Microbiology

Unit 4 · Lesson 1 · Culture media and pure cultures

The notes for Lesson 1 of Unit 4: chapters 4.1 to 4.2 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 4 | 2 chapters | Chapters 4.1 to 4.2

About these notes

These are the notes for **Lesson 1 · Culture media and pure cultures** of the University free n easy video lessons on Microbiology Unit 4, Microbial Nutrition, Growth and Genetics. They are chapters 4.1 to 4.2 of the unit book, unchanged, so a page here is the same page in the full book.

4.1 Nutritional requirements and culture media

Every microbe must take in the elements its cell is built from, a source of energy, and a source of electrons. Microbiologists sort organisms by where these come from, and they design culture media, the food we give microbes in the lab, to match. A good medium lets the wanted organism grow; a clever medium also stops unwanted ones or makes the wanted one easy to spot.

You need: the cell structure of bacteria from Unit 2, *Bacterial morphology and cell structure*.

What a cell needs

Most of a bacterial cell's dry weight comes from six elements: carbon, oxygen, nitrogen, hydrogen, phosphorus and sulfur (remember **CHONPS**). These are the **macronutrients**, needed in large amounts. Carbon builds every organic molecule. Nitrogen goes into amino acids and nucleotides. Phosphorus goes into nucleic acids, phospholipids and ATP. Sulfur goes into the amino acids cysteine and methionine.

Potassium, magnesium, calcium and iron are also needed in fair amounts: Mg^{2+} stabilises ribosomes and membranes, and iron sits in cytochromes. **Micronutrients** (trace elements) such

as zinc, copper, manganese, cobalt, molybdenum and nickel are needed in tiny amounts, often as cofactors of enzymes; tap water or impurities in other ingredients usually supply them.

Growth factors are organic compounds a cell needs but cannot make: some amino acids, purines and pyrimidines, and vitamins such as biotin or B12. An organism that needs a growth factor is called **fastidious** if it needs many of them. A mutant that has lost the ability to make one is an **auxotroph** (see 4.8).

Nutritional types

Three questions name an organism's nutritional type. Where does energy come from: light (**photo-**) or chemicals (**chemo-**)? Where do electrons come from: inorganic compounds (**litho-**) or organic compounds (**organo-**)? Where does carbon come from: CO_2 (**auto-**) or organic molecules (**hetero-**)? Join the answers with "-troph".

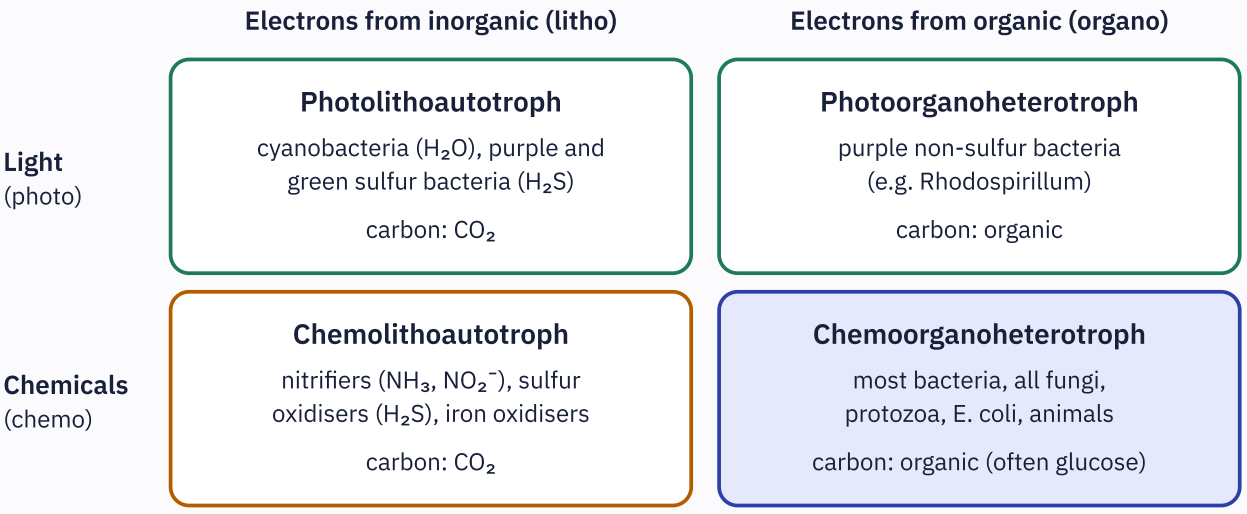


Figure 4.1.1 The four common nutritional types. The bottom-right box holds nearly every pathogen and every industrial heterotroph, which is why most lab media are built on sugar and peptone. Notice that the "auto" organisms are also the "litho" ones in the usual cases.

In practice the names are shortened: **photoautotroph**, **chemoautotroph** (or chemolithotroph), **photoheterotroph** and **chemoheterotroph**. Chemoheterotrophs take energy, electrons and carbon from the same organic molecule, for example glucose.

Culture media

A **culture medium** is a liquid (broth) or solid preparation that supplies the nutrients above at a suitable pH, usually around 7 for bacteria and 5 to 6 for fungi. Solid media are made by adding **agar**, a seaweed polysaccharide, at about 1.5% (15 g/L). Agar melts near 100 °C, sets around 40 °C, and almost no bacterium can digest it, so it stays solid at 37 °C incubation.

Media are classified in three ways:

- **By chemical composition.** A *defined* (synthetic) medium has every ingredient known in exact amount, such as glucose plus mineral salts for E. coli. A *complex* medium contains extracts whose exact make-up is unknown: peptone (digested protein), beef extract or yeast extract. Nutrient broth is complex.
- **By physical state.** Liquid (broth), semi-solid (about 0.5% agar, used to test motility) and solid (about 1.5% agar).
- **By purpose.** *Selective* media suppress unwanted organisms: mannitol salt agar's 7.5% NaCl allows staphylococci. *Differential* media make different organisms look different: on MacConkey agar, lactose fermenters form pink colonies and non-fermenters stay colourless. MacConkey is also selective, because bile salts and crystal violet suppress Gram-positive bacteria. *Enriched* media add blood or serum for fastidious organisms (blood agar, chocolate agar). *Enrichment* broths favour a rare organism in a mixed sample until it outnumbers the rest, such as selenite F broth for Salmonella from stool or food. *Reducing* media, such as thioglycollate broth, remove oxygen for anaerobes. *Transport* media keep a sample alive without letting it grow.

Blood agar is a good exam case because it is both enriched and differential: β-haemolysis clears the blood fully around a colony, α-haemolysis gives a green zone, and γ means no change.

WORKED EXAMPLE

Question. A lab uses this nutrient agar recipe per litre: peptone 5 g, beef extract 3 g, NaCl 5 g, agar 15 g. (a) How much of each do you weigh for 400 mL? (b) Is the medium defined or complex, and why? (c) What would you add, and at what level, to make it select for staphylococci? (4 marks)

1. Scale factor = 400 mL ÷ 1000 mL = 0.4.
2. Peptone 5 × 0.4 = 2.0 g; beef extract 3 × 0.4 = 1.2 g; NaCl 5 × 0.4 = 2.0 g; agar 15 × 0.4 = 6.0 g. Make up to 400 mL with water, adjust pH to about 7, autoclave at 121 °C for 15 min.
3. Complex: peptone and beef extract are mixtures whose exact chemical make-up is not known.
4. To select for staphylococci, raise NaCl to 7.5% (75 g/L). For 400 mL that is 0.4 × 75 = 30 g NaCl in total, replacing the 2.0 g. Adding mannitol with phenol red (as in mannitol salt agar) also makes it differential: S. aureus ferments mannitol and turns the medium yellow.

EXAM TRAPS

- Selective versus differential: selective decides *who grows*; differential decides *how they look*. MacConkey and mannitol salt agar are both.
- Enriched versus enrichment: enriched is a rich solid medium (blood agar); enrichment is a broth that tilts a mixed population towards one organism.
- Litho and auto are separate questions: one asks about electrons, the other about carbon.
- Agar is a solidifying agent, not a nutrient; few bacteria can break it down.

CHECK YOURSELF

– REMEMBER **Name the six macronutrient elements.** 2 marks

Carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur (CHONPS).

– REMEMBER **Define a growth factor and give two examples.** 2 marks

An organic compound the cell needs but cannot make, so it must be supplied: amino acids, purines, pyrimidines, vitamins such as biotin.

– UNDERSTAND **Why is MacConkey agar both selective and differential?** 3 marks

Bile salts and crystal violet stop Gram-positive bacteria (selective). Lactose with neutral red makes lactose fermenters pink and non-fermenters colourless (differential).

– UNDERSTAND **Classify Nitrosomonas, which oxidises ammonia and fixes CO_2.** 2 marks

Energy from a chemical, electrons from an inorganic compound (NH_3), carbon from CO_2: chemolithoautotroph.

– APPLY **How much agar and glucose for 250 mL of a medium with 1.5% agar and 0.5% glucose?** 2 marks

1.5% w/v = 1.5 g per 100 mL, so 3.75 g agar; 0.5% = 0.5 g per 100 mL, so 1.25 g glucose.

– APPLY **Suggest a medium to grow a strict anaerobe in broth.** 2 marks

Thioglycollate broth: sodium thioglycollate removes dissolved oxygen, and growth at the bottom of the tube shows an anaerobe.

– ANALYZE **A student's E. coli grows in nutrient broth but not in glucose-salts medium. What does this suggest?** 3 marks

The strain needs a growth factor that the complex medium supplies and the defined one lacks; it is probably an auxotroph. Testing defined medium with single added amino acids would find which one.

BEYOND THE SYLLABUS

Industry designs chemically defined media for recombinant protein production, because a defined medium gives the same batch every time and is easier to justify to regulators. Chromogenic media, where each species gives a different colony colour, are now common in clinical and food labs. Most environmental microbes still refuse to grow on any medium; culturomics and "iChip" style diffusion chambers try to change that. Search "chromogenic agar" and "the great plate count anomaly". This is not for the exam.

4.2 Pure culture methods and preservation of cultures

A **pure culture** (axenic culture) contains only one kind of microorganism, all descended from a single cell. Every identification test, every growth curve and every industrial fermentation starts from one, because a mixed culture gives mixed answers. The trick is to spread cells so thin that each lands alone and grows into its own colony, then keep that strain alive and unchanged for years. Others teach it in the lab course.

Getting a pure culture

All plating methods rest on one idea: one well-separated colony is assumed to come from one cell (strictly, one **colony-forming unit**, since a chain or clump can also give one colony).

- **Streak plate.** A sterile loop drags the sample across the agar in three or four sections, flaming or changing the loop between them, so each section gets fewer cells than the last.
- **Spread plate.** A small volume (usually 0.1 mL) of a diluted sample is spread over the surface with a sterile glass or plastic spreader. Colonies grow on the surface.
- **Pour plate.** Diluted sample is mixed with melted agar cooled to about 45 °C and poured. Colonies grow both on and inside the agar; heat-sensitive or strictly aerobic organisms may suffer.

- **Single-cell methods.** A micromanipulator under the microscope picks one cell. Flow sorting and limiting dilution in microwell plates do the same at scale.

Enrichment broths (see 4.1) come first when the organism is rare in the sample.

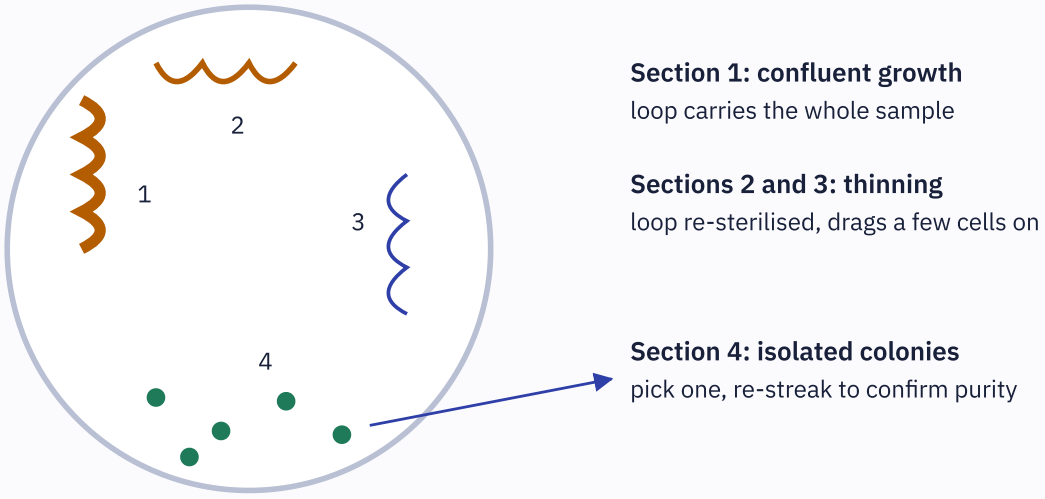


Figure 4.2.1 A four-quadrant streak plate after incubation. Cell number falls with each section, so only the last sections show single colonies. One colony is picked and streaked again before it is called pure.

Keeping it alive: preservation

The aim is to stop growth (so no mutations pile up and no contaminant enters) while keeping cells viable. Colder and drier means longer storage.

1. **Periodic subculture.** Transfer to fresh slants every few weeks and store at about 4 °C. Cheap, but each transfer risks contamination and selects mutants.
2. **Mineral oil overlay.** Sterile paraffin oil over a slant cuts oxygen and drying; works for months to a few years for many fungi and bacteria.
3. **Freezing in glycerol.** Cells in broth with about 15–20% glycerol are frozen at −80 °C. Glycerol is a cryoprotectant: it limits ice crystal damage. This is the everyday lab method.
4. **Liquid nitrogen.** Storage at −196 °C (or in the vapour phase above it) stops all metabolism; viability is kept for decades.
5. **Lyophilisation (freeze-drying).** Cells in a protectant such as skim milk are frozen, then water is removed under vacuum by sublimation. Sealed ampoules store at room temperature and are easy to post, which is why culture collections use them.

National collections hold reference strains: in India, MTCC (Microbial Type Culture Collection) at CSIR-IMTECH Chandigarh and NCIM at CSIR-NCL Pune; abroad, ATCC (USA) and DSMZ

(Germany).

WORKED EXAMPLE

Question. A soil suspension has about 10^8 bacteria per mL. You want 30 to 300 colonies on a spread plate of 0.1 mL. Which tenfold dilution should you plate? (3 marks)

1. Cells plated = concentration × dilution × 0.1 mL.
2. At 10^{-5}: $10^8 \times 10^{-5} \times 0.1 = 100$ colonies. At 10^{-6}: 10 colonies. At 10^{-4}: 1000 colonies.
3. Plate 10^{-5} (expect about 100) and, as a safety margin, 10^{-4} and 10^{-6} too, because the starting count is only an estimate.

EXAM TRAPS

- A colony comes from one CFU, which may be more than one cell; that is why you re-streak.
- Pour plates grow colonies inside the agar too; spread plates only on the surface.
- Lyophilisation removes water by sublimation from the frozen state, not by heating.
- Glycerol stocks belong at −80 °C; at −20 °C viability falls much faster.

CHECK YOURSELF

– REMEMBER **Define a pure culture.** 1 mark

A culture with only one kind of microorganism, descended from a single cell or CFU.

– REMEMBER **Name two Indian culture collections.** 2 marks

MTCC (CSIR-IMTECH, Chandigarh) and NCIM (CSIR-NCL, Pune).

– UNDERSTAND **Why does a streak plate give single colonies only in the last sections?** 2 marks

Each section is started by dragging a few cells from the previous one with a re-sterilised loop, so the number of cells falls step by step until cells land apart.

– APPLY **Choose a method to send 50 strains by post to another lab.** 2 marks

Lyophilised ampoules: dry, stable at room temperature, no cold chain needed.

– ANALYZE **Why is repeated subculture a poor way to keep an industrial strain for years?** 3 marks

Each transfer means growth, so mutations accumulate and faster-growing low-yield variants can take over; each transfer also risks contamination. Freezing or freeze-drying stops growth.

BEYOND THE SYLLABUS

Biotech companies keep a "master cell bank" and "working cell banks" of each production strain, frozen in many identical vials, so every batch starts from the same cells. Microfluidic droplets now isolate thousands of single cells at once. Search "cell banking ICH Q5D" and "droplet microfluidics single cell isolation". This is not for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Nutritional type	energy (photo/chemo) + electrons (litho/organo) + carbon (auto/hetero) + "troph"	4.1
Selective / differential medium	decides who grows / makes groups look different (MacConkey is both)	4.1
Pure culture	one kind of organism from one CFU; streak, spread or pour plate	4.2
Preservation	subculture at 4 °C; oil overlay; glycerol at −80 °C; liquid N_2 at −196 °C; lyophilisation	4.2

Glossary (terms used in this lesson)

Agar Seaweed polysaccharide used at about 1.5% to solidify media; few microbes digest it.

Auxotroph Mutant that needs a nutrient the wild type can make.

Complex medium Medium with ingredients of unknown exact composition, such as peptone.

Defined medium	Medium whose every component and amount is known.
Fermentation	Energy metabolism with no electron transport chain, in which an organic product accepts electrons from NADH.
Lyophilisation	Freeze-drying: freezing, then removing water by sublimation under vacuum.
Pure culture	Culture of one kind of organism from a single CFU.

The full unit book

Every chapter of Unit 4, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 4 · Microbial Nutrition, Growth and Genetics (one file, works offline).

Sources and further reading

Textbooks

- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: microbial nutrition, growth, metabolism and genetics chapters (from memory; check).
- Madigan and others, *Brock Biology of Microorganisms*, Pearson: reduction potentials, anaerobic respiration and phototrophy (from memory; check).
- Pelczar, Chan and Krieg, *Microbiology*, McGraw-Hill (Indian edition widely used) (from memory; check).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson (from memory; check).
- Shuler and Kargi, *Bioprocess Engineering: Basic Concepts*, for Monod and chemostat kinetics (from memory; check).

Open resources

- OpenStax, *Microbiology*, section 9.1 "How Microbes Grow": growth phases, 30–300 plate rule, direct and indirect counts. openstax.org/books/microbiology/pages/9-1-how-microbes-grow
- OpenStax, *Microbiology*, section 11.6 "How Asexual Prokaryotes Achieve Genetic Diversity": Griffith and Avery, Hfr and F′ cells, 100-minute E. coli transfer. openstax.org/books/microbiology/pages/11-6-how-asexual-prokaryotes-achieve-genetic-diversity

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