

Bacterial Culture

What bacteria need to grow, how their numbers rise and fall in a flask, the metabolic side-routes some of them take, how they swap genes, and how antibiotics exploit all of it.

Media & Nutrition Growth Curve Glyoxylate Cycle Entner-Doudoroff

Gene Transfer Antibiotics

Media

Physical Factors

Auxotrophy

Growth Curve

Metabolism

Genetics

1 Nutritional Requirements & Media

What a bacterium needs, and how we supply it in the lab

Every bacterium needs a carbon source, nitrogen, sulfur, phosphorus, trace elements (Mg, Ca, Fe, K, Zn, Mn...), and — for fastidious or auxotrophic strains — specific growth factors such as vitamins or amino acids.

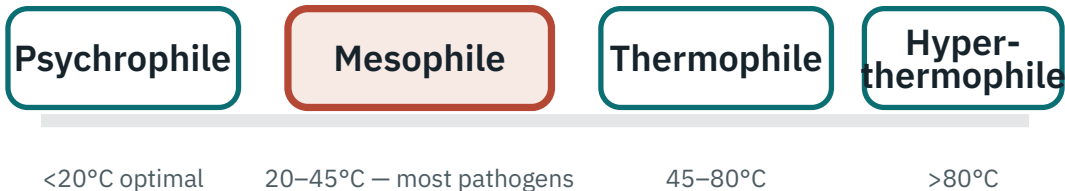
Media type	Composition	Use
Complex (undefined)	Extracts of unknown exact composition — beef/yeast extract, peptone	Routine culture, fastidious organisms (nutrient broth, blood agar)
Synthetic (defined)	Exact chemical composition, precise quantities	Studying specific nutritional requirements, auxotrophy
Selective	Inhibits unwanted organisms	MacConkey agar (bile salts inhibit

		Gram-positives)
Differential	Reveals a visible biochemical trait	Blood agar (haemolysis patterns)
Enriched	Extra nutrients added to a base medium	Chocolate agar for <i>Haemophilus</i> , <i>Neisseria</i>

2 Physical Factors of Bacterial Growth

pH, temperature, and the practical variables of culturing

Temperature classes



Factor	Effect
pH	Most pathogens are neutrophiles (6.5–7.5); acidophiles (<i>Lactobacillus</i>), alkaliphiles (<i>Vibrio cholerae</i> , ~8.5)
Oxygen requirement	Obligate aerobe / obligate anaerobe / facultative anaerobe / microaerophile / aerotolerant
Volume of media	Shallow, large surface area → more aerobic; deep, small surface → more anaerobic
Size of inoculum	Larger inoculum generally shortens the lag phase
Incubation time	Determines which growth phase is captured on sampling

3 Bacterial Auxotrophy

When a mutant loses a biosynthetic pathway

A **prototroph** (wild type) can synthesise every compound it needs from a minimal medium. An **auxotroph** has lost the ability to synthesise one particular compound (e.g. an amino acid) through mutation, and will only grow if that compound is supplied externally.

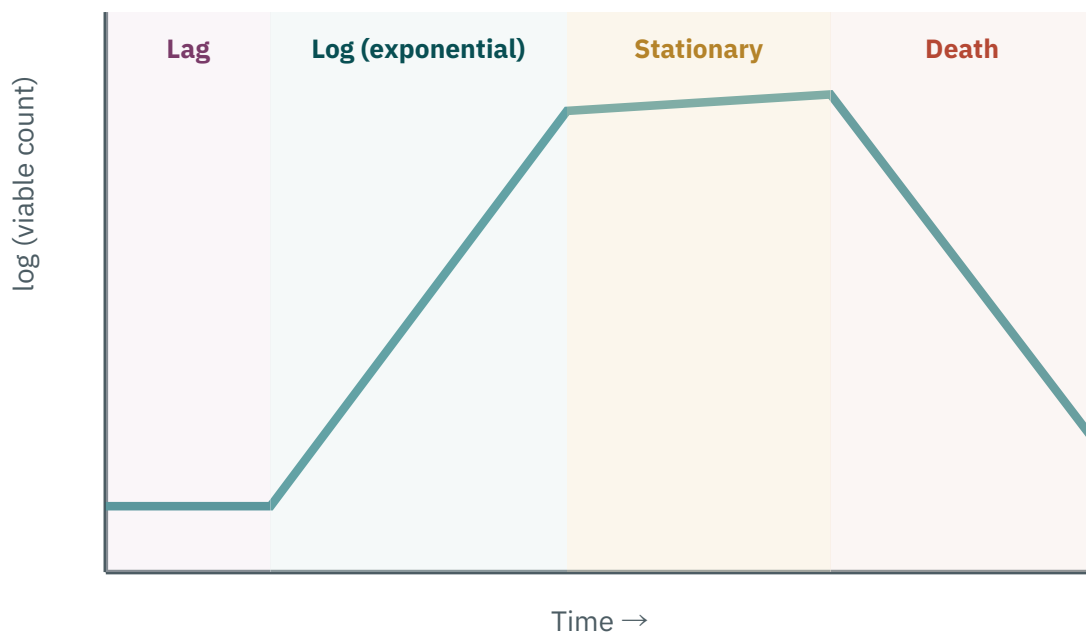
WHY AUXOTROPHS MATTER

Auxotrophic mutants are a classic genetics tool — replica plating onto minimal vs. supplemented media identifies which nutrient a mutant strain can no longer make, which in turn maps the function of the mutated gene.

4 Bacterial Growth Curve

Four phases in a closed (batch) culture

Log(viable count) vs. time



Phase	What's happening
Lag	Metabolic adaptation, enzyme/RNA synthesis — no division yet; duration depends on inoculum history
Log (exponential)	Constant doubling time; most uniform physiology; most antibiotic-susceptible phase
Stationary	Growth rate = death rate (nutrient depletion, waste build-up); secondary metabolites (toxins, antibiotics) often made here
Death (decline)	Death rate exceeds growth rate; some cells may persist in a viable-but-non-culturable state

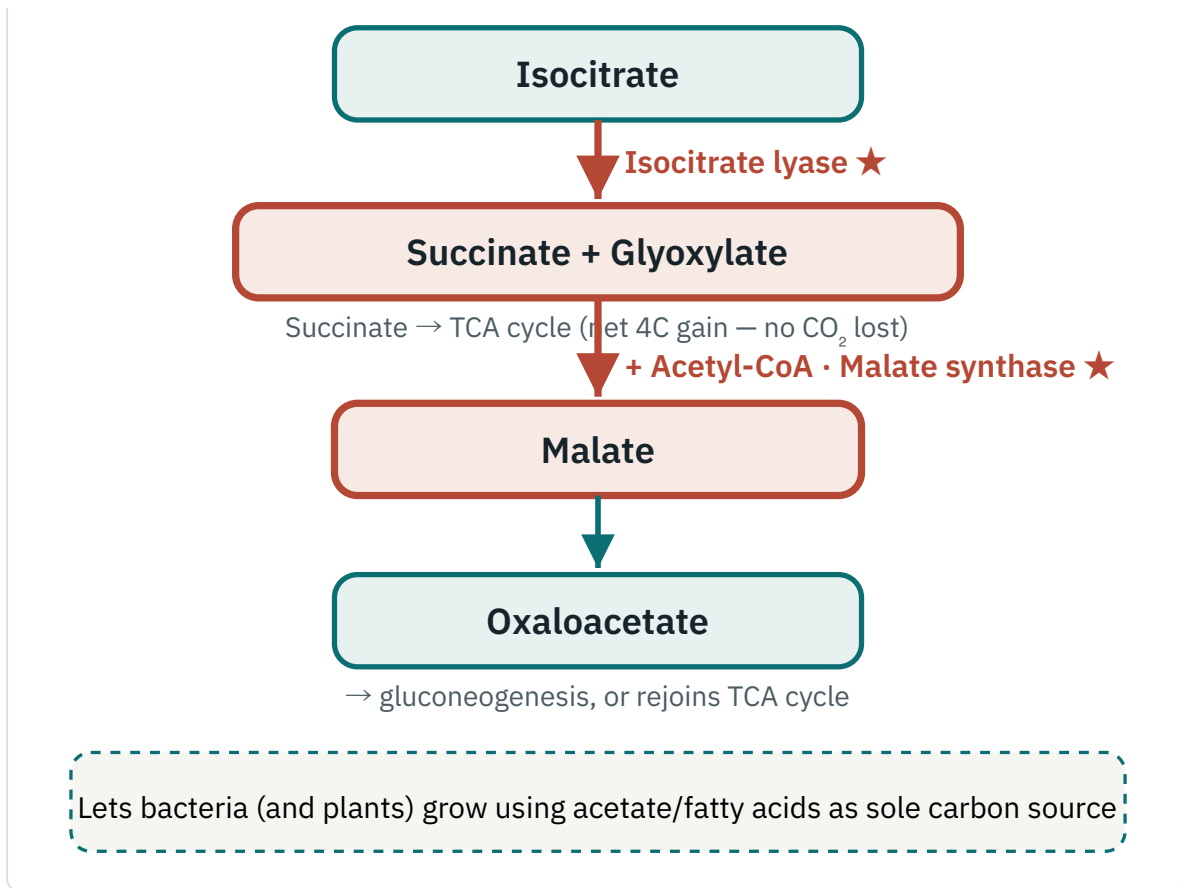
5 Bacterial Metabolism

Fermentation, the glyoxylate cycle, and the Entner–Doudoroff pathway

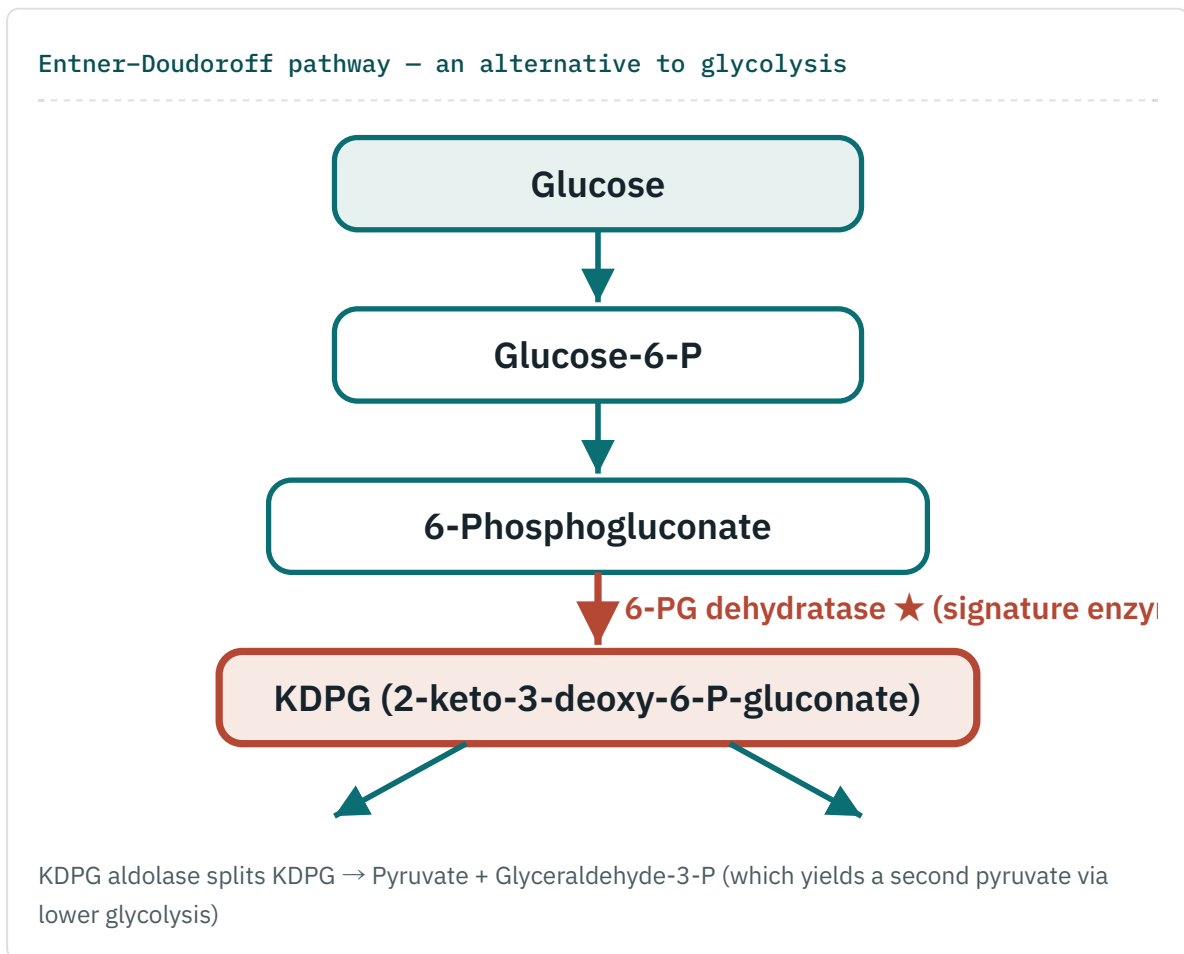
Fermentation uses an organic compound as both electron donor and acceptor (no respiration, substrate-level phosphorylation only) — the fermentation products are often used diagnostically.

Fermentation type	Products	Example organisms / test
Lactic acid	Lactate	<i>Streptococcus</i> , <i>Lactobacillus</i>
Mixed acid	Lactic, acetic, succinic, formic acid, ethanol, CO ₂ /H ₂	<i>E. coli</i> — Methyl Red positive
Butanediol	2,3-Butanediol	<i>Enterobacter</i> , <i>Klebsiella</i> — Voges-Proskauer positive

Glyoxylate cycle — a bypass around the TCA cycle



Entner-Doudoroff pathway – an alternative to glycolysis



WHY IT MATTERS

Found mainly in aerobic Gram-negatives such as *Pseudomonas* and *Zymomonas* (industrially important for ethanol fermentation). Net yield is only 1 ATP + 1 NADH + 1 NADPH per glucose — about half of EMP glycolysis — but it's the only route available to organisms lacking a complete glycolytic enzyme set.

6 Bacterial Genetics — Horizontal Gene Transfer

Three ways DNA moves between bacterial cells

Transformation, conjugation & transduction

TRANSFORMATION

Free DNA (from lysed cells) taken up directly by a "competent" recipient cell — no donor cell contact needed

Natural:

S. pneumoniae, *Neisseria*, *Bacillus* · Lab: CaCl_2 /heat-shock, electroporation
CONJUGATION Direct cell-to-cell contact via sex (F) pilus; F^+ donor transfers plasmid DNA to F^- recipient Hfr strains (F integrated into chromosome) transfer chromosomal genes too — the

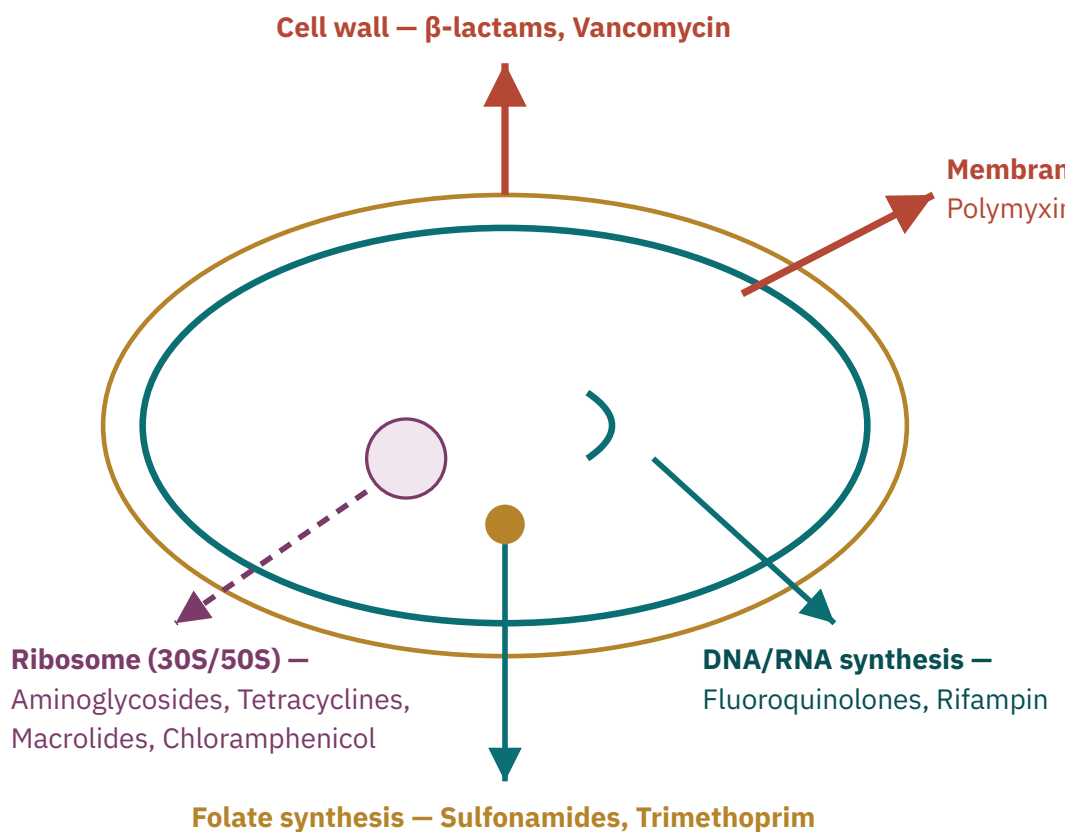
basis of interrupted-mating chromosome mapping
TRANSDUCTION DNA transferred by a bacteriophage vector
Generalized: lytic phage accidentally packages random host DNA — any gene can transfer
Specialized: imprecise prophage excision carries only genes adjacent to the integration site (e.g. λ phage in *E. coli*)

7 Bacterial Infection & Treatment

Chemotherapeutic agents and antibiotic mechanisms

A **chemotherapeutic agent** is any chemical used to treat disease with selective toxicity for the pathogen (includes fully synthetic drugs). An **antibiotic**, strictly, is a substance produced by one microorganism that inhibits or kills another — though the term is now used loosely for synthetic antimicrobials too.

Where antibiotics hit the bacterial cell



	Bactericidal	Bacteriostatic
Action	Kills bacteria directly, irreversibly	Halts growth/division; host immunity clears the rest
Examples	β -lactams, aminoglycosides, fluoroquinolones, vancomycin	Tetracyclines, chloramphenicol, macrolides, sulfonamides

SELECTIVE TOXICITY, AND A SYNERGY TRICK

Cell-wall and ribosome-targeted drugs work because human cells lack a cell wall and use a different (80S) ribosome. Sulfonamides and trimethoprim are each bacteriostatic alone, but together (co-trimoxazole) they block sequential steps of folate synthesis and become bactericidal — a classic example of antimicrobial synergy.

8 Exam Corner

Practice question set — end-semester format

B.Sc. Biochemistry — End-Semester Examination

Paper: Bacterial Culture

Full Marks: 30

Time: 1.5 Hrs

GROUP A — LONG ANSWER (ANSWER ANY TWO)

- Describe the bacterial growth curve with a labelled diagram, explaining the physiological events of each phase. **10**
- Describe the three mechanisms of horizontal gene transfer in bacteria — transformation, conjugation, and transduction. **10**
- Classify antibiotics by their site and mechanism of action, distinguishing bactericidal from bacteriostatic drugs with examples. **10**

GROUP B — SHORT NOTES (ANSWER ANY TWO)

- Complex vs. synthetic culture media **5**
- The glyoxylate cycle as a bypass of the TCA cycle **5**

