B.PHARM SEMESTER III

BP308T Pharmaceutical Microbiology

Unit I — Microorganisms in the Pharma Industry

FINAL UNIT NOTES

Chapters 1–3

Classification • Morphology • Growth • Culture Media • Industrial Applications

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CHAPTER 1 — Introduction to Microorganisms and Their Role in the Pharmaceutical Industry

(Unit I: Introduction and role of microorganisms in pharmaceutical industry)

1. Learning Objectives

After studying this chapter, you will be able to:

1. Define microbiology, microorganism, and pharmaceutical microbiology.
2. Name the main groups of microorganisms: bacteria, fungi, viruses, protozoa, and algae.
3. Explain the difference between prokaryotes and eukaryotes with examples.
4. Describe how microorganisms help make medicines (antibiotics, vitamins, vaccines, enzymes).
5. Explain the pharmaceutical importance of microorganisms — both useful and harmful roles.
6. Classify bacteria by shape, arrangement, and oxygen requirement.

2. Definitions

Term	Definition (simple)
Microbiology	The scientific study of living things that are too small to be seen with the naked eye.
Microorganism (microbe)	A living organism so tiny that you need a microscope to see it. Examples: bacteria, fungi, viruses.
Pharmaceutical microbiology	The branch of microbiology that deals with the role of microbes in making medicines, keeping medicines safe from contamination, and testing medicines.
Prokaryote	A simple cell with NO true nucleus — the genetic material floats free in the cell. Example: bacteria.
Eukaryote	A cell WITH a true nucleus (a protected room for genetic material) and membrane-bound structures. Examples: fungi, protozoa, human cells.
Pathogen	A microorganism that causes disease in humans, animals, or plants.
Sterile	Completely free of ALL living microorganisms (including spores).

3. Terminology (Key Terms)

Term	Meaning in plain words
Cocci	Round-shaped bacteria (singular: coccus).
Bacilli	Rod-shaped bacteria (singular: bacillus).
Spirilla	Spiral-shaped bacteria.
Vibrio	Comma-shaped (curved-rod) bacteria.
Colony	A visible group of millions of bacteria grown on a solid medium, all from one cell.
Culture	Growing microorganisms in a laboratory under controlled conditions.
Medium (plural: media)	The food/liquid/gel mixture on which microbes are grown.
Aerobe	A microbe that needs oxygen to grow.
Anaerobe	A microbe that grows WITHOUT oxygen (oxygen may even kill it).
Facultative anaerobe	A flexible microbe — can grow with or without oxygen.

Term	Meaning in plain words
Sporulation	Formation of a tough resting cell (spore) that survives heat, drying, and chemicals.
Contamination	Unwanted microorganisms entering a product, sample, or area.
Flora (microflora)	The normal, usually harmless microbes living on the human body.

4. Explanation

4.1 What are microorganisms?

> **Technical concept:** Microorganisms are living organisms with a size generally less than 0.1 mm, invisible to the unaided eye, including bacteria, fungi (yeasts and moulds), viruses, protozoa, and algae.

> **Simple explanation:** Imagine a world of tiny living things that are all around you — on your skin, in the air, in water, on every surface — but you cannot see a single one without a microscope. That is the world of microbes. A bacterium is about 1,000 times smaller than the dot at the end of this sentence.

Example: If you leave a slice of bread in a damp place for a week, green fuzzy growth appears — that is a fungus (mould) colony, visible only because millions of microbes grew together.

> **Key takeaway: Microbes are everywhere, invisible individually, and they can be either our helpers (making medicines) or our enemies (causing infections and spoiling medicines).**

4.2 Prokaryotes vs Eukaryotes

> **Technical concept:** Prokaryotic cells lack a membrane-bound nucleus and organelles; eukaryotic cells possess a true nucleus enclosed by a nuclear membrane plus organelles like mitochondria.

> **Simple explanation:** Think of a prokaryotic cell as a small open room — everything is in one space with no walls. A eukaryotic cell is like a big house with separate rooms — the nucleus is the "bedroom" where the genetic material sleeps safely behind walls. Bacteria are "open rooms" (prokaryotes); fungi and our own body cells are "houses with rooms" (eukaryotes).

Example: *E. coli* (a bacterium causing food poisoning) is a prokaryote. The yeast *Saccharomyces cerevisiae* (used in fermentation) is a eukaryote.

Diagram to draw (describe): Draw two large circles side by side. Left circle labelled "PROKARYOTE (Bacterium)": show free-floating twisted thread labelled "DNA (no nucleus)", small dots labelled "ribosomes", outer boundary labelled "cell wall + cell membrane". Right circle labelled "EUKARYOTE (Fungus/Human cell)": show an inner circle labelled "nucleus (DNA inside)", bean shapes labelled "mitochondria", outer boundary labelled "cell membrane". Add a caption: "Prokaryote = no true nucleus; Eukaryote = true nucleus."

> **Key takeaway: Bacteria = prokaryotes (simple, no nucleus). Fungi, protozoa, algae = eukaryotes (true nucleus). Viruses are not even cells — they are particles that can only multiply inside a host cell.**

4.3 The main groups of microorganisms

Group	Cell type	Size	Key feature	Pharma example
Bacteria	Prokaryote	~1–5 µm	Single cells, divide by binary fission	*Streptomyces* makes streptomycin antibiotic
Fungi (yeasts & moulds)	Eukaryote	Yeast ~5–10 µm; moulds form long threads	Yeasts are single cells; moulds grow as fuzzy filaments	*Penicillium* mould gave the world penicillin
Viruses	Not a cell	~0.02–0.3 µm	Only grow inside a living host cell	Used to make vaccines
Protozoa	Eukaryote	~10–50 µm	Single-celled animal-like microbes	Cause malaria (*Plasmodium*) — treated with drugs
Algae	Eukaryote	Variable	Plant-like, make food by photosynthesis	Agar (culture medium) comes from red algae

Analogy: Think of the microbial world like a zoo with different sections. The bacteria section has the tiniest, simplest animals. The fungi section has the yeast "birds" and mould "vines". The virus section has thieves that can only survive inside other animals' homes (host cells).

4.4 Microorganisms and medicines — the good side

Technical concept: Microbes are used as production organisms for antibiotics, vitamins, amino acids, enzymes, organic acids, vaccines, and biotechnological products (e.g., insulin).

Simple explanation: Many of our most important medicines are actually made BY microbes, or made WITH the help of microbes. The microbe is the tiny factory worker inside a big steel tank (fermenter) that produces the medicine.

Examples:

Medicine type	Microbe that produces it	Use
Penicillin	Mould *Penicillium chrysogenum*	Kills bacteria (antibiotic)
Streptomycin	Bacterium *Streptomyces griseus*	Treats tuberculosis (antibiotic)
Cephalosporins	Mould *Acremonium chrysogenum* (earlier *Cephalosporium*)	Treats bacterial infections (antibiotic)
Vitamin B12	Bacteria (*Propionibacterium*)	Treats anaemia
Citric acid	Mould *Aspergillus niger*	Used in medicines and food
Insulin (human)	Genetically modified *E. coli* or yeast	Treats diabetes
Vaccines	Weakened/killed microbes or their parts	Prevent diseases (polio, measles)

Key takeaway: **Without microbes, the pharmacy shelf would be nearly empty — antibiotics, vitamins, vaccines, and insulin all come from microbial work.**

4.5 The harmful side — why pharmacists must control microbes

Technical concept: Microorganisms contaminate pharmaceutical products during manufacturing, storage, and use, causing spoilage, loss of potency, and infections in patients, especially via non-sterile and sterile dosage forms.

Simple explanation: The same microbes that make medicines can also RUIN medicines. A cough syrup contaminated with bacteria can poison a child. An eye drop with fungus can blind a patient. That is why every medicine factory must fight contamination — and that fight is the subject of Units II and III.

Flowchart — the two faces of microbes in pharmacy:

```
MICROORGANISMS IN PHARMACY
        |
        ├── USEFUL (friends)
        |      ├── Make antibiotics (penicillin, streptomycin)
        |      ├── Make vitamins, enzymes, amino acids
        |      ├── Make vaccines
        |      └── Make insulin by genetic engineering
        |
        └── HARMFUL (enemies)
               ├── Contaminate medicines during manufacture
               ├── Spoil syrups, creams, tablets (bad smell, gas, colour change)
               └── Cause infections when injected/swallowed (especially dangerous
                   in sterile products like injections and eye drops)
```

4.6 Classification of bacteria (shapes, arrangements, oxygen needs)

Technical concept: Bacteria are classified morphologically (cocci, bacilli, spirilla, vibrio), by arrangement (strepto = chains, staphylo = clusters, diplo = pairs), by Gram staining, and by oxygen requirement (aerobic, anaerobic, facultative).

Simple explanation: Just as we describe people as tall/short, bacteria are described by their shape. Round ones are "cocci" (like balls), rod ones are "bacilli" (like sticks), spiral ones are "spirilla" (like springs).

Diagram to draw (describe): Draw four rows of simple sketches. Row 1: small circles in pairs, chains, and grape-like clusters — label "Cocci: diplococci (pairs), streptococci (chains), staphylococci (clusters)". Row 2: short rods, some in chains — label "Bacilli (rods): single, streptobacilli (chains)". Row 3: wavy spring shapes — label "Spirilla (spirals)". Row 4: comma shapes — label "Vibrio (comma/curved rods)".

Classification basis	Types	Examples
Shape	Cocci (round), Bacilli (rod), Spirilla (spiral), Vibrio (comma)	*Staphylococcus* (cocci), *E. coli* (bacilli)
Arrangement	Diplo (pairs), Strepto (chains), Staphylo (clusters)	*Streptococcus* (chains), *Staphylococcus* (clusters)
Oxygen need	Aerobe, Anaerobe, Facultative anaerobe	*Mycobacterium tuberculosis* (aerobe), *Clostridium* (anaerobe), *E. coli* (facultative)
Cell wall (Gram)	Gram-positive, Gram-negative	*Staphylococcus* (+), *E. coli* (−) — see Chapter 3

5. Infographic Concept Box

"THE MICROBE WORLD IN ONE PAGE" — a central circle labelled "MICROORGANISMS" with five spokes: Bacteria (prokaryote, ~1 µm, e.g., *E. coli*), Fungi (eukaryote, yeast + mould, e.g., *Penicillium*), Viruses (non-cellular, e.g., flu virus), Protozoa (eukaryote, e.g., *Plasmodium*), Algae (eukaryote, source of agar). Bottom banner split in two: green side "FRIENDS: antibiotics • vitamins • vaccines • insulin" and red side "ENEMIES: contamination • spoilage • infection". Corner box: "Prokaryote = no nucleus (bacteria); Eukaryote = true nucleus (fungi, protozoa)."

6. Pharmacy Relevance

A pharmacist must know microbes for three jobs: (1) **Production** — many drugs are manufactured using microbes in fermenters; (2) **Quality control** — every batch of medicine is tested to be sure it is not contaminated; (3) **Patient safety** — the pharmacist counsels patients on correct storage so microbes do not spoil the medicine at home. Sterile products (injections, eye drops) must be 100% microbe-free, and the pharmacist is part of the team that guarantees this.

7. India Context

India is often called the "pharmacy of the world" — a large share of the world's generic medicines and vaccines (for example, vaccines supplied globally by Indian manufacturers) are made here. Every Indian pharmaceutical factory therefore runs microbiology laboratories for quality control, and Indian pharmacopoeia (IP) sets microbial limit tests that products must pass. Job roles like "microbiologist — QC" in pharma companies are filled by pharmacy graduates who understand this subject.

8. Key Points

- Microbiology = study of organisms invisible to the naked eye.
- Bacteria are prokaryotes (no true nucleus); fungi, protozoa, algae are eukaryotes.
- Viruses are not cells — they multiply only inside host cells.
- Microbes are BOTH medicine-makers (antibiotics, vitamins, vaccines, insulin) and medicine-spoilers (contamination, infection).
- Bacteria are classified by shape (cocci, bacilli, spirilla, vibrio), arrangement, Gram reaction, and oxygen need.

9. Common Confusions

Confusion	Clarification
"All microbes are harmful."	Wrong. Most microbes are harmless or useful; only a small fraction cause disease.
"Bacteria and viruses are the same."	Wrong. Bacteria are living cells; viruses are non-cellular particles needing a host cell. Antibiotics kill bacteria but do NOT work on viruses.
"Yeast and mould are the same."	Both are fungi, but yeast is single-celled (round cells), mould grows as fuzzy filaments (hyphae).
"Sterile and clean mean the same."	Clean = reduced microbes; sterile = ZERO living microbes, including spores.

10. Common Mistakes

- Writing "Gram positive" for *E. coli* — it is Gram-negative (Chapter 3 explains the test).
- Calling a virus a "cell" — it has no cellular structure.
- Spelling: it is "cocci" (plural), "coccus" (singular); "bacilli" (plural), "bacillus" (singular).
- In exams, students list only harmful roles of microbes — always give BOTH useful and harmful roles for full marks.

11. Mnemonics

- **"Ben Fights Viruses, Protozoa, Algae"** — the five groups: **B**acteria, **F**ungi, **V**iruses, **P**rotozoa, **A**lgae.
- **"Coccus = Coconut (round), Bacillus = Baton (rod), Spirillum = Spring (spiral)"** — shapes.
- **"Staphylo = grapes in a bunch (clusters); Strepto = a string of beads (chains)"** — arrangements.

12. Exam Summary

Microorganisms are microscopic living entities studied in microbiology; pharmaceutical microbiology applies this science to drug manufacture, safety, and quality. The five groups are bacteria (prokaryotes), fungi, viruses, protozoa, and algae (eukaryotes except viruses, which are non-cellular). Microbes are pharmaceutically vital as producers of antibiotics (penicillin, streptomycin, cephalosporins), vitamins, enzymes, vaccines, and recombinant products like insulin — and simultaneously dangerous as contaminants that spoil medicines and infect patients. Bacteria are classified by shape (cocci, bacilli, spirilla, vibrio), arrangement (diplo, strepto, staphylo), oxygen requirement (aerobic, anaerobic, facultative), and Gram reaction.

13. One-Minute Revision

- 5 groups: Bacteria, Fungi, Viruses, Protozoa, Algae.
- Prokaryote (no nucleus) = bacteria. Eukaryote (true nucleus) = fungi, protozoa, algae.
- Viruses = non-cellular, need host cell.
- Shapes: cocci (round), bacilli (rod), spirilla (spiral), vibrio (comma).
- Friends: antibiotics, vitamins, vaccines, insulin. Enemies: contamination, spoilage, infection.

14. Exam Questions

Short-answer:

1. Define microbiology and pharmaceutical microbiology.
2. Differentiate between prokaryotes and eukaryotes with two examples each.
3. Name the five groups of microorganisms with one example each.
4. Classify bacteria based on shape with examples.

MCQs:

1. Which of the following is a prokaryote?
   a) Yeast b) *E. coli* c) Amoeba d) Mould — **Answer: b**
2. *Penicillium chrysogenum* is used in the production of:
   a) Streptomycin b) Penicillin c) Vitamin B12 d) Insulin — **Answer: b**
3. Vibrio-shaped bacteria look like:
   a) Round balls b) Rods c) Comma d) Spiral — **Answer: c**

1. Which organism is NOT cellular?
   a) Bacterium b) Virus c) Fungus d) Protozoan — **Answer: b**

Viva:

1. Why are viruses not considered true cells?
2. Give two antibiotics and the microbes that produce them.
3. What is the difference between contamination and infection?

Application-based:

1. A cough syrup bottle smells bad and has gas bubbles after one month of opening. Which microbial role does this show, and what should the pharmacist advise the patient? *(Spoilage role; advise checking expiry, storage, and not using spoiled medicine.)*
2. Why must injection vials be sterile while oral tablets only need to meet microbial limits? *(Injections bypass body defences and go directly into blood/tissues; even a few microbes can cause serious infection.)*

15. References

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CHAPTER 2 — Microscopy and Microbial Cultivation, Isolation and Enumeration

(Unit I: Introduction to microscope — compound and electron microscope; microbial cultivation, isolation and enumeration)

1. Learning Objectives

After studying this chapter, you will be able to:

1. Explain the principle and parts of a compound microscope and an electron microscope.
2. Compare compound and electron microscopes (magnification, resolution, specimen type).
3. Define culture media and classify media (general purpose, selective, differential, enriched).
4. Describe methods of microbial cultivation (aerobic and anaerobic).
5. Explain isolation techniques: streak plate, pour plate, spread plate.
6. Describe enumeration methods: plate count, membrane filtration, turbidity, and microscopic count.

2. Definitions

Term	Definition (simple)
Microscope	An instrument that makes tiny objects look bigger so we can see them.
Magnification	How many times bigger an object appears (e.g., 1000× means 1000 times bigger).
Resolution	The ability to see two very close points as two separate points — the sharpness or clarity of the image.
Culture medium	A specially prepared food mixture (liquid or gel) on which microbes are grown in the laboratory.
Inoculation	Placing microbes (the sample) onto or into the culture medium.
Incubation	Keeping the inoculated medium at the right temperature for the right time so microbes grow.
Isolation	Separating one type of microbe from a mixture so it grows alone as a pure culture.
Pure culture	A culture containing only ONE type of microorganism.
Enumeration	Counting the number of microorganisms in a sample.
CFU (Colony Forming Unit)	Each living bacterial cell (or group) that grows into one visible colony — the unit used when counting bacteria by plate methods.

3. Terminology (Key Terms)

Term	Meaning in plain words
Objective lens	The lens near the specimen that does most of the magnifying.
Ocular (eyepiece) lens	The lens you look through (usually 10×).
Oil immersion	Using a drop of special oil with the 100× lens to get maximum clarity.
Agar	A seaweed-based gelling agent that makes media solid (melts on heating, sets on cooling, microbes cannot eat it).
Streak plate	Zig-zag spreading of sample on agar to separate cells into single colonies.
Pour plate	Mixing sample INTO melted agar before it sets.

Term	Meaning in plain words
Spread plate	Spreading a drop of sample OVER the agar surface with a spreader.
Serial dilution	Diluting the sample step by step (1:10, 1:100, 1:1000...) so colonies become countable.
Turbidity	Cloudiness of a liquid culture — more cloudiness = more microbes.
Haemocytometer	A special glass slide with a grid for counting cells under the microscope.

4. Explanation

4.1 Why do we need microscopes?

> **Technical concept:** Most bacteria are 1–5 micrometres (µm) in size, below the resolving power of the naked eye (~0.1 mm = 100 µm), so magnification instruments are required.

> **Simple explanation:** Your eye can see a grain of sand but not a bacterium sitting on it. A bacterium is roughly 100 times smaller than what your eye can resolve. A microscope works like a super-powerful magnifying glass — it bends light (or electron beams) to make the tiny thing look big.

> **Analogy:** Reading a medicine label from across the room is impossible; you walk closer or use reading glasses. A microscope "walks you closer" to the microbe — 1,000 times closer.

4.2 Compound (light) microscope

> **Technical concept:** The compound microscope uses visible light and two sets of lenses (objective + ocular). Total magnification = objective magnification × ocular magnification (usually 10×), giving up to 1000× with the oil-immersion lens.

> **Simple explanation:** Light shines up through the specimen on a glass slide. Two lenses magnify it in two stages — like looking through one magnifying glass and then another. With the 100× oil lens and 10× eyepiece, you get 1000× — a bacterium looks 1,000 times bigger.

Parts and function (draw and label):

Diagram to draw (describe): Draw a side view of a compound microscope: at the top, the "eyepiece (ocular lens, 10×)"; below it a tube ("body tube"); a rotating disc holding three small cylinders ("revolving nosepiece with objective lenses: 10×, 40×, 100× oil"); below the lenses a flat platform ("stage — specimen slide kept here"); under the stage a light source ("illuminator") and a small lens assembly ("condenser — focuses light on specimen"); side knobs labelled "coarse and fine adjustment knobs"; the base labelled "base"; the curved arm labelled "arm". Arrows showing the light path travelling upward from illuminator through specimen and lenses to the eye.

Part	Function
Eyepiece (ocular)	You look through it; magnifies 10×
Objective lenses	Main magnification: 10× (low), 40× (high), 100× (oil immersion)
Stage	Platform holding the glass slide
Condenser	Focuses light onto the specimen

Part	Function
Iris diaphragm	Controls the amount of light
Coarse/fine knobs	Focus the image (coarse = rough, fine = sharp)
Oil immersion	A drop of cedarwood oil between 100× lens and slide prevents light scattering, giving the sharpest image

Total magnification examples: 10× objective × 10× eyepiece = 100×; 40× × 10× = 400×; 100× (oil) × 10× = 1000×.

> **Key takeaway:** **The compound microscope is the daily workhorse of every microbiology lab — used for examining stained bacteria (Chapter 3).**

4.3 Electron microscope

> **Technical concept:** The electron microscope uses a beam of electrons instead of light, with electromagnetic lenses. Because electrons have a much shorter wavelength than visible light, resolution is far higher (~0.1 nm). Two main types: Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM).

> **Simple explanation:** Light has a limit — it cannot show details smaller than its own wavelength, like trying to feel fine embroidery while wearing thick gloves. Electrons behave like ultra-fine fingers, so the electron microscope can "feel" details 1,000 times smaller than a light microscope can show — it can even show viruses.

Comparison table:

Feature	Compound (light) microscope	Electron microscope
Radiation used	Visible light	Electron beam
Magnification	Up to ~1,000–2,000×	Up to ~1,000,000× or more
Resolution	~0.2 µm (200 nm)	~0.1 nm (TEM) — about 2,000× better
Specimen	Living or dead; simple preparation	Dead only; complex preparation; vacuum needed
What you can see	Bacteria, fungi, protozoa	Viruses, cell ultrastructure (internal details)
Types	Simple student/lab model	TEM (internal structure), SEM (3D surface view)
Cost and size	Cheap, bench-top	Very expensive, room-sized

Diagram to draw (describe): Two panels. Left panel: "Light microscope" — a wavy arrow labelled "light rays" passing through simple lens shapes. Right panel: "Electron microscope (TEM)" — a straight column with a "electron gun (source)" at top, ring shapes labelled "electromagnetic lenses", a thin slice labelled "ultra-thin specimen", and a screen at the bottom labelled "fluorescent screen — image formed". Caption: "Electrons replace light; magnetic coils replace glass lenses."

> **Key takeaway:** **Light microscope = for bacteria (routine lab work). Electron microscope = for viruses and fine internal details (research and specialised labs).**

4.4 Culture media — the food for microbes

Technical concept: A culture medium supplies water, carbon source, nitrogen source, minerals, and growth factors needed for microbial growth. Media may be liquid (broth), solid (agar added), or semi-solid.

Simple explanation: To grow microbes in the lab, you must cook them a meal — a nutritious jelly or soup containing everything they need. Agar is the magic ingredient that turns the soup into jelly: it melts when hot, sets when cool, and microbes cannot digest it, so it stays solid while they eat the nutrients around it.

Classification of media:

Type	What it does	Example
General purpose / basal	Grows most common bacteria; no special selection	Nutrient agar, nutrient broth
Enriched	Basal medium + extra nutrients (blood, serum) for fastidious (fussy) bacteria	Blood agar
Selective	Contains chemicals that allow only wanted bacteria to grow, suppressing others	MacConkey agar (selects Gram-negative bacteria)
Differential	Contains indicators that make different bacteria look different (colour change)	MacConkey agar (lactose fermenters turn pink)
Transport	Keeps microbes alive during travel from patient to lab, without letting them multiply	Stuart's transport medium

Analogy: Media are like restaurant menus. A general-purpose medium is a buffet — everyone eats. A selective medium is an invitation-only dinner — only chosen guests enter. A differential medium gives each guest a different coloured badge so you can tell them apart.

4.5 Cultivation — growing microbes

Technical concept: Cultivation requires inoculating the medium and incubating at optimum temperature (usually 37°C for pathogens), pH, and atmosphere (aerobic or anaerobic) for 18–48 hours.

Simple explanation: Growing bacteria is like growing plants: you sow the seeds (inoculation), then keep the pot warm and moist (incubation at body temperature, 37°C, usually overnight). By morning, each invisible cell has multiplied into a visible colony.

Flowchart — cultivation steps:

```
Sample (throat swab / water / medicine)
        ↓  1. INOCULATION — transfer sample to sterile medium (loop/needle)
        ↓  2. INCUBATION — keep at 37°C for 18–24 h (upside down so
              condensation does not drip on colonies)
        ↓  3. OBSERVATION — examine colonies: size, shape, colour, smell
        ↓  4. IDENTIFICATION — staining + biochemical tests (Chapter 3)
```

Aerobic vs anaerobic cultivation: Most bacteria grow in normal air (aerobic cultivation — just incubate the plate). Anaerobes need oxygen removed — special jars (anaerobic jars with gas packs that absorb oxygen) or sealed containers are used.

4.6 Isolation techniques — getting a pure culture

> **Technical concept:** Isolation separates individual cells so each grows into a discrete colony; the three standard plating methods are streak plate, pour plate, and spread plate.

> **Simple explanation:** A sample (e.g., pond water) contains many types of bacteria mixed together — like a mixed crowd. Isolation is asking the crowd to stand far apart so you can meet each person individually. Each single cell left alone on agar grows into its own pure colony.

Method	How it is done	Best for
Streak plate	A wire loop spreads the sample in a zig-zag across agar; the loop is heated (sterilised) between streaks to thin out cells	Routine isolation of a pure colony from mixed sample
Pour plate	Sample is mixed INTO melted (cooled) agar, poured into a dish, allowed to set — colonies grow both on surface and inside agar	Counting microbes (enumeration) and growing anaerobes (inside agar = less oxygen)
Spread plate	A measured drop of diluted sample is spread evenly OVER the agar surface with a glass spreader	Counting microbes (enumeration); cells stay on surface

Diagram to draw (describe): Three petri dishes. Dish 1 "Streak plate": draw a zig-zag line divided into 4 quadrants with decreasing dots — label "loop flamed between quadrants; isolated colonies in last quadrant". Dish 2 "Pour plate": dots scattered through the agar layer (side view) — label "colonies on surface + embedded in agar". Dish 3 "Spread plate": evenly scattered dots on the surface — label "sample spread on surface; countable colonies".

4.7 Enumeration — counting microbes

> **Technical concept:** Enumeration estimates viable (living) cells in a sample, commonly by plate count (CFU), membrane filtration, turbidity measurement, or direct microscopic count.

> **Simple explanation:** You cannot count bacteria one by one — there are millions. So scientists use smart tricks: grow them into countable colonies, or measure how cloudy the liquid becomes.

Method	Principle	Counts	Example use
Plate count (viable count)	Dilute sample, plate it, count colonies; CFU/mL = colonies × dilution factor	Only LIVING cells	Testing microbial load in a syrup
Membrane filtration	Filter a large volume of liquid through a 0.45 µm membrane; microbes stay on the membrane, which is placed on agar and incubated	Living cells; good for very clean samples	Testing purified water for injection
Turbidimetry	Measure cloudiness (absorbance) of broth with a colorimeter/spectrophotometer	Living + dead cells (total)	Quick check of growth in a fermenter
Direct microscopic count (haemocytometer)	Count cells in a known tiny volume under the microscope using a grid slide	Living + dead cells	Counting yeast cells quickly

Worked example (plate count): 0.1 mL of a 1:10,000 dilution is spread on agar and 65 colonies grow. CFU/mL = (colonies ÷ volume plated) × dilution factor = (65 ÷ 0.1) × 10,000 = 650 × 10,000 = **6,500,000 CFU/mL (6.5×10^6 CFU/mL)**.

Key takeaway: **Plate count and membrane filtration count only living cells (viable count) — the methods that matter for pharmaceutical quality control. Turbidity and microscopic counts are faster but count dead cells too.**

5. Infographic Concept Box

"FROM SAMPLE TO COUNT — THE MICROBIOLOGY WORKFLOW" — a horizontal 5-step strip: (1) SAMPLE (swab/water/medicine icon) → (2) MICROSCOPE (see them: compound 1000×, electron 1,000,000×) → (3) CULTURE (feed them: agar plate at 37°C overnight) → (4) ISOLATE (separate them: streak/pour/spread plate) → (5) COUNT (count them: plate count = CFU, membrane filter, turbidity). Below, a "MEDIA MENU" mini-table: General (buffet), Selective (invitation only), Differential (colour badges), Enriched (VIP nutrients). Side note: "Agar = seaweed jelly microbes cannot eat."

6. Pharmacy Relevance

Every pharmaceutical QC laboratory uses exactly these techniques daily: microscopes to examine contaminants, culture media to grow them, isolation to identify the culprit organism, and plate counts/membrane filtration to check that raw materials, water, and finished products meet microbial limits. Without cultivation and enumeration, no medicine batch can be released as safe.

7. India Context

Indian pharmaceutical companies and testing laboratories follow the Indian Pharmacopoeia (IP) for microbial limit tests, which are based on the plate-count and membrane-filtration methods described here. During monsoon, when humidity rises, Indian QC labs increase environmental monitoring (air and surface counts) because microbial contamination risks go up — a direct application of enumeration methods.

8. Key Points

- Compound microscope: light + glass lenses, up to 1000× (oil immersion); routine lab use.
- Electron microscope: electron beam, up to ~1,000,000×; shows viruses and ultrastructure; TEM = internal view, SEM = surface view.
- Media types: general purpose, enriched, selective, differential, transport; agar = solidifying agent from seaweed.
- Isolation: streak plate (zig-zag), pour plate (mixed into agar), spread plate (spread on surface).
- Enumeration: plate count (CFU — viable only), membrane filtration (viable, for clean liquids), turbidity and haemocytometer (total count, living + dead).

9. Common Confusions

Confusion	Clarification
"Magnification and resolution are the same."	Magnification = bigger image; resolution = sharper image. A blurry photo enlarged is bigger but not clearer.
"Selective and differential media are the same."	Selective = chooses WHO grows; differential = shows WHICH is which by colour. (MacConkey agar is both.)

Confusion	Clarification
"Pour plate and spread plate are the same."	Pour = sample mixed INTO agar; spread = sample spread ONTO agar surface.
"Turbidity counts only living cells."	No — cloudiness comes from living AND dead cells. Only plate methods give viable (living) counts.

10. Common Mistakes

- Forgetting the oil drop with the 100× objective — without oil the image is poor.
- Writing "CFU" for a turbidity result — CFU applies only to plate/membrane (viable) counts.
- In the plate-count calculation, forgetting to divide by the volume plated (0.1 mL) — a very common numerical error.
- Saying agar is "food for bacteria" — agar is only the solidifying agent; the nutrients are the peptone/beef extract in the medium.

11. Mnemonics

- **"My Cat Eats Small Tuna"** — Microscope types by power: **M**agnification order — **C**ompound (light), **E**lectron; **S**EM = **S**urface, **T**EM = **T**hrough (internal).
- **"Streak, Pour, Spread — Zigzag, Inside, On top"** — how each plating method places the sample.
- **"SEED"** for media: **S**elective, **E**nriched, **E**veryday (general purpose), **D**ifferential.

12. Exam Summary

Microscopes make microbes visible: the compound microscope (light, glass lenses, up to 1000× with oil immersion) is the routine laboratory instrument, while the electron microscope (electron beam, up to ~10^6×; TEM for internal structure, SEM for surface) reveals viruses and ultrastructure. Microbes are cultivated on culture media — general purpose, enriched, selective, differential, and transport types — solidified with agar, inoculated and incubated (usually 37°C, 18–24 h). Isolation into pure cultures uses streak, pour, and spread plate techniques. Enumeration uses the plate count (CFU/mL, viable cells only), membrane filtration (for low-count liquids like water), turbidimetry, and direct microscopic (haemocytometer) counts.

13. One-Minute Revision

- Compound microscope: 1000× (oil), routine. Electron: ~10^6×, viruses, TEM internal / SEM surface.
- Media: general, enriched, selective, differential, transport. Agar = solidifier.
- Isolation: streak (zigzag), pour (into agar), spread (on agar).
- Counting: plate count = CFU (living only); membrane filter for water; turbidity = quick but counts dead too.
- Formula: CFU/mL = (colonies ÷ mL plated) × dilution factor.

14. Exam Questions

Short-answer:

1. Draw a labelled diagram of a compound microscope and state the function of any four parts.
2. Differentiate between compound and electron microscopes (any four points).
3. Classify culture media with one example each.
4. Describe the streak plate technique for isolation of bacteria.
5. What is CFU? Calculate CFU/mL if 45 colonies grow from 0.1 mL of a 1:1000 dilution. *(Answer: (45 ÷ 0.1) × 1000 = 450,000 = 4.5 × 10^5 CFU/mL.)*

MCQs:

1. Total magnification with 100× oil objective and 10× eyepiece is:
   a) 100× b) 400× c) 1000× d) 10,000× — **Answer: c**
2. Agar is obtained from:
   a) Bacteria b) Seaweed (algae) c) Fungi d) Plants — **Answer: b**
3. MacConkey agar is an example of:
   a) Enriched medium b) Selective and differential medium c) Transport medium d) General purpose only — **Answer: b**
4. Which enumeration method counts ONLY viable cells?
   a) Turbidity b) Haemocytometer c) Plate count d) All of these — **Answer: c**
5. SEM is used to study:
   a) Internal cell structure b) Surface (3D) structure c) Living cells in colour d) Bacterial movement — **Answer: b**

Viva:

1. Why is oil used with the 100× objective?
2. Why are plates incubated upside down?
3. When would you choose membrane filtration over pour plate?

Application-based:

1. A QC lab must test highly purified water expected to have very few microbes. Which enumeration method is best and why? *(Membrane filtration — a large volume can be filtered to trap even a few cells, which pour plate would miss.)*
2. Two plates from the same sample show 320 and 28 colonies. Which count is more reliable and why? *(28 is too few — statistically unreliable; ideally 30–300 colonies per plate. The 320 plate is crowded; the correct practice is to use the dilution giving 30–300 colonies.)*

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CHAPTER 3 — Bacterial Identification: Staining Techniques, IMViC Tests and Microbial Antibiotics

(Unit I: Identification of bacteria using staining techniques — simple, Gram's & acid-fast staining — and biochemical tests (IMViC); antibiotics produced by microbiology — production and uses: streptomycin, cephalosporin)

1. Learning Objectives

After studying this chapter, you will be able to:

1. Explain the principle and procedure of simple staining, Gram staining, and acid-fast staining.
2. Differentiate Gram-positive and Gram-negative bacteria based on cell-wall structure.
3. Describe the four IMViC tests and interpret their results.
4. Explain how staining + biochemical tests identify an unknown bacterium.
5. Describe the microbial production (fermentation) of streptomycin and cephalosporin.
6. State the uses of streptomycin and cephalosporins.

2. Definitions

Term	Definition (simple)
Staining	Colouring bacteria with dyes so they become visible under the microscope (bacteria are otherwise colourless).
Smear	A thin layer of bacterial sample spread on a glass slide, dried and heat-fixed before staining.
Mordant	A chemical that fixes the dye firmly to the cell (like a "glue" for the colour).
Decolouriser	A chemical (alcohol/acetone) that washes dye OUT of some bacteria but not others — the key step of Gram staining.
Counterstain	A second, contrasting dye applied at the end so decolourised cells become visible in a different colour.
Biochemical test	A test that identifies bacteria by the chemicals they make or the food they can digest (e.g., do they make acid? gas?).
IMViC	A group of four classic biochemical tests: **I**ndole, **M**ethyl red, **V**oges–Proskauer, **C**itrate — used mainly to identify intestinal (enteric) bacteria.
Antibiotic	A chemical produced by a microorganism that kills or stops the growth of other microorganisms.

3. Terminology (Key Terms)

Term	Meaning in plain words
Crystal violet	The purple primary dye in Gram staining.
Safranin	The red/pink counterstain in Gram staining.
Carbol fuchsin	The red primary dye in acid-fast staining.
Acid-fast	Bacteria that hold the red dye even after washing with acid-alcohol (e.g., *Mycobacterium*).
Peptidoglycan	The mesh-like wall material of bacteria; thick in Gram-positive, thin in Gram-negative.
Mycolic acid	A waxy substance in the wall of acid-fast bacteria that resists decolourisation.
Fermentation (industrial)	Growing microbes in large tanks to manufacture useful products like antibiotics.

Term	Meaning in plain words
Broad-spectrum	An antibiotic effective against many types of bacteria (both Gram-positive and Gram-negative).

4. Explanation

4.1 Why stain bacteria?

> **Technical concept:** Bacterial cells are transparent and have a refractive index close to water, making them nearly invisible under the bright-field microscope; staining increases contrast.

> **Simple explanation:** Trying to see an unstained bacterium under the microscope is like trying to see a clear glass marble in a glass of water — it is there, but invisible. Staining paints the marble so you can see its shape, size, and arrangement.

Flowchart — general staining procedure:

```
Bacterial sample
   ↓ 1. SMEAR — spread a thin film on a clean glass slide
   ↓ 2. AIR-DRY — let it dry completely
   ↓ 3. HEAT-FIX — pass the slide quickly through a flame 2–3 times
   |     (kills bacteria + sticks them to the slide; do NOT overheat)
   ↓ 4. STAIN — apply dye(s) as per the technique
   ↓ 5. WASH — rinse gently with water
   ↓ 6. OBSERVE — dry, add oil, examine under 100× oil-immersion lens
```

4.2 Simple staining

> **Technical concept:** Simple staining uses a single basic dye (methylene blue, crystal violet, or safranin) for a short time to reveal cell shape, size, and arrangement.

> **Simple explanation:** This is the "one-colour paint job" — dip the slide in one dye for one minute, wash, and look. It shows you the SHAPE of the bacteria (round? rod?) but cannot tell types apart.

Example: A methylene-blue-stained smear of curd shows purple rod-shaped bacteria (*Lactobacillus*).

> **Key takeaway:** **Simple staining = morphology only (shape, size, arrangement). For identification, we need differential stains.**

4.3 Gram staining — the most important stain in microbiology

> **Technical concept:** Gram staining is a differential staining technique based on differences in bacterial cell-wall structure. Gram-positive bacteria have a thick peptidoglycan layer that retains crystal violet; Gram-negative bacteria have a thin peptidoglycan layer plus an outer membrane, so the dye washes out and they take the pink counterstain.

> **Simple explanation:** Imagine two types of sponges. A thick dense sponge (Gram-positive wall) soaks up purple ink and will not let it go even when rinsed with alcohol. A thin sponge with a slippery coat (Gram-negative wall) loses the purple ink when rinsed, so we re-paint it pink to see it. The colour at the end tells you which wall type the bacterium has.

The four steps (with the science of each):

Step	Reagent	Time	What happens
1. Primary stain	Crystal violet (purple)	~1 min	ALL bacteria turn purple
2. Mordant	Gram's iodine	~1 min	Iodine forms a big dye–iodine complex inside cells — "locks" the purple in
3. Decolouriser	Alcohol/acetone	~10–20 seconds (critical!)	Gram-positive: thick wall traps the complex → stay purple. Gram-negative: thin wall + outer membrane dissolve → purple washes out → become colourless
4. Counterstain	Safranin (pink/red)	~30 sec–1 min	Colourless (Gram-negative) cells turn pink; purple cells are unaffected

Result: Gram-positive = **purple**; Gram-negative = **pink/red**.

Diagram to draw (describe): Two panels side by side showing cell-wall cross-sections as layered bars. Left panel "GRAM-POSITIVE (purple)": a very thick brick-wall layer labelled "thick peptidoglycan (traps dye)". Right panel "GRAM-NEGATIVE (pink)": a thin brick layer labelled "thin peptidoglycan" with an extra wavy line on top labelled "outer membrane (dissolved by alcohol — dye escapes)". Below, two microscope views: purple round clusters labelled "*Staphylococcus aureus* — Gram-positive cocci"; pink rods labelled "*E. coli* — Gram-negative bacilli".

Examples:

Gram-positive (purple)	Gram-negative (pink)
Staphylococcus aureus (cocci, clusters)	*Escherichia coli* (rods)
Streptococcus (cocci, chains)	*Salmonella* (rods)
Bacillus (rods, spore-forming)	*Pseudomonas aeruginosa* (rods)
Mycobacterium is weakly Gram-positive ("ghost" cells) — needs acid-fast stain	*Vibrio cholerae* (comma-shaped)

Pharmacy relevance: Gram staining is the FIRST step when a contaminated medicine or a patient's infection is investigated — the colour result immediately narrows down which antibiotic may work, because many antibiotics act differently on the two wall types.

> **Common mistake alert:** Decolourising too long washes purple out of Gram-positive cells too (false pink); too short leaves Gram-negative cells purple (false purple). Timing of the alcohol step decides everything.

4.4 Acid-fast staining (Ziehl–Neelsen stain)

> **Technical concept:** Acid-fast staining identifies bacteria with waxy, mycolic-acid-rich cell walls (mainly *Mycobacterium* species) that resist decolourisation with acid-alcohol after staining with hot carbol fuchsin.

> **Simple explanation:** Some bacteria wear a "wax raincoat" (mycolic acid) that normal stains cannot penetrate. We force red dye in with heat/steam, then try to wash it out with strong acid-alcohol. Ordinary bacteria lose the red; the wax-coated ones hold it fast — they are "acid-fast" (fast = firm, holding tight).

Steps: (1) Flood smear with carbol fuchsin (red) + gentle heating/steaming ~5 min → all cells red. (2) Decolourise with acid-alcohol → non-acid-fast cells become colourless; acid-fast keep red. (3) Counterstain with methylene blue → non-acid-fast turn blue.

Result: Acid-fast = **red** bacilli on a blue background; non-acid-fast = blue.

Example: Sputum smear of a tuberculosis patient shows red rod-shaped *Mycobacterium tuberculosis* — this test is the classic rapid diagnosis of TB.

Diagram to draw (describe): A microscope field: blue background with scattered thin red rods labelled "Acid-fast bacilli (red) — *Mycobacterium tuberculosis*"; nearby blue rods labelled "non-acid-fast bacteria (blue)". Add a callout: "Waxy mycolic-acid wall = raincoat that keeps the red dye."

4.5 IMViC — biochemical identification tests

> **Technical concept:** IMViC is a set of four biochemical tests used to differentiate members of the Enterobacteriaceae (intestinal bacteria), based on metabolic products: indole production, mixed-acid fermentation (methyl red), butylene-glycol fermentation (Voges–Proskauer), and citrate utilisation.

> **Simple explanation:** Staining tells you the shape and wall type, but two different bacteria can look identical. IMViC asks each bacterium four "food questions" — what it eats and what waste it makes — and the pattern of answers reveals its identity, like a four-question quiz that distinguishes twins.

Test	What it detects	Positive result	*E. coli*	*Klebsiella*
I — Indole	Enzyme tryptophanase splits tryptophan → indole; add Kovac's reagent	Red ring on top	**+**	–
M — Methyl Red	Mixed-acid fermentation makes the broth strongly acidic (pH < 4.4); methyl red indicator turns red	Red colour	**+**	–
V — Voges–Proskauer	Butylene-glycol pathway makes acetoin; Barritt's reagents give red colour	Red colour	–	**+**
C — Citrate	Bacterium uses citrate as its only carbon source; indicator (bromothymol blue) turns from green to blue	Blue colour	–	**+**

Memory pattern: *E. coli* = **+ + – –** ("E. coli is plus-plus-minus-minus"); *Klebsiella* = **– – + +** (the exact opposite).

Flowchart — identifying an unknown Gram-negative rod from a contaminated syrup:

```
Unknown bacterium from sample
   ↓ Gram stain → pink rods (Gram-negative)
   ↓ Grow on MacConkey agar → pink colonies (lactose fermenter)
   ↓ IMViC tests:
   ├── Indole +, MR +, VP –, Citrate –  →  E. coli
   └── Indole –, MR –, VP +, Citrate +  →  Klebsiella pneumoniae
   ↓ Report + take action on the contaminated batch
```

4.6 Antibiotics produced by microbiology — streptomycin and cephalosporin

> **Technical concept:** Antibiotics are secondary metabolites produced by microbes (mainly *Streptomyces* bacteria and *Penicillium*/*Acremonium* moulds) during industrial fermentation, then extracted and purified.

> **Simple explanation:** In nature, microbes make antibiotics as chemical weapons to kill competing microbes nearby. Humans discovered this war, learned to grow the winning microbe in giant tanks, and harvest its weapon as medicine.

Streptomycin

- **Producer:** The soil bacterium *Streptomyces griseus* (discovered by Selman Waksman — the scientist who coined the word "antibiotic").
- **Class:** Aminoglycoside.
- **Production (simple flow):** Spores of *S. griseus* → seed tank (small starter culture) → large production fermenter with soybean meal/glucose medium, aerated and stirred, ~25–28°C, several days → antibiotic accumulates in the broth → mycelium filtered off → streptomycin extracted from the filtrate, purified, and converted to a stable salt.
- **Uses:** Treatment of **tuberculosis** (combined with other anti-TB drugs), plague, and certain severe Gram-negative infections. Given by injection (it is not absorbed well orally).

Cephalosporins

- **Producer:** The mould *Acremonium chrysogenum* (originally named *Cephalosporium acremonium* — hence the name).
- **Class:** Beta-lactam antibiotics, chemically related to penicillin.
- **Production (simple flow):** Mould spores → seed culture → production fermenter (aerobic, ~25°C) → cephalosporin C accumulates → extracted from broth → chemically modified in the factory to make the many cephalosporin drugs used today (cephalexin, cefixime, ceftriaxone, etc.).
- **Uses:** Broad-spectrum treatment of respiratory, urinary, skin, and surgical infections; many are the first-choice antibiotics in hospitals. Available as oral capsules and injections.

Comparison table:

Feature	Streptomycin	Cephalosporins
Producer microbe	Bacterium *Streptomyces griseus*	Mould *Acremonium chrysogenum*
Antibiotic class	Aminoglycoside	Beta-lactam (like penicillin)
Main use	Tuberculosis, plague	Broad-spectrum bacterial infections
Given as	Injection	Oral and injection forms

5. Infographic Concept Box

> **"IDENTIFY THE CULPRIT — BACTERIAL DETECTIVE KIT"** — a detective-style panel with three magnifier icons: (1) STAIN: "Simple = shape only; Gram = purple (+) vs pink (−) — the alcohol step decides; Acid-fast = red rods on blue (TB test)". (2) IMViC QUIZ: "*E. coli* = ++−−, *Klebsiella* = −−++". (3) ANTIBIOTIC FACTORY: soil microbe → fermenter tank → medicine bottle; "Streptomycin ← *Streptomyces griseus* (TB); Cephalosporins ← *Acremonium* mould (broad-spectrum)". Bottom strip: "Stain first, then biochemical tests — colour narrows it down, chemistry confirms it."

6. Pharmacy Relevance

Staining and IMViC are the core tools of pharmaceutical QC microbiology: when a raw material, water sample, or finished product shows contamination, the microbiologist stains the isolate and runs biochemical tests to name the organism — the name decides the corrective action (e.g., finding *E. coli* means faecal contamination and batch rejection). Knowledge of antibiotic production helps pharmacists understand why streptomycin must be injected, why cephalosporins are broad-spectrum, and how fermentation manufactures these drugs.

7. India Context

India runs one of the world's largest TB-control programmes, and the acid-fast (sputum) test of *Mycobacterium tuberculosis* is performed daily in thousands of Indian diagnostic labs — the same Ziehl–Neelsen technique in this chapter. Indian pharmaceutical companies are among the world's biggest producers of cephalosporin antibiotics and anti-TB drugs including streptomycin, so fermentation-based antibiotic manufacture is a major domestic industry employing pharmacy graduates.

8. Key Points

- Staining makes invisible bacteria visible; always smear → dry → heat-fix first.
- Gram stain: crystal violet → iodine → alcohol (critical step) → safranin; Gram-positive = purple (thick wall), Gram-negative = pink (thin wall + outer membrane).
- Acid-fast stain: carbol fuchsin + heat → acid-alcohol → methylene blue; acid-fast = red (waxy mycolic-acid wall, e.g., TB bacillus).
- IMViC = Indole, Methyl red, Voges–Proskauer, Citrate; *E. coli* ++−−, *Klebsiella* −−++.
- Streptomycin ← *Streptomyces griseus* (aminoglycoside, TB, injection). Cephalosporins ← *Acremonium chrysogenum* mould (beta-lactam, broad-spectrum).

9. Common Confusions

Confusion	Clarification
"Gram-positive means the bacterium is positively charged."	No — "positive" only means it RETAINS the purple dye.
"Acid-fast and Gram-positive are the same."	No — acid-fast is a separate property due to waxy mycolic acid; the test is different.
"IMViC identifies all bacteria."	No — it mainly differentiates enteric (intestinal) Gram-negative rods.
"Streptomycin and penicillin are the same type."	No — streptomycin is an aminoglycoside (from bacteria); penicillin/cephalosporins are beta-lactams (from moulds).

10. Common Mistakes

- Over-decolourising in Gram staining → false Gram-negative result. Watch the clock (10–20 seconds).
- Writing IMViC results without the organism name — always link the pattern (++−−) to the bacterium (*E. coli*).
- Confusing the producer: streptomycin comes from a BACTERIUM (*Streptomyces*), cephalosporin from a MOULD (*Acremonium*).
- Forgetting heat-fixing before staining — cells wash off the slide.

11. Mnemonics

- **"Purple People Stay, Pink People Go"** — Gram-positive STAY purple; Gram-negative GO pink (lose purple, take pink).
- **"I'M ViC"** — **I**ndole, **M**ethyl red, **V**oges–Proskauer, **C**itrate.
- **"E. coli Eats Plus-Plus"** — *E. coli* = ++−− (first two positive).
- **"StreptoMYcin from StreptoMYces; CephaloSPORIN from CephaloSPORIUM (mould)"** — match the names.

12. Exam Summary

Bacterial identification starts with staining: simple staining shows morphology; Gram staining (crystal violet → iodine → alcohol → safranin) differentiates bacteria by cell-wall structure into Gram-positive (purple, thick peptidoglycan) and Gram-negative (pink, thin peptidoglycan + outer membrane); acid-fast staining (carbol fuchsin + heat → acid-alcohol → methylene blue) detects waxy-walled mycobacteria as red bacilli — the classic TB test. The IMViC set (indole, methyl red, Voges–Proskauer, citrate) biochemically differentiates enteric bacteria (*E. coli* ++−− vs *Klebsiella* −−++). Industrially, the bacterium *Streptomyces griseus* produces streptomycin (aminoglycoside, used in tuberculosis, given by injection) and the mould *Acremonium chrysogenum* produces cephalosporin C, the parent of broad-spectrum cephalosporin antibiotics — both manufactured by large-scale fermentation.

13. One-Minute Revision

- Gram: purple = positive (thick wall); pink = negative (thin wall). Alcohol step is critical.
- Acid-fast: red on blue = *Mycobacterium* (TB test, waxy wall).
- IMViC: I, M, V, C. *E. coli* ++−−; *Klebsiella* −−++.
- Streptomycin ← *Streptomyces griseus* (TB, injection). Cephalosporins ← *Acremonium* mould (broad-spectrum).

14. Exam Questions

Short-answer:

1. Describe the principle and procedure of Gram staining. How do you interpret the result?
2. Explain acid-fast staining with reference to *Mycobacterium tuberculosis*.
3. What is IMViC? Give the expected results for *E. coli* and *Klebsiella*.
4. Describe the production and uses of streptomycin.
5. Write the production and uses of cephalosporins.

MCQs:

1. The decolouriser used in Gram staining is:
   a) Iodine b) Alcohol/acetone c) Safranin d) Carbol fuchsin — **Answer: b**
2. Gram-negative bacteria appear _____ after Gram staining:
   a) Purple b) Pink/red c) Blue d) Green — **Answer: b**
3. Acid-fastness is due to the presence of:
   a) Peptidoglycan b) Mycolic acid (wax) c) Teichoic acid d) Lipid bilayer — **Answer: b**
4. In IMViC, *E. coli* gives:
   a) −−++ b) ++−− c) +−+− d) −++− — **Answer: b**

1. Streptomycin is produced by:
   a) *Penicillium chrysogenum* b) *Streptomyces griseus* c) *Acremonium chrysogenum* d) *Aspergillus niger* — **Answer: b**
2. Cephalosporins belong to which antibiotic class?
   a) Aminoglycoside b) Beta-lactam c) Macrolide d) Tetracycline — **Answer: b**

Viva:

1. Why is iodine called a mordant in Gram staining?
2. What happens if you forget the counterstain in Gram staining? *(Gram-negative cells remain colourless/invisible.)*
3. Why is streptomycin given by injection and not orally?

Application-based:

1. A sputum sample from a coughing patient shows red bacilli on a blue background after staining. Name the technique, the likely organism, and the disease. *(Acid-fast/Ziehl–Neelsen staining; Mycobacterium tuberculosis; tuberculosis.)*
2. A QC lab isolates pink rods from a contaminated tablet batch. IMViC shows ++−−. Identify the organism and state what its presence suggests. *(E. coli; suggests faecal/water contamination — the batch fails microbial limits.)*

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