Microbiology

Unit 2 · Lesson 1 · The bacterial cell and its wall

The notes for Lesson 1 of Unit 2: chapters 2.1 to 2.2 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 1 of 3 | 2 chapters | Chapters 2.1 to 2.2

About these notes

These are the notes for **Lesson 1 · The bacterial cell and its wall** of the University free n easy video lessons on Microbiology Unit 2, Bacteria, Fungi, Algae and Protozoa. They are chapters 2.1 to 2.2 of the unit book, unchanged, so a page here is the same page in the full book.

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.

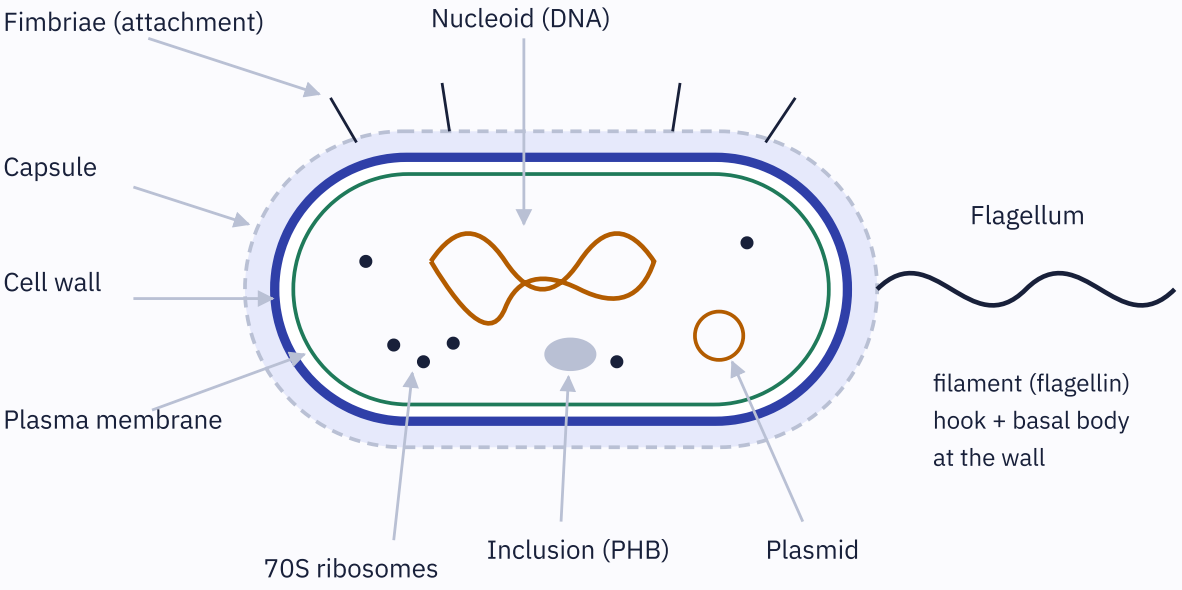


Figure 2.1.1 A rod-shaped bacterium in section. From outside in: capsule (dashed), thick cell wall, plasma membrane, then a cytoplasm with no nucleus. Notice that the DNA lies free in a nucleoid and the plasmid is a separate small circle.

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 μm^{-1}**. Including the two end caps adds $2\pi r^2$ to S and raises the ratio a little, to 5 μm^{-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.

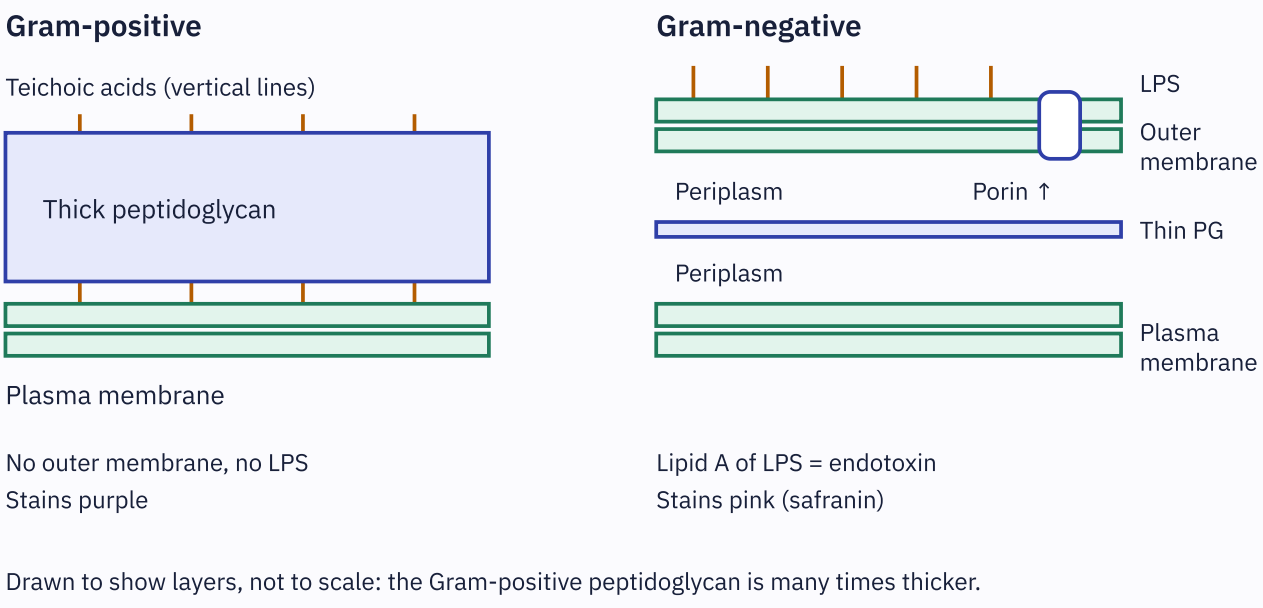


Figure 2.2.1 The two envelopes, outside at the top. The Gram-positive cell has one thick peptidoglycan layer threaded with teichoic acids; the Gram-negative cell has an outer membrane with LPS and porins, a periplasm, and only a thin peptidoglycan layer.

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.

Key terms of this lesson

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

Glossary (terms used in this lesson)

Capsule Organised, attached layer of the glycocalyx that resists phagocytosis.

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.

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.

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.

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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