

Classification of Microorganisms

How microbes are named, identified, and controlled — the domains of life, the tools that tell one bacterium from another, how we kill or tame them, and the anatomy that makes a bacterial cell a bacterial cell.

Identification Techniques

Sterilization

Man-Microbe Interactions

Food-Borne Infection

Bacterial Structure

Gram & AFB Staining

Classification

Identification

Sterilization

Interactions

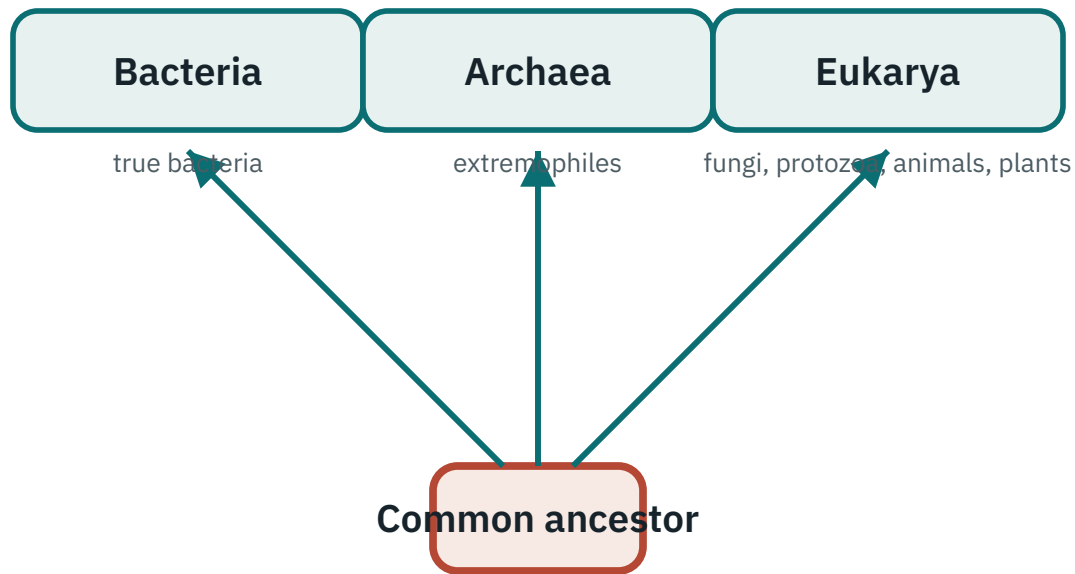
Food-Borne

Morpl

1 Classification of Microorganisms

The three domains of life, and where viruses sit outside them

Woese's three-domain system (rRNA-based)



WHERE DO VIRUSES FIT?

Nowhere in this tree — viruses are acellular, obligate intracellular parasites with no independent metabolism, so they sit outside the three-domain classification entirely. Taxonomic hierarchy for cellular life runs Domain → Kingdom → Phylum → Class → Order → Family → Genus → Species, with organisms named by binomial nomenclature (e.g. *Escherichia coli*).

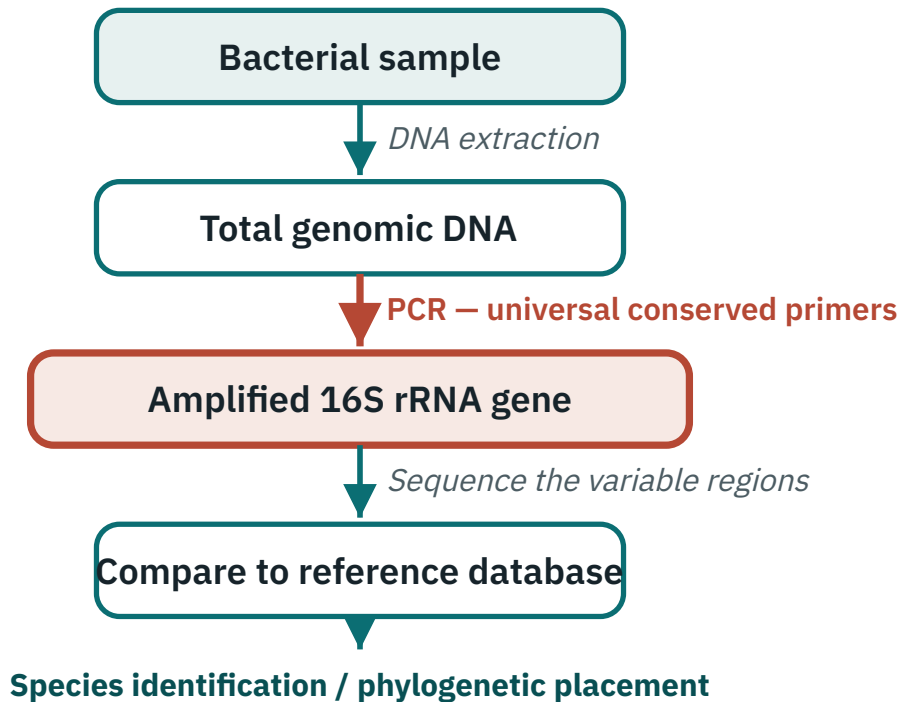
2 Techniques for Identification

Microscopic, biochemical, and molecular methods

Method	Best for
Bright-field microscopy	Routine, stained specimens
Dark-field microscopy	Thin, unstained organisms (e.g. <i>Treponema pallidum</i>)
Phase-contrast	Living, unstained cells
Fluorescence microscopy	Fluorescent dyes/antibody-tagged targets
Electron microscopy (TEM/SEM)	Ultrastructure, viruses

Biochemical tests exploit metabolic differences: catalase ($\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{H}_2\text{O}$, splits *Staphylococcus* from *Streptococcus*), oxidase, coagulase (*S. aureus*), and the IMViC panel (Indole, Methyl red, Voges-Proskauer, Citrate) that separates the Enterobacteriaceae.

16S rRNA sequencing – the molecular gold standard



WHY 16S rRNA?

The gene is present in essentially all bacteria, is large enough to be informative, and contains both highly conserved regions (for universal primer binding) and variable regions (for species-level discrimination) — and critically, it works even on organisms that cannot be cultured.

3 Sterilization, Disinfection & Pasteurization

Physical and chemical methods of microbial control

The spectrum of microbial killing

Sterilization**Disinfection****Sanitization/Cleaning**

kills ALL life, incl. spores most pathogens, spores may survive reduces microbial load

Method	Type	Typical use
Autoclave (121°C, 15 psi, 15–20 min)	Physical — moist heat	Sterilization of media, instruments; kills spores
Hot air oven (160–180°C, 1–2 h)	Physical — dry heat	Glassware; less penetrating than moist heat
Pasteurization (72°C/15s or 63°C/30min)	Physical — mild heat	Milk/beverages — reduces pathogens, does not sterilize
Filtration (0.22 µm membrane)	Physical — removal	Heat-labile solutions, antibiotics, vaccines
UV radiation	Physical — non-ionizing	Surface/air disinfection; forms thymine dimers
Gamma/ionizing radiation	Physical — ionizing	Disposable medical equipment, food irradiation
Alcohols (70% ethanol/isopropanol)	Chemical	Skin antisepsis — denatures protein
Glutaraldehyde, formaldehyde	Chemical — aldehyde	High-level disinfection of equipment
Chlorine, iodine	Chemical — halogen	Water treatment, antisepsis — oxidising
Ethylene oxide gas	Chemical — alkylating	Heat-sensitive medical devices

Routes of entry: respiratory (droplets), gastrointestinal (faecal-oral), skin/mucous membrane (contact, breaks in barrier), genitourinary (sexual), parenteral (injection, vector bite), transplacental (vertical). **Habitat organs** host distinct normal flora — skin (*S. epidermidis*), oral cavity (viridans streptococci), colon (the largest microbial population in the body — *E. coli*, *Bacteroides*), vagina (*Lactobacillus*, maintaining acidic pH); the lower respiratory tract is normally sterile.

Interaction	Effect (A / B)	Example
Mutualism	+ / + (obligatory)	Gut flora synthesise vitamin K/B12; host provides nutrients
Commensalism	+ / 0	Normal skin flora
Syntropism	+ / + (cross-feeding)	One organism's waste is another's substrate — biofilms
Proto-cooperation	+ / + (facultative)	Mutual benefit, but neither partner is obligatorily dependent
Predation	+ / –	Protozoa consuming bacteria
Parasitism	+ / –	Pathogen harming host for its own benefit
Competition	– / –	Two species competing for the same nutrient/space
Amensalism	– / 0	<i>Penicillium</i> inhibiting nearby bacteria, unaffected itself

5 Food-Borne Infections

Live pathogen vs. pre-formed toxin

	Food-borne infection	Food-borne intoxication
What's ingested	Live, viable pathogen	Pre-formed toxin (organism need not be viable)

Onset	Slower — organism must multiply in host	Rapid, often within hours
Examples	<i>Salmonella</i> , <i>Campylobacter</i> , <i>Listeria</i> , pathogenic <i>E. coli</i>	<i>S. aureus</i> enterotoxin, <i>C. botulinum</i> toxin, <i>B. cereus</i> emetic toxin

PREVENTION

Proper hand hygiene, adequate cooking temperatures, refrigeration outside the 4–60°C "danger zone," pasteurization of dairy, safe water/sanitation, and avoiding cross-contamination between raw and cooked foods remain the core controls across nearly every food-borne pathogen.

6 Bacterial Classification by Morphology

Shape and arrangement — the first clue under the microscope

Shapes and arrangements

COCCUS



single



diplococcus



streptococcus



staphylococcus



tetrad



sarcina

BACILLUS



single rod



streptobacilli



coccobacillus

SPIRAL



vibrio (comma)



spirillum (rigid)



spirochete (flexible)

PLEOMORPHIC BACTERIA

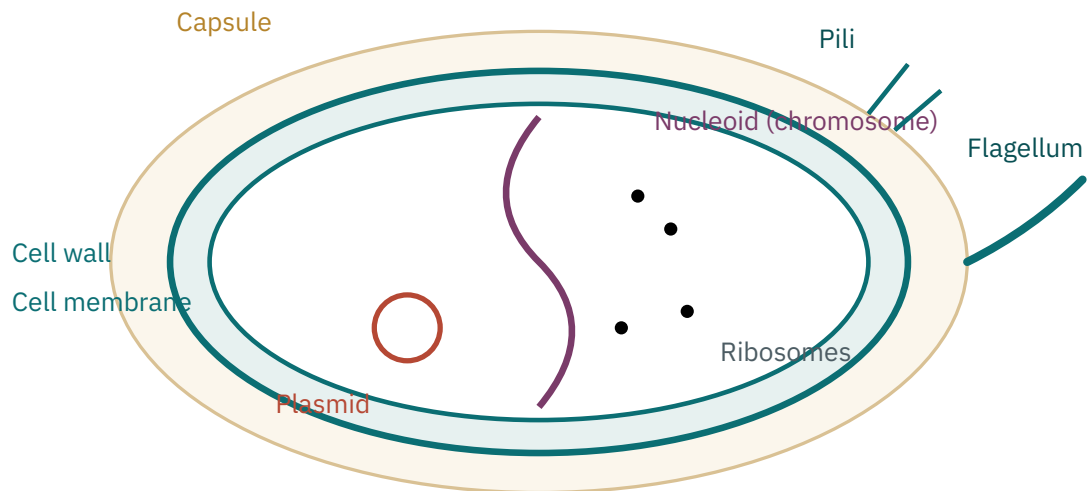
Mycoplasma lacks a cell wall entirely, so it takes on variable shapes — the

exception that proves how much the wall normally dictates bacterial morphology.

7 Bacterial Structure

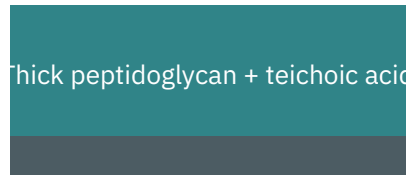
Cell envelope, appendages, genetic material, and survival forms

A generalised bacterial cell



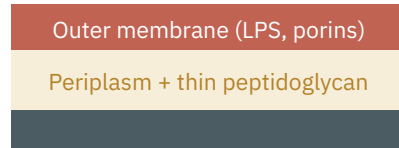
Gram-positive vs. Gram-negative cell envelope

GRAM-POSITIVE



Cell (plasma) membrane

GRAM-NEGATIVE



Cell (plasma) membrane

WHY IT MATTERS FOR GRAM STAINING

Thick peptidoglycan traps the crystal violet–iodine complex when dehydrated by decolouriser → stays purple.

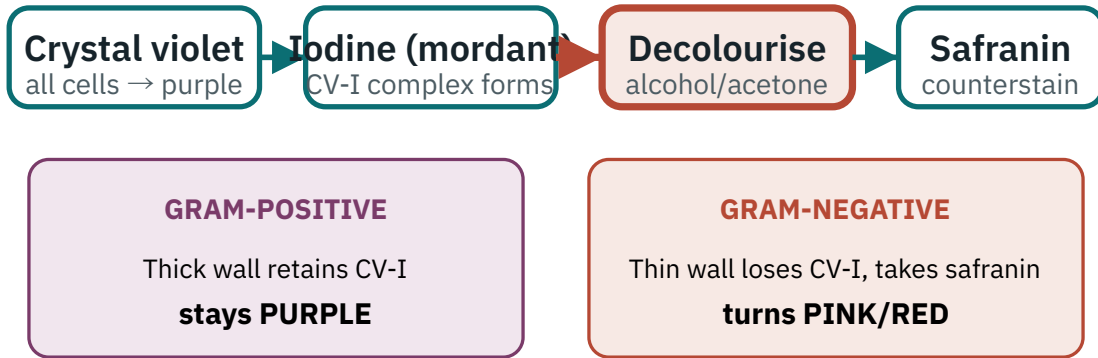
Thin peptidoglycan + dissolving outer membrane → loses it → pink.

Structure	Role
Flagella	Locomotion (protein flagellin); monotrichous/lophotrichous/amphitrichous/peritrichous arrangements
Pili / fimbriae	Adhesion to surfaces (fimbriae); DNA transfer via sex/F pilus during conjugation
Chromosome	Single circular dsDNA, supercoiled, in the nucleoid — no nuclear membrane
Plasmid	Extrachromosomal circular DNA — often carries antibiotic resistance/virulence genes
Endospore	Dormant, highly resistant survival form (<i>Bacillus</i> , <i>Clostridium</i>) — not a reproductive structure
Cyst	Protective dormant form in some protozoa (<i>Entamoeba</i> , <i>Giardia</i>) aiding faecal-oral transmission

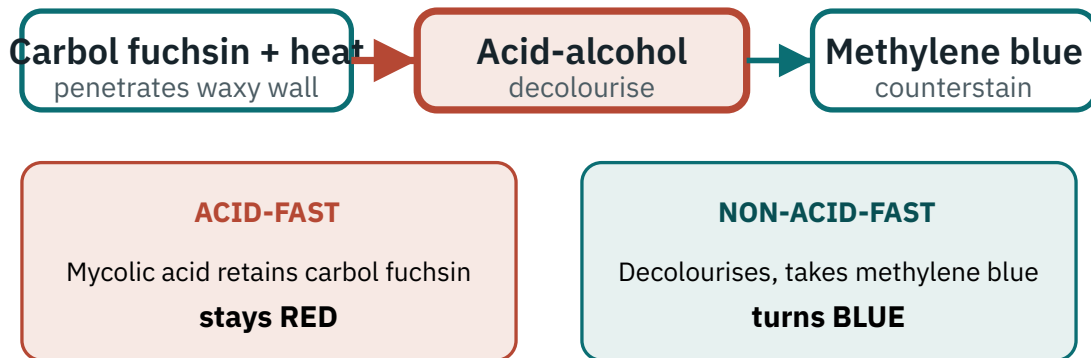
8 Staining Techniques

Gram stain and acid-fast stain — reading the cell wall through colour

Gram staining



Acid-fast (Ziehl-Neelsen) staining



WHY ACID-FAST STAINING EXISTS

Mycobacterium species (tuberculosis, leprosy) have a mycolic-acid-rich waxy cell wall that barely takes up Gram stain at all — acid-fast staining was specifically developed to identify these organisms via their unique resistance to acid-alcohol decolourisation.

9 Exam Corner

Practice question set — end-semester format

B.Sc. Biochemistry — End-Semester Examination

Paper: Classification of Microorganisms

Full Marks: 30

Time: 1.5 Hrs

GROUP A – LONG ANSWER (ANSWER ANY TWO)

1. Describe the Gram staining procedure and explain, with reference to cell wall structure, why Gram-positive and Gram-negative bacteria stain differently. **10**
2. Describe the structure of a generalised bacterial cell, including the cell envelope, appendages, and genetic material. **10**
3. Discuss the physical and chemical methods used for sterilization and disinfection, distinguishing sterilization from disinfection and pasteurization. **10**

GROUP B – SHORT NOTES (ANSWER ANY TWO)

- a. 16S rRNA sequencing for bacterial identification **5**
- b. Positive and negative man-microbe interactions **5**
- c. Food-borne infection vs. food-borne intoxication **5**