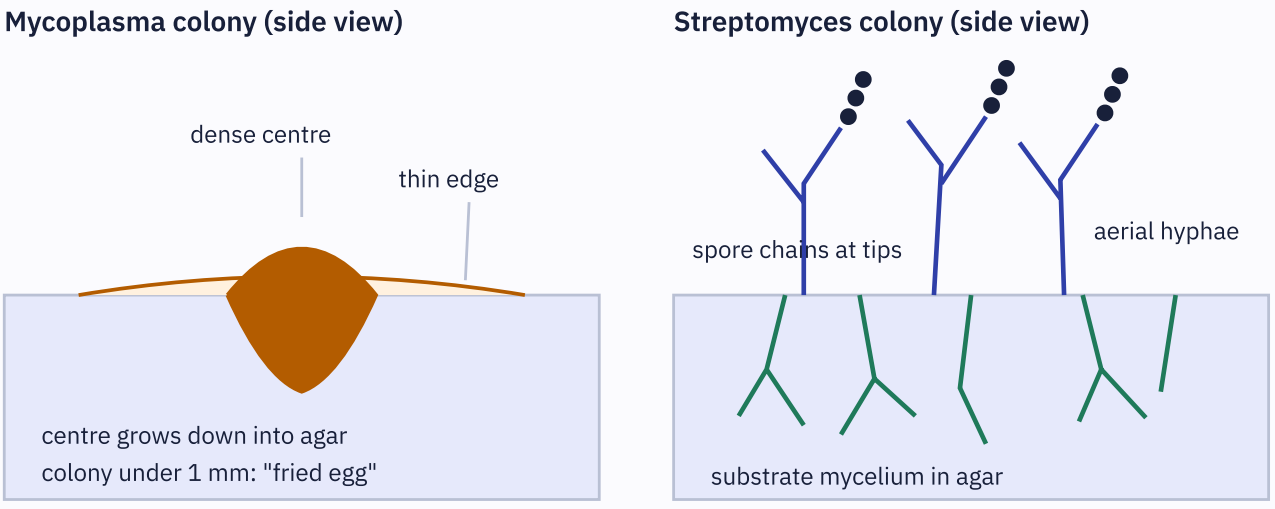
Actinomycetes: filamentous bacteria

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.

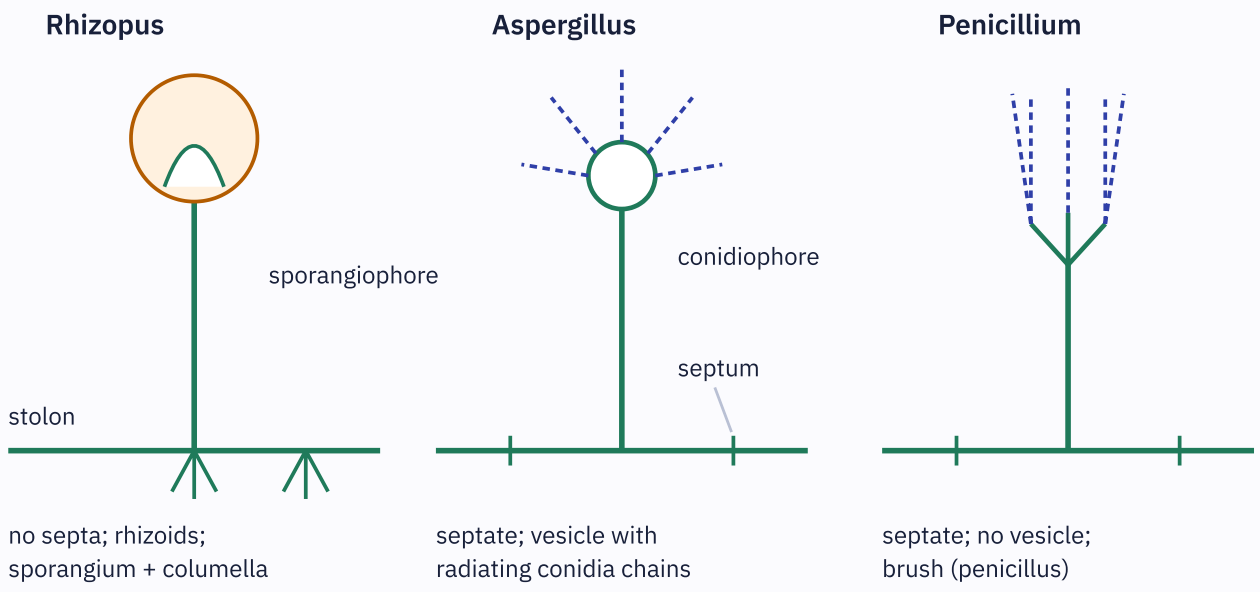


Figure 2.4.1 Three common moulds under the low-power microscope, as seen in a lactophenol cotton blue mount. Dashed lines are chains of conidia. Notice the two things to check first: are there septa, and are spores enclosed in a sac (*Rhizopus*) or free on a swollen vesicle (*Aspergillus*) or on brush-like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

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
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

Glossary (terms used in this lesson)

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.

Ascus Sac in which ascomycete fungi form their sexual spores, usually eight.

Basidium Club-shaped cell bearing basidiospores, usually four, on its outside.

Conidia Asexual fungal or actinomycete spores formed free, not inside a sac.

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.

Ergosterol	The main sterol of fungal membranes; target of many antifungals.
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.

The full unit book

Every chapter of Unit 2, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 2 · Bacteria, Fungi, Algae and Protozoa (one file, works offline).

Sources and further reading

Textbooks

- Madigan, Bender, Buckley, Sattley and Stahl, *Brock Biology of Microorganisms*, Pearson: cell structure, cell walls, archaea and extremophiles (from memory; check edition).
- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: bacterial structure, fungi, algae and protists (from memory; check edition).
- Pelczar, Chan and Krieg, *Microbiology*, Tata McGraw-Hill: the classic Indian course text for morphology and the microbial groups (from memory; check).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson: eukaryotic microbes, helminths and food-borne disease (from memory; check).
- Ananthanarayan and Paniker's *Textbook of Microbiology* and Paniker's *Textbook of Medical Parasitology*, Universities Press / Jaypee: Indian context for mycology, protozoa and helminths (from memory; check publishers).

Open resources used

- OpenStax *Microbiology*, "Unique Characteristics of Prokaryotic Cells", as adapted on LibreTexts (CC BY): wall thicknesses, LPS parts and named inclusions. bio.libretexts.org
- Takai and co-workers, "Cell proliferation at 122 °C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation", *PNAS* 105 (2008), abstract: *Methanopyrus kandleri* strain 116 growth limit of 122 °C at 20 MPa. ui.adsabs.harvard.edu
- US Centers for Disease Control and Prevention, "About Cysticercosis": eggs as the infective stage, and why eating pork cannot cause cysticercosis. cdc.gov

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