Microbiology

Unit 1 · Lesson 2 · Sort, name and identify microbes

The notes for Lesson 2 of Unit 1: chapters 1.3 to 1.5 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 2 of 4 | 3 chapters | Chapters 1.3 to 1.5

About these notes

These are the notes for **Lesson 2 · Sort, name and identify microbes** of the University free n easy video lessons on Microbiology Unit 1, Foundations: Microbial World and Microscopy. They are chapters 1.3 to 1.5 of the unit book, unchanged, so a page here is the same page in the full book.

1.3 Microbial diversity and types of microorganisms

Microbes are the most numerous and varied living things on Earth. One estimate puts the number of prokaryotic cells on the planet at 4–6 × 10^{30} (Whitman and colleagues, 1998), living everywhere from the soda water of Lonar Lake in Maharashtra to the human gut. This chapter is the map; each group gets its own chapter in Units 2 and 3.

The types of microorganisms

GROUP	CELL TYPE	KEY FEATURES	EXAMPLE
Bacteria	Prokaryotic	Peptidoglycan wall; cocci, rods, spirals	*Lactobacillus* in curd
Archaea	Prokaryotic	No peptidoglycan; ether-linked lipids; many in extreme or oxygen-free places	Methane makers in biogas plants
Fungi	Eukaryotic	Chitin wall; absorb food; yeasts are single cells, moulds grow as threads (hyphae)	*Saccharomyces*, *Penicillium*
Algae	Eukaryotic	Chlorophyll; usually cellulose wall	*Chlorella*, diatoms
Protozoa	Eukaryotic	No wall; usually motile; ingest food	*Plasmodium*, *Entamoeba*

GROUP	CELL TYPE	KEY FEATURES	EXAMPLE
Viruses	Not cells	DNA or RNA in a protein coat; multiply only inside host cells	SARS-CoV-2, rabies virus
Viroids, prions	Not cells	Viroid: naked circular RNA; prion: misfolded protein	Potato spindle tuber viroid

Helminthes") because their eggs and larvae are identified under the microscope; see Unit 2, *Protozoa and helminths*.

Ways in which microbes differ

Microbes differ in size (Figure 1.3.1), in shape (Unit 2), in where they get energy and carbon (light or chemicals, CO_2 or organic matter; Unit 4), in their need for oxygen, and in habitat, up to the extremophiles of hot springs and salt pans (Unit 2). Most of this diversity has never been grown in a lab: plate counts of soil or sea water recover only a small fraction (often quoted as about 1%) of the cells seen under the microscope. This gap is called the great plate count anomaly, and DNA-based methods (Chapter 1.5) were developed to see past it.

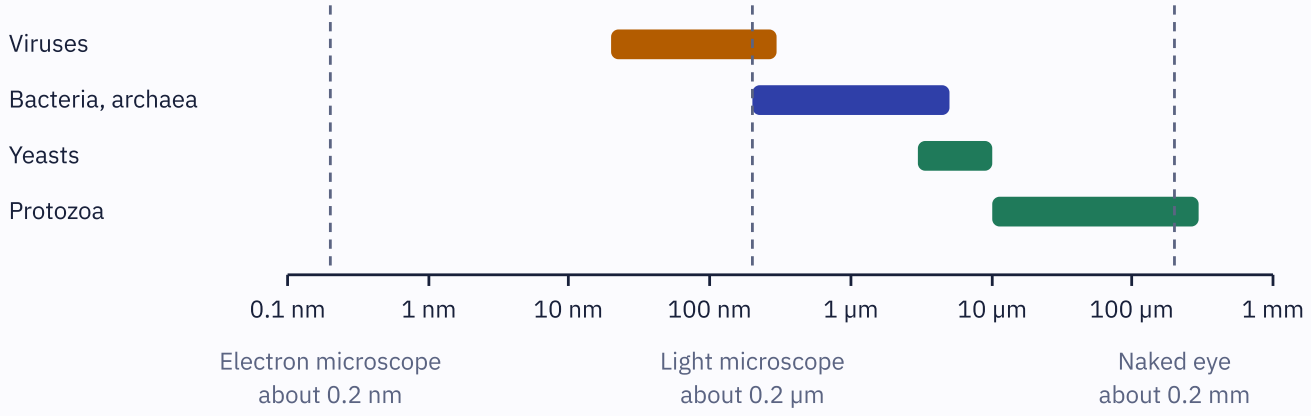


Figure 1.3.1 Typical size ranges on a logarithmic scale (each tick is ten times the last), with the smallest detail each way of seeing can resolve (dashed lines). Most viruses fall below the light-microscope limit and need the electron microscope; bacteria sit just above it; the largest protozoa reach the limit of the naked eye.

WORKED EXAMPLE

Question (6 marks). Name the group of each organism and say whether it is prokaryotic, eukaryotic or acellular: (a) *Spirulina*, sold as "blue-green alga" tablets; (b) *Saccharomyces cerevisiae*; (c) *Plasmodium falciparum*; (d) *Methanobrevibacter smithii* from the human gut; (e) tobacco mosaic virus; (f) *Chlorella*.

1. First ask: is it a cell? Only (e) is not; a virus is acellular.
2. Next ask: does the cell have a nucleus? (a) *Spirulina* does not: it is a cyanobacterium, a prokaryote, whatever the label says. (d) is also a prokaryote; it makes methane and lacks peptidoglycan, so it is an archaeon.
3. Then sort the eukaryotes by wall and nutrition: (b) wall of glucan and chitin, absorbs food: a fungus (yeast). (c) no wall, parasite of red blood cells: a protozoan. (f) chlorophyll and a cellulose wall: a green alga.

Answer: (a) bacterium, prokaryotic; (b) fungus, eukaryotic; (c) protozoan, eukaryotic; (d) archaeon, prokaryotic; (e) virus, acellular; (f) alga, eukaryotic.

EXAM TRAPS

- "Blue-green algae" are cyanobacteria: prokaryotes, not algae.
- Viruses are not prokaryotes. They are not cells at all: no ribosomes and no metabolism of their own.
- Yeasts and moulds are both fungi; some fungi switch between the two forms.

CHECK YOURSELF

– REMEMBER **List the major groups of microorganisms with one example each.** 4 marks

Bacteria (*E. coli*), archaea (methanogens), fungi (yeast), algae (*Chlorella*), protozoa (*Plasmodium*), viruses (SARS-CoV-2).

– UNDERSTAND **What is the great plate count anomaly?** 2 marks

Far fewer colonies grow on plates than cells are seen under the microscope, so most environmental microbes have never been cultured.

– APPLY **Using Figure 1.3.1, which can be resolved with a light microscope: SARS-CoV-2 (about 100 nm), *Mycoplasma* (about 0.3 µm), a yeast cell (5 µm)?** 3 marks

The yeast clearly. *Mycoplasma* only just, as a dot without detail. SARS-CoV-2 is below the 0.2 µm limit and needs an electron microscope.

– ANALYZE **A microbe has a nucleus and a wall, no chlorophyll, and grows as branched threads. Name its group and the feature that rules out each alternative.** 4 marks

A mould (fungus). No chlorophyll rules out algae; the nucleus rules out actinomycetes, which are thread-forming bacteria; the wall rules out protozoa.

– UNDERSTAND **Why are helminths taught in microbiology although they are not microbes?** 2 marks

They cause infections that are diagnosed by finding their microscopic eggs or larvae in samples.

BEYOND THE SYLLABUS

Projects such as the Earth Microbiome Project map diversity by sequencing DNA straight from soil, water and gut samples. One surprise is a large group of very small bacteria, the candidate phyla radiation, known almost only from their genomes. Giant viruses such as Mimivirus, described in 2003, are larger than some bacteria. None of this is for the exam.

1.4 Classification, taxonomy and identification of microbes

Taxonomy is the science of sorting living things into groups. It has three parts: classification (arranging organisms into groups by their similarities), nomenclature (giving each group its correct name) and identification (deciding which named group an unknown organism belongs to). Microbes are hard to classify because many look alike under the microscope and most reproduce without sex, so microbial taxonomy has moved from shape and staining to biochemistry, and now to DNA sequences.

You need: the prokaryote–eukaryote difference from Chapter 1.2.

Ranks and names

Organisms are placed in ranks, each inside the next: domain, phylum, class, order, family, genus, species (left of Figure 1.4.1). Below species, microbiologists use the **strain**, a pure culture descended from one isolate (*E. coli* K-12), the **serovar** or serotype, strains that differ in surface antigens (*E. coli* O157:H7), and the **biovar**, strains that differ in biochemistry.

Names follow the binomial system of Linnaeus: the genus with a capital letter, then the species epithet in lower case, both in italics (underlined when handwritten), as in *Escherichia coli*. After the first use, the genus may be shortened to *E. coli*. Names of bacteria and archaea follow the International Code of Nomenclature of Prokaryotes. A new species name becomes valid only when it is published in, or listed by, the *International Journal of Systematic and Evolutionary Microbiology*, and its type strain (the reference culture that carries the name) is deposited in public culture collections; India's include MTCC in Chandigarh and NCIM in Pune.

Names change as knowledge improves. In 2021 the phylum names were brought under the code, so Proteobacteria became Pseudomonadota and Firmicutes became Bacillota. Many books and question papers still use the old names; use the one your syllabus uses and mention the other.

From two kingdoms to three domains

Linnaeus recognised two kingdoms, plants and animals, and microbes were forced into one or the other. Ernst Haeckel (1866) added a third, Protista, for microorganisms. Robert Whittaker's five-kingdom system (1969) used cell type, cell number and mode of nutrition to give Monera (all prokaryotes), Protista, Fungi, Plantae and Animalia.

Carl Woese compared the sequences of small-subunit ribosomal RNA (16S rRNA in prokaryotes, 18S in eukaryotes) across many organisms. In 1977 he found that the prokaryotes formed two groups as different from each other as either is from eukaryotes, and in 1990 he and his colleagues proposed three domains, Bacteria, Archaea and Eukarya, above the level of kingdom. rRNA was a good choice because every cell has it, it does the same job everywhere, and it contains both slowly changing (conserved) and faster changing (variable) stretches, so it can compare distant and close relatives alike.

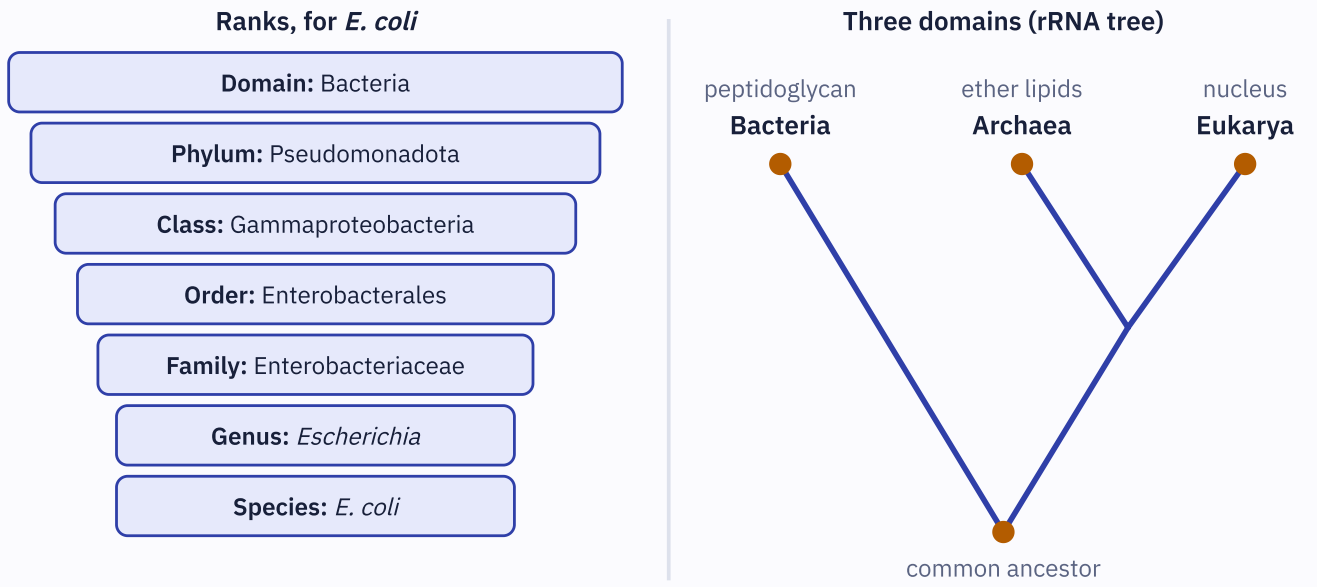


Figure 1.4.1 Left: each rank sits inside the one above it. Right: Woese's tree from rRNA sequences, with one hallmark above each domain name. Notice that Archaea and Eukarya share a branch point: archaea are more closely related to eukaryotes than to bacteria, even though archaea and bacteria are both prokaryotes.

Three ways to classify

Phenetic classification groups organisms by overall similarity in many observable characters (shape, staining, growth, biochemistry), all weighted equally. Its quantitative form is numerical taxonomy, developed by Sneath and Sokal: score 50 or more characters as + or −, calculate a similarity coefficient for every pair of strains, and join the most similar strains first into a tree-like diagram called a dendrogram. For two strains, let a = characters positive in both, b and c = characters positive in only one, and d = characters negative in both. The simple matching coefficient S_SM and the Jaccard coefficient S_J are:

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S_SM = (a + d) / (a + b + c + d)
S_J  = a / (a + b + c)
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S_J leaves out shared negatives, because two strains can fail the same test for unrelated reasons. **Phylogenetic classification** groups organisms by evolutionary descent, read from gene sequences (16S rRNA, and now whole genomes). **Polyphasic taxonomy**, today's standard for describing a new species, combines phenotypic, chemical and genetic data (Chapter 1.5).

Identification

Identification uses only the few characters that separate known groups. A dichotomous key asks a series of two-way questions: Gram-positive or Gram-negative? Cocci or rods? Catalase-positive or negative? Each answer leads to the next question until one name remains. Gram-

positive cocci in clusters that are catalase-positive are staphylococci; if they are also coagulase-positive, the organism is *Staphylococcus aureus*. The standard reference is Bergey's Manual. *Bergey's Manual of Determinative Bacteriology* is the identification guide; *Bergey's Manual of Systematic Bacteriology* describes classification and relationships, and continues online as *Bergey's Manual of Systematics of Archaea and Bacteria*.

WORKED EXAMPLE

Question (8 marks). Three isolates from curd were scored on ten tests. Calculate S_SM and S_J for each pair, and say which two isolates join first in a dendrogram.

TEST	1	2	3	4	5	6	7	8	9	10
A	+	+	−	+	−	+	+	−	−	+
B	+	+	−	+	−	+	−	−	−	+
C	−	+	+	−	+	−	+	+	−	−

1. **A and B.** Both positive in tests 1, 2, 4, 6, 10: a = 5. A only in test 7: b = 1, c = 0. Both negative in 3, 5, 8, 9: d = 4. S_SM = (5 + 4)/10 = 0.90; S_J = 5/(5 + 1 + 0) = 0.83.
2. **A and C.** a = 2 (tests 2, 7); b = 4 (1, 4, 6, 10); c = 3 (3, 5, 8); d = 1 (9). S_SM = 3/10 = 0.30; S_J = 2/9 = 0.22.
3. **B and C.** a = 1 (test 2); b = 4 (1, 4, 6, 10); c = 4 (3, 5, 7, 8); d = 1 (9). S_SM = 2/10 = 0.20; S_J = 1/9 = 0.11.
4. **Cluster.** A and B are most similar by either coefficient, so they join first, at 0.90 (S_SM). C then joins the A–B group at the average of its two values: (0.30 + 0.20)/2 = 0.25.

Conclusion: A and B are probably strains of one species; C is a different organism. A real study would score many more tests and confirm the grouping with 16S rRNA sequencing.

EXAM TRAPS

- Bergey's *Determinative* manual is for identification; the *Systematic* manual is for classification. Examiners ask the difference.
- Write *Escherichia coli* or *E. coli*: capital genus, small species, italic or underlined. "E. Coli" loses marks.
- Classification builds the groups; identification places an unknown into a group that already exists.
- State which coefficient you used: S_SM counts shared negatives (d), S_J does not, so their values differ.
- Domains are above kingdoms, not another name for them.

CHECK YOURSELF

– REMEMBER **List the taxonomic ranks from domain to species.** 2 marks

Domain, phylum, class, order, family, genus, species.

– REMEMBER **Name Whittaker's five kingdoms.** 2 marks

Monera, Protista, Fungi, Plantae, Animalia.

– UNDERSTAND **Why is 16S rRNA suited to building a tree of all cellular life?** 3 marks

All cells have it; it has the same function everywhere; its conserved regions allow alignment across distant groups while its variable regions separate close relatives.

– APPLY **Two strains are both positive in 6 tests, both negative in 2, and differ in 2 (each positive in one). Find S_SM and S_J.** 3 marks

a = 6, b = 1, c = 1, d = 2. S_SM = (6 + 2)/10 = 0.80; S_J = 6/(6 + 1 + 1) = 0.75.

– ANALYZE **An isolate is a Gram-positive coccus in clusters, catalase-positive and coagulase-negative. What can you conclude, and why is the key not finished?** 4 marks

It is a coagulase-negative staphylococcus, not *S. aureus*. Several species fit (for example *S. epidermidis*, *S. saprophyticus*), so further tests such as novobiocin sensitivity are needed.

– UNDERSTAND **How does a strain differ from a species?** 2 marks

A species is a group of similar strains; a strain is one pure-culture line within a species, descended from a single isolate.

BEYOND THE SYLLABUS

Since 2018 the Genome Taxonomy Database (GTDB) has reclassified bacteria and archaea from whole-genome trees, and in 2022 the SeqCode was launched so that microbes known only from DNA sequences can receive formal names. Uncultured organisms are given provisional "*Candidatus*" names. Search "GTDB taxonomy" or "SeqCode prokaryotes" to read further. This is not for the exam.

1.5 Biochemical and molecular characterization of microbes

Two bacteria can look identical after a Gram stain; their enzymes and genes tell them apart. Biochemical characterization tests what an organism does: which sugars it ferments, which enzymes it makes, which products it forms. Molecular characterization reads what it is: the base composition of its DNA, the sequence of marker genes, or its whole genome. Together with shape and staining they form the polyphasic approach of Chapter 1.4.

Biochemical tests

Most tests detect one enzyme or pathway, usually by a colour change. They use a pure culture 18–24 hours old, because mixed or old cultures give confusing results.

TEST	WHAT IT DETECTS	POSITIVE RESULT	EXAMPLE
Catalase	Enzyme splitting H_2O_2 into water and oxygen	Bubbles with 3% H_2O_2	*Staphylococcus* +, *Streptococcus* –
Oxidase	Cytochrome c oxidase	Purple colour within seconds	*Pseudomonas* +, *E. coli* –
Sugar fermentation	Acid, and gas, from a sugar	Phenol red turns yellow; gas in a Durham tube	*E. coli*: lactose, acid and gas
Indole	Tryptophan split to indole	Red ring with Kovács' reagent	*E. coli* +
Methyl red (MR)	Mixed-acid fermentation, strong acid	Red	*E. coli* +
Voges–Proskauer (VP)	Acetoin, from butanediol fermentation	Pink-red with α-naphthol and KOH	*Klebsiella pneumoniae* +

TEST	WHAT IT DETECTS	POSITIVE RESULT	EXAMPLE
Citrate	Citrate used as the only carbon source	Simmons citrate agar turns blue	*Klebsiella* +, *E. coli* –
Urease	Urea split to ammonia and CO_2	Pink (alkaline)	*Proteus* +
H_2S	Sulfur compounds reduced to H_2S	Black precipitate in TSI agar	Most *Salmonella* +
Coagulase	Enzyme that clots plasma	Clot	*S. aureus* +

Indole, MR, VP and citrate together are the IMViC tests, used to separate coliform bacteria: *E. coli* gives + + – – and *Klebsiella pneumoniae* – – + +.

Kits, machines and chemical fingerprints

Miniaturised kits run many tests at once. The API 20E strip for enteric bacteria has 20 small cups of dried substrates. With the oxidase test, the 21 results are split into seven groups of three; a positive scores 1, 2 or 4 by its place in the group, and the seven group sums form a profile number that is looked up in the maker's database (worked example 1). Automated systems such as VITEK 2 read colour changes in sealed cards by machine.

Chemotaxonomy uses chemical make-up instead of reactions. Fatty acid methyl ester (FAME) analysis profiles membrane fatty acids by gas chromatography. MALDI-TOF mass spectrometry identifies a colony in minutes from the mass pattern of its abundant proteins, mostly ribosomal proteins of about 2–20 kDa, matched against a reference library; many hospital labs now use it in place of long biochemical panels.

Molecular characterization

G + C content. The mol% G + C of DNA is (G + C) ÷ (A + T + G + C) × 100. Strains of one species differ little, so a large difference rules out a close relationship, though similar values do not prove one. It is measured from the melting temperature T_m, at which half the DNA strands have separated: G·C pairs have three hydrogen bonds and A·T pairs two, so G + C-rich DNA melts higher. In standard saline citrate, Marmur and Doty's relation is $T_m = 69.3 + 0.41 \times (\text{mol\% G + C})$, with T_m in °C.

DNA–DNA hybridization. DNA from two strains is melted, mixed and allowed to re-pair. At least 70% re-pairing was the classic test for the same species.

16S rRNA gene sequencing. The gene is about 1,500 base pairs long, with nine variable regions (V1–V9) between conserved ones (Figure 1.5.1). Universal primers bind the conserved ends,

PCR copies the gene, and the sequence is compared with databases such as NCBI BLAST or EzBioCloud. As a rule of thumb, 98.7% identity or more suggests the same species, and below about 95% a different genus. The gene is too conserved to separate some close species: *E. coli* and *Shigella* are nearly identical in 16S.

Genomes and typing. Average nucleotide identity (ANI) between two whole genomes, 95–96% or more for the same species, has largely replaced DNA–DNA hybridization. To tell strains apart in an outbreak, labs use multilocus sequence typing (MLST, sequences of seven housekeeping genes), pulsed-field gel electrophoresis and, increasingly, whole-genome sequencing.

The as-yet-unculturables. Culture-independent methods work on DNA or RNA taken straight from a sample: 16S amplicon sequencing lists which groups are present; shotgun metagenomics sequences all the DNA and rebuilds genomes from it; fluorescence in situ hybridization (FISH) lights up chosen groups with fluorescent rRNA probes (Chapter 1.7); and quantitative PCR counts copies of a target gene.

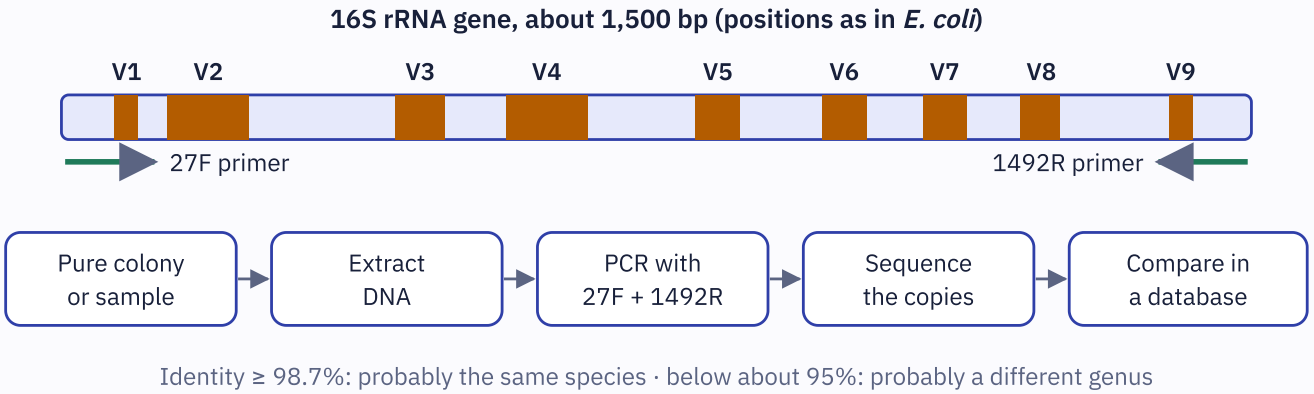


Figure 1.5.1 Top: the variable regions V1–V9 (orange) lie between conserved stretches (blue), where the universal primers bind. Bottom: the steps from colony to name. Notice that the primers sit in conserved DNA, so the same pair copies the gene from almost any bacterium, while the variable regions carry the information that tells species apart.

WORKED EXAMPLE 1: AN API 20E PROFILE

Question (5 marks). An oxidase-negative, lactose-fermenting rod gives these API 20E results, in strip order: ONPG +, ADH −, LDC +, ODC +, CIT −, H_2S −, URE −, TDA −, IND +, VP −, GEL −, GLU +, MAN +, INO −, SOR +, RHA +, SAC −, MEL +, AMY −, ARA +, OX −. Find the profile number.

1. Split into groups of three; score 1, 2, 4 for a positive in the first, second, third place.
2. ONPG, ADH, LDC = 1 + 0 + 4 = 5. ODC, CIT, H_2S = 1 + 0 + 0 = 1. URE, TDA, IND = 0 + 0 + 4 = 4. VP, GEL, GLU = 0 + 0 + 4 = 4.
3. MAN, INO, SOR = 1 + 0 + 4 = 5. RHA, SAC, MEL = 1 + 0 + 4 = 5. AMY, ARA, OX = 0 + 2 + 0 = 2.
4. Profile number: 5 144 552. The database lookup gives *E. coli*, as the key results already suggest: ONPG and indole positive; citrate, H_2S, urease and VP negative.

WORKED EXAMPLE 2: 16S IDENTITY AND G + C

Question (5 marks). An isolate's 16S rRNA gene, 1,460 bp after alignment, differs from its closest type strain at 22 positions. Its DNA has T_m = 91.0 °C in standard saline citrate. Find (a) the percentage identity, (b) whether it is likely to be the same species, (c) its mol% G + C.

1. (a) Identity = (1,460 − 22) ÷ 1,460 × 100 = 1,438 ÷ 1,460 × 100 = 98.49%.
2. (b) 98.49% is below 98.7%, so it is probably a different species of the same genus (it is well above 95%). Confirm with ANI from genome sequences.
3. (c) mol% G + C = (T_m − 69.3) ÷ 0.41 = (91.0 − 69.3) ÷ 0.41 = 21.7 ÷ 0.41 = 52.9%.

EXAM TRAPS

- Catalase on colonies from blood agar can give a false positive, because red blood cells contain catalase.
- Do the oxidase test with a platinum loop or wooden stick; iron in a nichrome loop can give a false positive.
- MR and VP are usually opposite for one organism: mixed-acid and butanediol fermentation are alternative routes.
- Low 16S identity is strong evidence of a different species; high identity is weak evidence of the same one.
- Similar G + C content does not prove a relationship; very different values rule one out.

CHECK YOURSELF

– REMEMBER **What do the letters IMViC stand for, and what is the IMViC pattern of *E. coli*?** 3 marks

Indole, Methyl red, Voges–Proskauer, Citrate (the "i" is for easy saying). *E. coli* is + + – –.

– UNDERSTAND **Why does G + C-rich DNA melt at a higher temperature?** 2 marks

A G·C pair is held by three hydrogen bonds and an A·T pair by two, and G·C pairs also stack more strongly, so more heat is needed to separate the strands.

– APPLY **DNA from an isolate melts at 85.0 °C in standard saline citrate. Find its mol% G + C.** 2 marks

(85.0 – 69.3) ÷ 0.41 = 15.7 ÷ 0.41 = 38.3%.

– APPLY **In an API 20E strip, ODC –, CIT +, H_2S +. What digit does this group give?** 1 mark

0 + 2 + 4 = 6.

– ANALYZE **Two isolates share 99.6% 16S identity but their genomes have an ANI of 91%. Are they the same species? Explain.** 3 marks

No. ANI below 95–96% shows different species. The 16S gene changes too slowly to separate close species, so its high identity does not decide the question.

– UNDERSTAND **How can we study microbes that cannot be cultured?** 3 marks

Extract DNA or RNA directly from the sample and use 16S amplicon sequencing, shotgun metagenomics, FISH with rRNA probes or quantitative PCR.

BEYOND THE SYLLABUS

Whole-genome sequencing is now routine in public-health labs; during COVID-19, India's INSACOG network tracked SARS-CoV-2 variants this way. Portable nanopore sequencers read DNA in the field, and resistance-gene databases let labs predict drug resistance from a genome (Unit 5, *Antimicrobial resistance*). Search "MALDI-TOF identification" or "shotgun metagenomics" to read more. This is not for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Three domains	Bacteria, Archaea, Eukarya (Woese, from rRNA sequences)	1.4
Similarity coefficients	$S_{SM} = (a + d)/(a + b + c + d)$; $S_J = a/(a + b + c)$	1.4
IMViC	Indole, methyl red, Voges–Proskauer, citrate; *E. coli* + + − −	1.5
G + C from melting point	mol% G + C = $(T_m - 69.3) / 0.41$	1.5
Same-species rules of thumb	16S identity ≥ 98.7%; ANI ≥ 95–96%; DNA–DNA hybridization ≥ 70%	1.5

Glossary (terms used in this lesson)

ANI Average nucleotide identity between two genomes; 95–96% or more suggests one species.

Polyphasic taxonomy Classification that combines phenotypic, chemical and genetic data.

Serovar Strains of a species that differ in their surface antigens.

Strain A pure-culture line within a species, descended from one isolate.

Taxonomy The science of classification, nomenclature and identification.

Type strain The reference culture that carries a species name, kept in public culture collections.

The full unit book

Every chapter of Unit 1, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 1 · Foundations: Microbial World and Microscopy (one file, works offline).

Sources and further reading

- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: history, cell structure, microscopy, staining and taxonomy (from memory; check the edition you are prescribed).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson (from memory; check).
- Madigan and colleagues, *Brock Biology of Microorganisms*, Pearson (from memory; check).
- Pelczar, Chan and Krieg, *Microbiology*, Tata McGraw-Hill, 5th edition (from memory; check).
- *Ananthanarayan and Paniker's Textbook of Microbiology*, Universities Press: staining methods as used in Indian medical laboratories (from memory; check).
- Dubey and Maheshwari, *A Textbook of Microbiology*, S. Chand (from memory; check).
- Whitman, Coleman and Wiebe (1998), "Prokaryotes: the unseen majority", *Proceedings of the National Academy of Sciences*: the source of the 4–6 × 10^{30} estimate in Chapter 1.3 (from memory; check).
- OpenStax, *Microbiology* (2016), Chapter 2, "How We See the Invisible World", read in the SUNY and Lumen Learning edition: courses.lumenlearning.com/suny-microbiology. Used for light-microscope magnification limits and TEM section thickness.
- Central TB Division, Government of India, Technical and Operational Guidelines, Annexure 1 (Ziehl–Neelsen sputum smear method and grading): tbcindia.mohfw.gov.in.

Syllabus: the University free n easy universal syllabus, built from top universities' syllabi.

Built for the University free n easy YouTube channel from the universal syllabus of Microbiology (Oct 2026). Written with top universities' syllabi and standard textbooks; check a formula against your prescribed book before an exam, and report mistakes on the channel.