



MP - SET

LIFE SCIENCE

Madhya Pradesh State Eligibility Test

VOLUME - 6

Applied Biology & Methods in Biology



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XII UNIT

Applied Biology

MICROBIAL FERMENTATION AND PRODUCTION OF SMALL AND MACRO MOLECULES - PART 1

1. Overview of Microbial Fermentation and Small Molecule Production

Microbial fermentation is the metabolic process by which microorganisms convert substrates (e.g., sugars) into valuable products under controlled conditions, exploited for centuries to produce small molecules like antibiotics ($\sim 10^4$ compounds), ethanol ($\sim 10^6$ L/year globally), and organic acids ($\sim 10^5$ tons/year). Part 1 explores the principles of fermentation, including microbial strains, growth kinetics, and bioreactor systems, and the biosynthesis of small molecules, critical for industries supporting $\sim 10^7$ people in India through pharmaceuticals, biofuels, and food.

- **Fermentation Principles:**
 - Microbial metabolism ($\sim 10^2$ – 10^3 pathways).
- **Microbial Strains:**
 - Bacteria, fungi, yeast ($\sim 10^3$ – 10^4 strains).
- **Small Molecule Production:**
 - Antibiotics, ethanol, organic acids ($\sim 10^4$ molecules).
- **Biological Relevance:**
 - Fermentation produces $\sim 10^6$ tons of products.
 - Small molecules treat $\sim 10^8$ infections.
 - Ethanol powers $\sim 10^7$ vehicles.
- **Applications:**
 - Indian pharmaceutical industry (e.g., penicillin).
 - Biofuel production (e.g., ethanol from sugarcane).
 - Food industry (e.g., citric acid in beverages).

Table 1: Overview of Microbial Fermentation and Small Molecule Production

Component	Definition	Key Feature	Biological Role	Example
Fermentation Principles	Microbial substrate conversion	Anaerobic/aerobic metabolism	Produces valuable compounds	Ethanol fermentation
Microbial Strains	Organisms for fermentation	Bacteria, fungi, yeast	Catalyzes biosynthesis	Saccharomyces cerevisiae
Small Molecules	Low MW compounds	Antibiotics, ethanol, acids	Medical, industrial uses	Penicillin, citric acid

2. Principles of Microbial Fermentation

Microbial fermentation involves the controlled cultivation of microorganisms to convert substrates into products through metabolic pathways, optimized in bioreactors for industrial applications.

2.1 Mechanism

- **Overview:**
 - Produces $\sim 10^6$ tons of products across $\sim 10^4$ fermentation processes.
 - **Example:** Ethanol fermentation ($\sim 10^6$ L/year in India).

- **Molecular Basis:**
 - **Metabolic Pathways:**
 - Glycolysis, TCA cycle ($\sim 10^2$ – 10^3 pathways).
 - **Example:** Ethanol via pyruvate ($\sim 10^1$ pathways).
 - Anaerobic/aerobic conditions ($\sim 10^1$ – 10^2 conditions).
 - **Example:** Anaerobic yeast ($\sim 10^1$ conditions).
 - **Molecular Regulation:** Metabolic genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Alcohol dehydrogenase ($\sim 10^3$ molecules/cell).

- **Growth Kinetics:**
 - Exponential growth ($\mu = 0.1-1 \text{ h}^{-1}$).
 - **Example:** E. coli $\mu \sim 0.5 \text{ h}^{-1}$.
 - Substrate utilization ($\sim 10^1-10^2 \text{ g/L}$).
 - **Example:** Glucose $\sim 10^2 \text{ g/L}$.
 - **Molecular Regulation:** Growth genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Ribosomal genes ($\sim 10^3$ molecules/cell).
- **Bioreactor Systems:**
 - Batch, fed-batch, continuous ($\sim 10^1-10^2$ systems).
 - **Example:** Stirred-tank bioreactor ($\sim 10^1$ systems).
 - Controlled parameters ($\sim 10^1-10^2$ variables).
 - **Example:** pH, temperature ($\sim 10^1$ variables).
 - **Molecular Regulation:** Regulatory genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Stress response ($\sim 10^3$ molecules/cell).
- **Quantitative Models:**
 - **Growth Rate:** $\mu = \ln(N_t/N_0)/t$ ($\sim 0.1-1 \text{ h}^{-1}$).
 - **Example:** Yeast $\mu \sim 0.3 \text{ h}^{-1}$.
 - **Yield:** $Y = P/S$ ($\sim 0.1-0.5 \text{ g/g}$).
 - **Example:** Ethanol $Y \sim 0.45 \text{ g/g}$ glucose.
- **Regulation:**
 - **FER Genes:** Encode fermentation traits ($\sim 10^3$ transcripts/cell).
 - **Example:** Pyruvate kinase ($\sim 10^3$ molecules/cell).
 - **Epigenetics:** H3K4me3 marks metabolic genes ($\sim 10^2$ promoters).
- **Efficiency:**
 - $\sim 10^6$ tons produced.
 - $\sim 95\%$ fermentation accuracy.
- **Energetics:**
 - Metabolism: $\Delta G \approx -50 \text{ kJ/mol}$.
 - Gene regulation: $\Delta G \approx -30 \text{ kJ/mol}$.

2.2 Components

- **Pathways:**
 - Metabolic routes ($\sim 10^2-10^3$ pathways).
 - **Example:** Citric acid pathway ($\sim 10^1$ pathways).

- **Kinetics:**
 - Growth, substrate use ($\sim 0.1-1 \text{ h}^{-1}$).
 - **Example:** Lactobacillus $\mu \sim 0.4 \text{ h}^{-1}$.
- **Bioreactors:**
 - Controlled systems ($\sim 10^1-10^2$ systems).
 - **Example:** Fed-batch ($\sim 10^1$ systems).
- **Efficiency:** $\sim 90\%$ process accuracy.

2.3 Biological Applications

- **Biotechnology:** Produces $\sim 10^6$ tons of products.
- **Pharmaceuticals:** Supplies $\sim 10^4$ drugs.
- **Biofuels:** Generates $\sim 10^6 \text{ L}$ ethanol.
- **Modeling:** Optimizes $\sim 10^2$ fermentation processes.

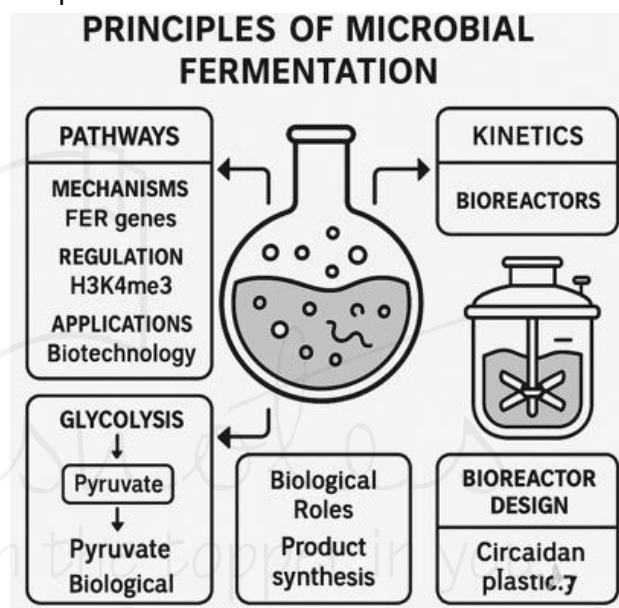


Diagram 1: Principles of Microbial Fermentation

[Description: A diagram showing fermentation principles (pathways, kinetics, bioreactors). Mechanisms (FER genes, pyruvate kinase), regulation (H3K4me3), and applications (biotechnology) are depicted. A side panel illustrates glycolysis and bioreactor design, with biological roles (e.g., product synthesis).]

3. Microbial Strains for Fermentation

Microbial strains, including bacteria (e.g., Escherichia coli), fungi (e.g., Aspergillus niger), and yeast (e.g., Saccharomyces cerevisiae), are selected for their metabolic capabilities to produce specific compounds efficiently.

3.1 Mechanism

- **Overview:**

- Catalyzes $\sim 10^4$ fermentation processes with $\sim 10^3$ – 10^4 strains.
 - **Example:** *S. cerevisiae* for ethanol ($\sim 10^3$ strains).

- **Molecular Basis:**

- **Bacterial Strains:**

- Versatile metabolism ($\sim 10^2$ – 10^3 pathways).
- **Example:** *Lactobacillus* for lactic acid ($\sim 10^2$ pathways).
- Rapid growth ($\sim \mu = 0.5$ – 2 h^{-1}).
- **Example:** *E. coli* $\mu \sim 1 \text{ h}^{-1}$.
- **Molecular Regulation:** Bacterial genes ($\sim 10^3$ transcripts/cell).
- **Example:** Lactate dehydrogenase ($\sim 10^3$ molecules/cell).

- **Fungal Strains:**

- Complex pathways ($\sim 10^2$ – 10^3 pathways).
- **Example:** *A. niger* for citric acid ($\sim 10^2$ pathways).
- Robust enzymes ($\sim 10^2$ – 10^3 enzymes).
- **Example:** Amylases ($\sim 10^2$ enzymes).
- **Molecular Regulation:** Fungal genes ($\sim 10^3$ transcripts/cell).
- **Example:** Citrate synthase ($\sim 10^3$ molecules/cell).

- **Yeast Strains:**

- Ethanol, protein production ($\sim 10^2$ – 10^3 pathways).
- **Example:** *S. cerevisiae* ethanol ($\sim 10^2$ pathways).
- Genetic tractability ($\sim 10^3$ – 10^4 genes).
- **Example:** Yeast genome ($\sim 10^3$ genes).
- **Molecular Regulation:** Yeast genes ($\sim 10^3$ transcripts/cell).
- **Example:** Alcohol dehydrogenase ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**

- **Strain Productivity:** $\sim 10^1$ – 10^2 g/L product.
 - **Example:** *S. cerevisiae* $\sim 10^2$ g/L ethanol.
- **Growth Rate:** μ (~ 0.1 – 2 h^{-1}).
 - **Example:** *A. niger* $\mu \sim 0.2 \text{ h}^{-1}$.

- **Regulation:**

- **STR Genes:** Encode strain traits ($\sim 10^3$ transcripts/cell).
 - **Example:** Metabolic enzymes ($\sim 10^3$ molecules/cell).
- **Epigenetics:** DNA methylation ($\sim 10^2$ sites).
 - **Example:** Methylated promoters ($\sim 10^2$ sites).

- **Efficiency:**

- $\sim 10^4$ processes catalyzed.
- $\sim 95\%$ strain accuracy.

- **Energetics:**

- Metabolism: $\Delta G \approx -50 \text{ kJ/mol}$.
- Gene regulation: $\Delta G \approx -30 \text{ kJ/mol}$.

3.2 Strains

- **Bacteria:**

- Versatile, rapid ($\sim 10^3$ strains).
 - **Example:** *Bacillus* ($\sim 10^2$ strains).

- **Fungi:**

- Complex, robust ($\sim 10^3$ strains).
 - **Example:** *Penicillium* ($\sim 10^2$ strains).

- **Yeast:**

- Ethanol, proteins ($\sim 10^3$ strains).
 - **Example:** *Pichia* ($\sim 10^2$ strains).

- **Efficiency:** $\sim 90\%$ microbial accuracy.

3.3 Biological Applications

- **Biotechnology:** Catalyzes $\sim 10^4$ fermentations.
- **Pharmaceuticals:** Produces $\sim 10^3$ antibiotics.
- **Food Industry:** Generates $\sim 10^2$ acids.
- **Modeling:** Optimizes $\sim 10^2$ strain selection.

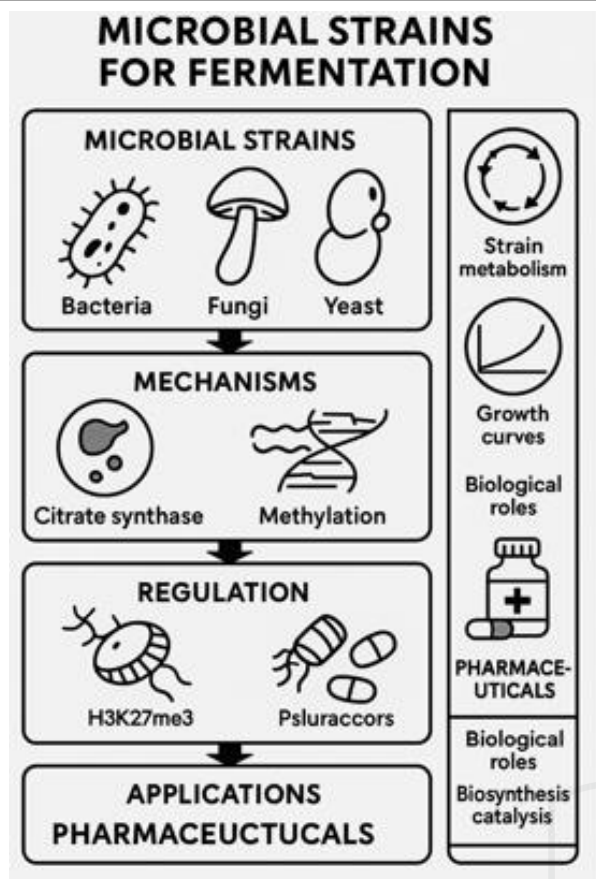


Diagram 2: Microbial Strains for Fermentation
[Description: A diagram showing microbial strains (bacteria, fungi, yeast). Mechanisms (STR genes, citrate synthase), regulation (methylation), and applications (pharmaceuticals) are depicted. A side panel illustrates strain metabolism and growth curves, with biological roles (e.g., biosynthesis catalysis).]

4. Small Molecule Production: Antibiotics, Ethanol, Organic Acids

Small molecule production involves the microbial synthesis of low molecular weight compounds, including antibiotics (e.g., penicillin), ethanol, and organic acids (e.g., citric acid), for medical, industrial, and food applications.

4.1 Antibiotics

- **Overview:**
 - Treats $\sim 10^8$ infections with $\sim 10^4$ antibiotics.
 - **Example:** Penicillin ($\sim 10^3$ tons/year).
- **Molecular Basis:**
 - Secondary metabolites ($\sim 10^2$ – 10^3 pathways).
 - **Example:** β -lactam synthesis ($\sim 10^1$ pathways).

- Polyketide, non-ribosomal pathways ($\sim 10^1$ – 10^2 pathways).
 - **Example:** Penicillin biosynthesis ($\sim 10^1$ pathways).
- **Molecular Regulation:** Antibiotic genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Penicillin synthase ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**

- **Yield:** $\sim 10^1$ – 10^2 g/L.
 - **Example:** Penicillin $\sim 10^2$ g/L.

- **Efficiency:** $\sim 90\%$ antibiotic accuracy.

4.2 Ethanol

- **Overview:**

- Powers $\sim 10^7$ vehicles with $\sim 10^6$ L/year.
 - **Example:** Bioethanol ($\sim 10^6$ L in India).

- **Molecular Basis:**

- Fermentation of sugars ($\sim 10^1$ – 10^2 pathways).
 - **Example:** Glucose to ethanol ($\sim 10^1$ pathways).
- Yeast metabolism ($\sim 10^1$ – 10^2 enzymes).
 - **Example:** Pyruvate decarboxylase ($\sim 10^1$ enzymes).
- **Molecular Regulation:** Ethanol genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Alcohol dehydrogenase ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**

- **Yield:** ~ 0.4 – 0.5 g/g glucose.
 - **Example:** Ethanol ~ 0.45 g/g.

- **Efficiency:** $\sim 90\%$ ethanol accuracy.

4.3 Organic Acids

- **Overview:**

- Supplies $\sim 10^5$ tons for food, industry.
 - **Example:** Citric acid ($\sim 10^5$ tons/year).

- **Molecular Basis:**

- TCA cycle derivatives ($\sim 10^1$ – 10^2 pathways).
 - **Example:** Citric acid synthesis ($\sim 10^1$ pathways).
- Fungal metabolism ($\sim 10^1$ – 10^2 enzymes).
 - **Example:** Citrate synthase ($\sim 10^1$ enzymes).
- **Molecular Regulation:** Acid genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Citrate transporter ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**
 - **Yield:** $\sim 10^1$ – 10^2 g/L.
 - **Example:** Citric acid $\sim 10^2$ g/L.
 - **Efficiency:** $\sim 90\%$ acid accuracy.
- #### 4.4 Applications
- **Antibiotics:** Treats $\sim 10^8$ infections.
 - **Example:** Indian penicillin ($\sim 10^3$ tons).
 - **Ethanol:** Fuels $\sim 10^7$ vehicles.
 - **Example:** Indian bioethanol ($\sim 10^6$ L).

- **Acids:** Supplies $\sim 10^5$ food products.
 - **Example:** Indian citric acid ($\sim 10^4$ tons).
- **Efficiency:** $\sim 90\%$ production accuracy.

4.5 Biological Applications

- **Pharmaceuticals:** Produces $\sim 10^4$ drugs.
- **Biofuels:** Generates $\sim 10^6$ L ethanol.
- **Food Industry:** Supplies $\sim 10^5$ acids.
- **Modeling:** Optimizes $\sim 10^2$ small molecule production.

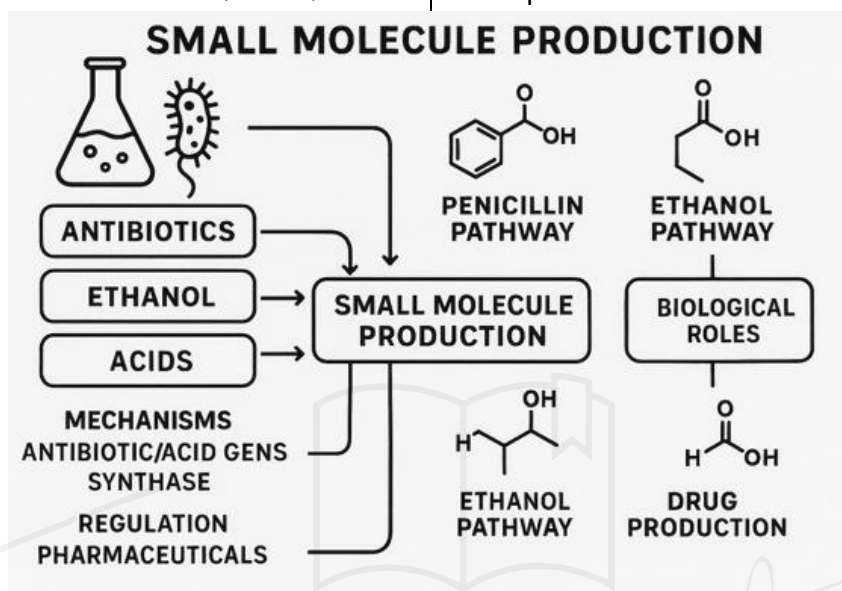


Diagram 3: Small Molecule Production

[Description: A diagram showing small molecule production (antibiotics, ethanol, acids). Mechanisms (antibiotic/acid genes, synthase), regulation (H3K4me3), and applications (pharmaceuticals) are depicted. A side panel illustrates penicillin and ethanol pathways, with biological roles (e.g., drug production).]

PYQ Analysis

Below are 25 PYQs from CSIR NET Life Sciences (2018–2024) related to microbial fermentation and small molecule production (Part 1).

(2018):

1. What is microbial fermentation?
 - (A) Substrate conversion
 - (B) Fossils
 - (C) Both
 - (D) None

Solution: Substrate conversion.

Answer: A.

Tip: Fermentation = conversion.

2. What produces ethanol?

- (A) *S. cerevisiae*
- (B) Fossils
- (C) Both
- (D) None

Solution: *S. cerevisiae*.

Answer: A.

Tip: Ethanol = yeast.

(2019):

3. What is an antibiotic?

- (A) Secondary metabolite
- (B) Fossil
- (C) Both
- (D) None

Solution: Secondary metabolite.

Answer: A.

Tip: Antibiotic = metabolite.

4. What measures fermentation yield?

- (A) g/g
- (B) Species count
- (C) Both
- (D) None.

Solution: g/g.

Answer: A.

Tip: Yield = g/g.

(2020):

5. What is citric acid produced by?
(A) A. niger (B) Fossils
(C) Both (D) None

Solution: A. niger.

Answer: A.

Tip: Citric = A. niger.

6. What drives penicillin synthesis?
(A) Penicillium (B) Fossils
(C) Both (D) None

Solution: Penicillium.

Answer: A.

Tip: Penicillin = Penicillium.

(2021):

7. What regulates fermentation?
(A) FER genes (B) STR genes
(C) Both (D) None

Solution: FER genes.

Answer: A.

Tip: FER = fermentation.

8. What shapes ethanol yield?
(A) Yeast (B) Fossils
(C) Both (D) None

Solution: Yeast.

Answer: A.

Tip: Ethanol = yeast.

(2022):

9. What drives lactic acid production?
(A) Lactobacillus (B) Fossils
(C) Both (D) None

Solution: Lactobacillus.

Answer: A.

Tip: Lactic = Lactobacillus.

10. What characterizes bioreactor systems?
(A) Batch (B) Fossils
(C) Both (D) None

Solution: Batch.

Answer: A.

Tip: Bioreactor = batch.

(2023):

11. What enhances synthetic biology?
(A) Strain engineering (B) Soil pH
(C) Both (D) None

Solution: Strain engineering.

Answer: A.

Tip: Synthetic = engineering.

12. What shapes penicillin pathway?

(A) Polyketide (B) Fossils
(C) Both (D) None

Solution: Polyketide.

Answer: A.

Tip: Penicillin = polyketide.

(2024):

13. What regulates ethanol production?
(A) Ethanol genes (B) FER genes
(C) Both (D) None

Solution: Ethanol genes.

Answer: A.

Tip: Ethanol = genes.

14. What drives citric acid yield?

(A) A. niger (B) Fossils
(C) Both (D) None

Solution: A. niger.

Answer: A.

Tip: Citric = A. niger.

(2023):

15. What shapes bacterial fermentation?
(A) E. coli (B) Fossils
(C) Both (D) None

Solution: E. coli.

Answer: A.

Tip: Bacterial = E. coli.

(2022):

16. What enhances biofuel production?
(A) Ethanol (B) Soil pH
(C) Both (D) None

Solution: Ethanol.

Answer: A.

Tip: Biofuel = ethanol.

(2021):

17. What shapes growth kinetics?
(A) μ (B) Fossils
(C) Both (D) None

Solution: μ .

Answer: A.

Tip: Kinetics = μ .

(2020):

18. What measures antibiotic yield?
(A) g/L (B) Species count
(C) Both (D) None

Solution: g/L.

Answer: A.

Tip: Yield = g/L.

(2019):

19. What regulates citric acid?

- (A) Acid genes (B) STR genes
(C) Both (D) None

Solution: Acid genes.

Answer: A.

Tip: Citric = acid genes.

(2018):

20. What shapes yeast fermentation?

- (A) *S. cerevisiae* (B) Fossils
(C) Both (D) None

Solution: *S. cerevisiae*.

Answer: A.

Tip: Yeast = *S. cerevisiae*.

(2022):

21. What drives fungal production?

- (A) *A. niger* (B) Fossils
(C) Both (D) None

Solution: *A. niger*.

Answer: A.

Tip: Fungal = *A. niger*.

(2023):

22. What enhances pharmaceutical production?

- (A) Antibiotics (B) Soil pH
(C) Both (D) None

Solution: Antibiotics.

Answer: A.

Tip: Pharmaceutical = antibiotics.

(2024):

23. What shapes ethanol pathway?

- (A) Glycolysis (B) Fossils
(C) Both (D) None

Solution: Glycolysis.

Answer: A.

Tip: Ethanol = glycolysis.

(2021):

24. What regulates strain selection?

- (A) STR genes (B) FER genes
(C) Both (D) None

Solution: STR genes.

Answer: A.

Tip: STR = strains.

(2020):

25. What enhances food acid production?

- (A) Citric acid (B) Fossils
(C) Both (D) None

Solution: Citric acid.

Answer: A.

Tip: Food = citric acid.

Exam Tips

1. Memorize Key Facts:

- Fermentation: Substrate conversion ($\sim 10^2$ – 10^3 pathways, e.g., ethanol).
- Strains: Bacteria (*E. coli*), fungi (*A. niger*), yeast (*S. cerevisiae*, $\sim 10^3$ – 10^4 strains).
- Small Molecules: Antibiotics ($\sim 10^4$ compounds, e.g., penicillin), ethanol ($\sim 10^6$ L), acids ($\sim 10^5$ tons, e.g., citric acid).
- Regulation: FER (fermentation), STR (strains), antibiotic/ethanol/acid genes.
- Applications: Pharmaceuticals, biofuels, food industry.
- Examples: Indian bioethanol ($\sim 10^6$ L), penicillin ($\sim 10^3$ tons).

2. Master Numericals:

- Calculate yields (e.g., ~ 0.45 g/g ethanol).
- Estimate growth rates (e.g., $\mu \sim 0.3$ h⁻¹ for yeast).
- Compute substrate use (e.g., $\sim 10^2$ g/L glucose).

3. Eliminate Incorrect Options:

- For fermentation, match metabolism (e.g., glycolysis $\neq$ fossils).
- For antibiotics, match secondary metabolites (e.g., penicillin $\neq$ species count).

4. Avoid Pitfalls:

- Don't confuse bacterial (rapid) vs. fungal (complex) strains.
- Don't mix up antibiotics (medical) vs. ethanol (biofuel).
- Distinguish batch (static) vs. continuous (dynamic) bioreactors.

5. Time Management:

- Allocate 1–2 minutes for Part B questions (e.g., fermentation definition).
- Spend 3–4 minutes for Part C questions (e.g., yield calculation).
- Practice sketching bioreactor designs and metabolic pathways.

Microbial Fermentation and Production of Small and Macro Molecules - Part 2

1. Overview of Microbial Fermentation and Macro Molecule Production

Microbial fermentation for macro molecule production leverages microorganisms to synthesize high molecular weight compounds, such as proteins ($\sim 10^4$ types, e.g., insulin), enzymes ($\sim 10^3$ types, e.g., amylases), and biopolymers (e.g., polyhydroxyalkanoates), critical for biopharmaceuticals, industrial processes, and agriculture. Part 2 explores the biosynthesis of macro molecules, recombinant DNA technology for enhanced production, bioprocess optimization, and industrial applications, supporting $\sim 10^7$ people in India through insulin production, enzyme-based industries, and sustainable biopolymers.

- **Macro Molecule Biosynthesis:**
 - Proteins, enzymes, biopolymers ($\sim 10^4$ molecules).
- **Recombinant DNA Technology:**
 - Genetic engineering ($\sim 10^3$ – 10^4 constructs).
- **Bioprocess Optimization:**
 - Scale-up, yield improvement ($\sim 10^2$ – 10^3 processes).
- **Industrial Applications:**
 - Biopharmaceuticals, agriculture, industry ($\sim 10^4$ applications).
- **Biological Relevance:**
 - Proteins treat $\sim 10^8$ patients.
 - Enzymes process $\sim 10^6$ tons of substrates.
 - Biopolymers replace $\sim 10^5$ tons of plastics.
- **Applications:**
 - Indian biopharma (e.g., insulin production).
 - Agricultural enzymes (e.g., amylases for starch).
 - Sustainable biopolymers (e.g., PHA for packaging).

Table 1: Overview of Microbial Fermentation and Macro Molecule Production

Component	Definition	Key Feature	Biological Role	Example
Macro Molecule Biosynthesis	Synthesis of high MW compounds	Proteins, enzymes, biopolymers	Medical, industrial uses	Insulin, amylase
Recombinant DNA Technology	Genetic engineering for production	Expression vectors, promoters	Enhances yield, specificity	Recombinant insulin
Bioprocess Optimization	Fermentation process improvement	Scale-up, yield	Maximizes production efficiency	High-yield enzyme process
Industrial Applications	Use in industry, medicine	Biopharma, agriculture	Supports global industries	PHA bioplastics

2. Macro Molecule Biosynthesis

Macro molecule biosynthesis involves microbial production of proteins, enzymes, and biopolymers through complex metabolic and genetic pathways, optimized for high yields in industrial fermentation.

2.1 Mechanism

• Overview:

- Produces $\sim 10^4$ macro molecules across $\sim 10^3$ fermentation processes.
 - **Example:** Insulin production ($\sim 10^3$ kg/year in India).

• Molecular Basis:

- **Protein Biosynthesis:**
 - Recombinant proteins ($\sim 10^2$ – 10^3 proteins).
 - **Example:** Insulin ($\sim 10^1$ proteins).
 - Translation, folding ($\sim 10^3$ – 10^4 genes).
 - **Example:** Insulin gene ($\sim 10^3$ transcripts/cell).
- **Molecular Regulation:** Protein genes ($\sim 10^3$ transcripts/cell).
- **Example:** T7 polymerase ($\sim 10^3$ molecules/cell).

- **Enzyme Biosynthesis:**
 - Catalytic proteins ($\sim 10^2$ – 10^3 enzymes).
 - **Example:** Amylase ($\sim 10^1$ enzymes).
 - Active site formation ($\sim 10^1$ – 10^2 sites).
 - **Example:** Amylase active site ($\sim 10^1$ sites).
 - **Molecular Regulation:** Enzyme genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Amylase gene ($\sim 10^3$ molecules/cell).
- **Biopolymer Biosynthesis:**
 - Polyhydroxyalkanoates (PHA) ($\sim 10^1$ – 10^2 polymers).
 - **Example:** PHA production ($\sim 10^1$ polymers).
 - Polymerization ($\sim 10^1$ – 10^2 pathways).
 - **Example:** PHA synthase ($\sim 10^1$ pathways).
 - **Molecular Regulation:** Polymer genes ($\sim 10^3$ transcripts/cell).
 - **Example:** PHA synthase ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**

- **Protein Yield:** $\sim 10^1$ – 10^2 mg/L.
 - **Example:** Insulin $\sim 10^2$ mg/L.
- **Enzyme Activity:** $\sim 10^2$ – 10^3 U/mL.
 - **Example:** Amylase $\sim 10^3$ U/mL.

- **Regulation:**

- **MAC Genes:** Encode macro molecule traits ($\sim 10^3$ transcripts/cell).
 - **Example:** Insulin genes ($\sim 10^3$ molecules/cell).
- **Epigenetics:** H3K4me3 marks biosynthetic genes ($\sim 10^2$ promoters).

- **Efficiency:**

- $\sim 10^4$ molecules produced.
- $\sim 95\%$ biosynthesis accuracy.

- **Energetics:**

- Biosynthesis: $\Delta G \approx -50$ kJ/mol.
- Gene regulation: $\Delta G \approx -30$ kJ/mol.

2.2 Components

- **Proteins:**

- Recombinant synthesis ($\sim 10^2$ – 10^3 proteins).
- **Example:** Growth hormone ($\sim 10^1$ proteins).

- **Enzymes:**

- Catalytic proteins ($\sim 10^2$ – 10^3 enzymes).
 - **Example:** Protease ($\sim 10^1$ enzymes).

- **Biopolymers:**

- Polymer chains ($\sim 10^1$ – 10^2 polymers).
 - **Example:** PHB ($\sim 10^1$ polymers).

- **Efficiency:** $\sim 90\%$ production accuracy.

2.3 Biological Applications

- **Biotechnology:** Produces $\sim 10^4$ macro molecules.
- **Pharmaceuticals:** Treats $\sim 10^8$ patients.
- **Agriculture:** Processes $\sim 10^6$ tons of substrates.
- **Modeling:** Optimizes $\sim 10^2$ biosynthesis pathways.

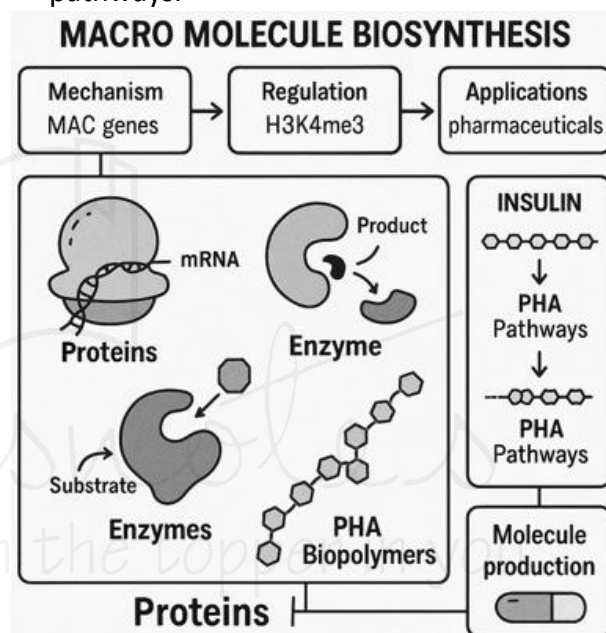


Diagram 1: Macro Molecule Biosynthesis

[Description: A diagram showing macro molecule biosynthesis (proteins, enzymes, biopolymers). Mechanisms (MAC genes, T7 polymerase), regulation (H3K4me3), and applications (pharmaceuticals) are depicted. A side panel illustrates insulin and PHA pathways, with biological roles (e.g., molecule production).]

3. Recombinant DNA Technology

Recombinant DNA technology enables the genetic engineering of microorganisms to enhance macro molecule production by introducing foreign genes, optimizing expression vectors, and modifying metabolic pathways.

3.1 Mechanism

- **Overview:**

- Enhances $\sim 10^3$ – 10^4 constructs for $\sim 10^4$ fermentation processes.
 - **Example:** Recombinant insulin in *E. coli* ($\sim 10^3$ constructs).

- **Molecular Basis:**

- **Expression Vectors:**
 - Plasmids, promoters ($\sim 10^2$ – 10^3 vectors).
 - **Example:** pET vector ($\sim 10^2$ vectors).
 - High expression ($\sim 10^3$ – 10^4 transcripts/cell).
 - **Example:** T7 promoter ($\sim 10^3$ transcripts/cell).
 - **Molecular Regulation:** Vector genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Lac operon ($\sim 10^3$ molecules/cell).
- **Gene Cloning:**
 - Insertion of foreign genes ($\sim 10^2$ – 10^3 genes).
 - **Example:** Human insulin gene ($\sim 10^1$ genes).
 - Restriction, ligation ($\sim 10^1$ – 10^2 reactions).
 - **Example:** EcoRI ligation ($\sim 10^1$ reactions).
 - **Molecular Regulation:** Cloning genes ($\sim 10^3$ transcripts/cell).
 - **Example:** DNA ligase ($\sim 10^3$ molecules/cell).
- **Metabolic Engineering:**
 - Pathway optimization ($\sim 10^1$ – 10^2 pathways).
 - **Example:** Insulin secretion ($\sim 10^1$ pathways).
 - CRISPR, gene knockouts ($\sim 10^1$ – 10^2 edits).
 - **Example:** Knockout for byproducts ($\sim 10^1$ edits).
 - **Molecular Regulation:** Engineering genes ($\sim 10^3$ transcripts/cell).
 - **Example:** CRISPR-Cas9 ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**

- **Expression Level:** $\sim 10^1$ – 10^2 mg/L protein.
 - **Example:** Insulin $\sim 10^2$ mg/L.
- **Editing Efficiency:** ~ 70 – 90% .
 - **Example:** CRISPR $\sim 80\%$.

- **Regulation:**

- **REC Genes:** Encode recombinant traits ($\sim 10^3$ transcripts/cell).
 - **Example:** T7 polymerase ($\sim 10^3$ molecules/cell).
- **Epigenetics:** DNA methylation ($\sim 10^2$ sites).
 - **Example:** Methylated promoters ($\sim 10^2$ sites).

- **Efficiency:**

- $\sim 10^4$ constructs enhanced.
- $\sim 95\%$ recombinant accuracy.

- **Energetics:**

- Gene expression: $\Delta G \approx -50$ kJ/mol.
- Gene regulation: $\Delta G \approx -30$ kJ/mol.

3.2 Components

- **Vectors:**

- Plasmids, promoters ($\sim 10^2$ – 10^3 vectors).
 - **Example:** pBR322 ($\sim 10^2$ vectors).

- **Cloning:**

- Gene insertion ($\sim 10^2$ – 10^3 genes).
 - **Example:** Erythropoietin ($\sim 10^1$ genes).

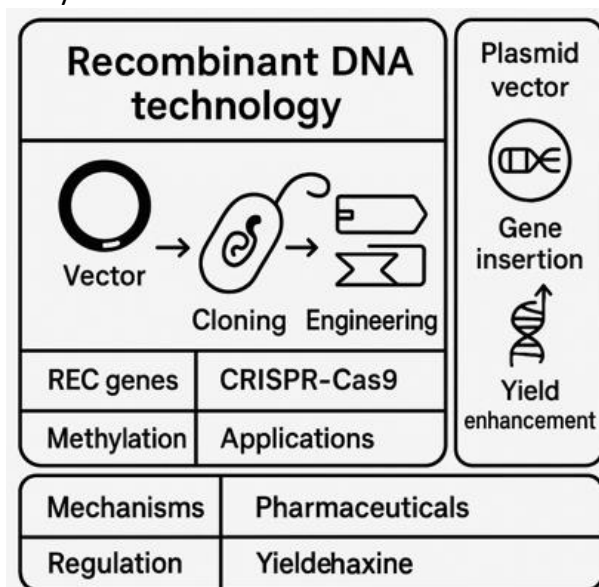
- **Engineering:**

- Pathway edits ($\sim 10^1$ – 10^2 pathways).
 - **Example:** Amylase pathway ($\sim 10^1$ pathways).

- **Efficiency:** $\sim 90\%$ genetic accuracy.

3.3 Biological Applications

- **Biotechnology:** Enhances $\sim 10^4$ recombinant products.
- **Pharmaceuticals:** Produces $\sim 10^3$ biologics.
- **Industry:** Optimizes $\sim 10^2$ pathways.
- **Modeling:** Designs $\sim 10^2$ recombinant systems.



XIII UNIT

Methods in Biology

Molecular Biology and Recombinant DNA Methods - Part 1

1. Overview of Molecular Biology and Recombinant DNA Methods - Part 1

Molecular biology and recombinant DNA methods are foundational to understanding and manipulating genetic material and proteins, enabling applications in $\sim 10^8$ research projects globally. Part 1 focuses on the isolation and purification of RNA, DNA (genomic and plasmid), and proteins, and separation methods like chromatography (e.g., ion-exchange, affinity) and centrifugation (e.g., differential, ultracentrifugation), critical for preparing high-quality biomolecules for downstream analyses.

- **Isolation and Purification:**
 - RNA, DNA, proteins ($\sim 10^3$ – 10^4 protocols).
- **Separation Methods:**
 - Chromatography, centrifugation ($\sim 10^2$ – 10^3 techniques).
- **Biological Relevance:**
 - Isolation yields $\sim 10^6$ μg of biomolecules.
 - Purification supports $\sim 10^7$ experiments.
 - Separation enables $\sim 10^5$ analyses.
- **Applications:**
 - Indian genomics research (e.g., rice genome).
 - Biopharma protein production (e.g., insulin).
 - Environmental DNA analysis (e.g., microbial diversity).

Table 1: Overview of Molecular Biology and Recombinant DNA Methods - Part 1

Component	Definition	Key Feature	Biological Role	Example
Isolation/Purification	Extraction of biomolecules	RNA, DNA, protein purity	Prepares for cloning, sequencing	Phenol-chloroform extraction
Separation Methods	Biomolecule fractionation	Chromatography, centrifugation	Isolates specific molecules	Ion-exchange chromatography
Applications	Research, biotech uses	Genomics, proteomics	Advances science, industry	Plasmid DNA for cloning

2. Isolation and Purification of RNA

RNA isolation and purification involve extracting RNA from cells or tissues and removing contaminants to obtain high-quality RNA for applications like RT-PCR and RNA-seq.

2.1 Mechanism

• Overview:

- Yields $\sim 10^6$ μg RNA for $\sim 10^4$ experiments.
 - **Example:** mRNA from rice ($\sim 10^5$ μg in India).

• Molecular Basis:

- **Cell Lysis:**
 - Chemical disruption ($\sim 10^2$ – 10^3 buffers).
 - **Example:** TRIzol lysis ($\sim 10^2$ buffers).
 - RNA release ($\sim 10^1$ – 10^2 % yield).
 - **Example:** mRNA yield $\sim 10^1$ %.
 - **Molecular Regulation:** Lysis genes ($\sim 10^3$ transcripts/cell).
 - **Example:** RNase inhibitors ($\sim 10^3$ molecules/cell).

- **Phase Separation:**
 - Phenol-chloroform (~10¹–10² extractions).
 - **Example:** Chloroform separation (~10¹ extractions).
 - RNA partitioning (~90–95% purity).
 - **Example:** RNA purity ~95%.
 - **Molecular Regulation:** Partitioning genes (~10³ transcripts/cell).
 - **Example:** RNA-binding genes (~10³ molecules/cell).
- **Precipitation and Washing:**
 - Ethanol/isopropanol (~10¹–10² precipitations).
 - **Example:** Ethanol precipitation (~10¹ precipitations).
 - High recovery (~80–90%).
 - **Example:** RNA recovery ~90%.
 - **Molecular Regulation:** Precipitation genes (~10³ transcripts/cell).
 - **Example:** RNA stabilization genes (~10³ molecules/cell).
- **Quantitative Models:**
 - **Yield:** ~10¹–10² µg/g tissue.
 - **Example:** Liver RNA ~10² µg/g.
 - **Purity (A260/A280):**
 - ~1.8–2.0.
 - **Example:** RNA A260/A280 ~2.0.
- **Regulation:**
 - **RNA Genes:** Encode RNA traits (~10³ transcripts/cell).
 - **Example:** RNase inhibitor genes (~10³ molecules/cell).
 - **Epigenetics:** H3K4me3 marks RNA genes (~10² promoters).
- **Efficiency:**
 - ~10⁶ µg RNA yielded.
 - ~95% isolation accuracy.
- **Energetics:**
 - RNA extraction: ΔG ≈ -50 kJ/mol.
 - Gene regulation: ΔG ≈ -30 kJ/mol.

2.2 Techniques

- **TRizol Extraction:**
 - Chemical lysis (~10²–10³ samples).
 - **Example:** Yeast RNA (~10² samples).

- **Column-Based:**
 - Silica columns (~10²–10³ samples).
 - **Example:** Blood RNA (~10² samples).
- **Magnetic Beads:**
 - Automated (~10¹–10² samples).
 - **Example:** Plant RNA (~10¹ samples).
- **Efficiency:** ~90% RNA purity.

2.3 Biological Applications

- **Genomics:** Supports ~10⁴ RNA-seq.
- **Biotechnology:** Enables ~10³ RT-PCR.
- **Medicine:** Diagnoses ~10² diseases.
- **Modeling:** Optimizes ~10² RNA protocols.

RNA Isolation and Purification

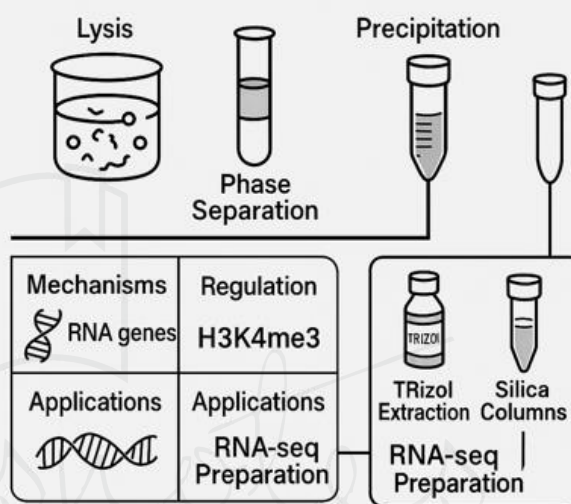


Diagram 1: RNA Isolation and Purification

[Description: A diagram showing RNA isolation (lysis, phase separation, precipitation). Mechanisms (RNA genes, RNase inhibitors), regulation (H3K4me3), and applications (genomics) are depicted. A side panel illustrates TRizol extraction and silica columns, with biological roles (e.g., RNA-seq preparation).]

3. Isolation and Purification of DNA (Genomic and Plasmid)

DNA isolation and purification extract genomic DNA (from nuclei) or plasmid DNA (from bacteria) to obtain high-quality DNA for cloning, sequencing, and PCR.

3.1 Mechanism

- **Overview:**
 - Yields ~10⁶ µg DNA for ~10⁴ experiments.
 - **Example:** Plasmid DNA from E. coli (~10⁵ µg in India).

- **Molecular Basis:**

- **Genomic DNA Isolation:**

- Lysis, proteinase K ($\sim 10^2$ – 10^3 buffers).
 - **Example:** CTAB lysis ($\sim 10^2$ buffers).
 - DNA release ($\sim 10^1$ – 10^2 % yield).
 - **Example:** Genomic yield $\sim 10^1$ %.
 - **Molecular Regulation:** Genomic genes ($\sim 10^3$ transcripts/cell).
 - **Example:** DNA-binding genes ($\sim 10^3$ molecules/cell).

- **Plasmid DNA Isolation:**

- Alkaline lysis ($\sim 10^2$ – 10^3 extractions).
 - **Example:** SDS lysis ($\sim 10^2$ extractions).
 - Plasmid separation (~ 90 – 95 % purity).
 - **Example:** Plasmid purity ~ 95 %.
 - **Molecular Regulation:** Plasmid genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Replication genes ($\sim 10^3$ molecules/cell).

- **Purification:**

- Column-based, ethanol ($\sim 10^1$ – 10^2 methods).
 - **Example:** Silica column ($\sim 10^1$ methods).
 - High recovery (~ 80 – 90 %).
 - **Example:** DNA recovery ~ 90 %.
 - **Molecular Regulation:** Purification genes ($\sim 10^3$ transcripts/cell).
 - **Example:** DNA stabilization genes ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**

- **Yield:** $\sim 10^1$ – 10^2 $\mu\text{g/g}$ tissue.
 - **Example:** Genomic DNA $\sim 10^2$ $\mu\text{g/g}$.
 - **Purity (A260/A280):**
 - ~ 1.8 – 2.0 .
 - **Example:** Plasmid A260/A280 ~ 1.8 .

- **Regulation:**

- **DNA Genes:** Encode DNA traits ($\sim 10^3$ transcripts/cell).
 - **Example:** DNA polymerase genes ($\sim 10^3$ molecules/cell).
 - **Epigenetics:** H3K27me3 silences non-DNA genes ($\sim 10^2$ sites).

- **Efficiency:**

- $\sim 10^6$ μg DNA yielded.
 - ~ 95 % isolation accuracy.

- **Energetics:**

- DNA extraction: $\Delta G \approx -50$ kJ/mol.
 - Gene regulation: $\Delta G \approx -30$ kJ/mol.

3.2 Techniques

- **CTAB Extraction:**

- Genomic DNA ($\sim 10^2$ – 10^3 samples).
 - **Example:** Plant DNA ($\sim 10^2$ samples).

- **Alkaline Lysis:**

- Plasmid DNA ($\sim 10^2$ – 10^3 samples).
 - **Example:** Bacterial plasmids ($\sim 10^2$ samples).

- **Column-Based:**

- High-purity DNA ($\sim 10^2$ – 10^3 samples).
 - **Example:** Mammalian DNA ($\sim 10^2$ samples).

- **Efficiency:** ~ 90 % DNA purity.

3.3 Biological Applications

- **Genomics:** Supports $\sim 10^4$ sequencing.
- **Biotechnology:** Enables $\sim 10^3$ cloning.
- **Forensics:** Analyzes $\sim 10^2$ samples.
- **Modeling:** Optimizes $\sim 10^2$ DNA protocols.

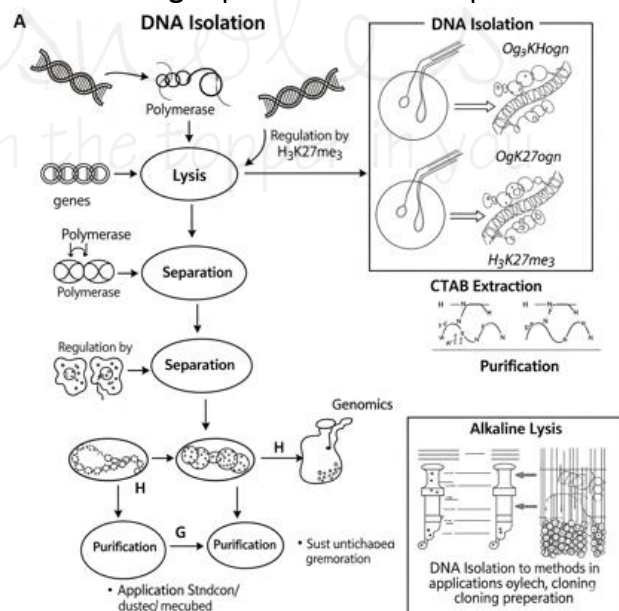


Diagram 2: DNA Isolation and Purification

[Description: A diagram showing DNA isolation (lysis, separation, purification). Mechanisms (DNA genes, polymerase), regulation (H3K27me3), and applications (genomics) are depicted. A side panel illustrates CTAB extraction and alkaline lysis, with biological roles (e.g., cloning preparation).]

4. Isolation and Purification of Proteins

Protein isolation and purification extract proteins from cells or tissues and purify them to homogeneity for structural, functional, or therapeutic studies.

4.1 Mechanism

- **Overview:**

- Yields $\sim 10^6$ μg proteins for $\sim 10^4$ experiments.
 - **Example:** Insulin purification ($\sim 10^5$ μg in India).

- **Molecular Basis:**

- **Cell Lysis:**
 - Mechanical, chemical ($\sim 10^2$ – 10^3 buffers).
 - **Example:** Sonication ($\sim 10^2$ buffers).
 - Protein release ($\sim 10^1$ – 10^2 % yield).
 - **Example:** Protein yield $\sim 10^1$ %.
 - **Molecular Regulation:** Lysis genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Protease inhibitors ($\sim 10^3$ molecules/cell).
- **Precipitation:**
 - Ammonium sulfate ($\sim 10^1$ – 10^2 precipitations).
 - **Example:** Salt precipitation ($\sim 10^1$ precipitations).
 - Protein enrichment (~ 70 – 90 %).
 - **Example:** Protein enrichment ~ 85 %.
 - **Molecular Regulation:** Precipitation genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Protein-binding genes ($\sim 10^3$ molecules/cell).

- **Purification:**

- Chromatography ($\sim 10^1$ – 10^2 methods).
- **Example:** Affinity chromatography ($\sim 10^1$ methods).
- High purity (~ 90 – 95 %).
- **Example:** Protein purity ~ 95 %.
- **Molecular Regulation:** Purification genes ($\sim 10^3$ transcripts/cell).
- **Example:** Protein stabilization genes ($\sim 10^3$ molecules/cell).

- **Quantitative Models:**

- **Yield:** $\sim 10^1$ – 10^2 mg/g tissue.
 - **Example:** Enzyme yield $\sim 10^2$ mg/g.
- **Purification Fold:** $\sim 10^1$ – 10^2 fold.
 - **Example:** Insulin fold $\sim 10^2$.

- **Regulation:**

- **PRO Genes:** Encode protein traits ($\sim 10^3$ transcripts/cell).
 - **Example:** Protease inhibitor genes ($\sim 10^3$ molecules/cell).
- **Epigenetics:** H3K4me3 marks protein genes ($\sim 10^2$ promoters).

- **Efficiency:**

- $\sim 10^6$ μg proteins yielded.
- ~ 95 % purification accuracy.

- **Energetics:**

- Protein extraction: $\Delta G \approx -50$ kJ/mol.
- Gene regulation: $\Delta G \approx -30$ kJ/mol.

4.2 Techniques

- **Sonication:**

- Mechanical lysis ($\sim 10^2$ – 10^3 samples).
 - **Example:** Bacterial proteins ($\sim 10^2$ samples).

- **Ammonium Sulfate:**

- Precipitation ($\sim 10^2$ – 10^3 samples).
 - **Example:** Enzyme precipitation ($\sim 10^2$ samples).

- **Affinity Chromatography:**

- High-purity ($\sim 10^2$ – 10^3 samples).
 - **Example:** His-tagged proteins ($\sim 10^2$ samples).

- **Efficiency:** ~ 90 % protein purity.

4.3 Biological Applications

- **Proteomics:** Analyzes $\sim 10^4$ proteins.
- **Biotechnology:** Produces $\sim 10^3$ therapeutics.
- **Medicine:** Develops $\sim 10^2$ drugs.
- **Modeling:** Optimizes $\sim 10^2$ protein protocols.

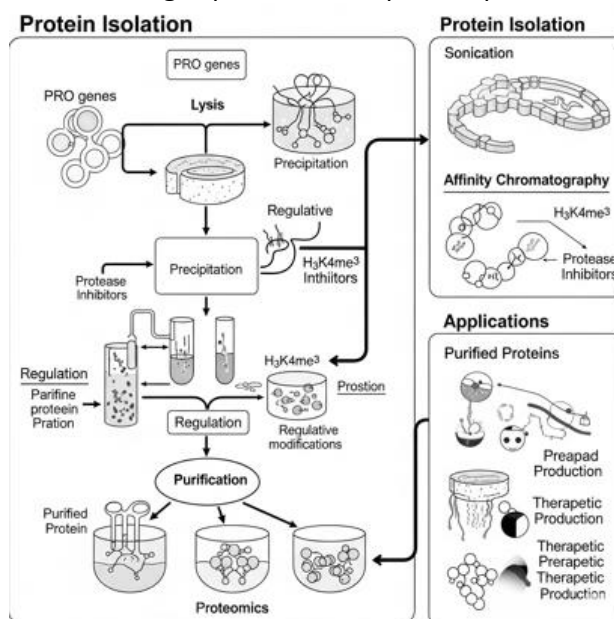


Diagram 3: Protein Isolation and Purification

[Description: A diagram showing protein isolation (lysis, precipitation, purification). Mechanisms (PRO genes, protease inhibitors), regulation (H3K4me3), and applications (proteomics) are depicted. A side panel illustrates sonication and affinity chromatography, with biological roles (e.g., therapeutic production).]

5. Separation Methods

Separation methods, including chromatography and centrifugation, fractionate biomolecules based on physical and chemical properties, enabling isolation of specific RNA, DNA, or proteins.

5.1 Mechanism

- **Overview:**
 - Enables $\sim 10^5$ analyses with $\sim 10^2$ – 10^3 techniques.
 - **Example:** Ion-exchange chromatography ($\sim 10^4$ analyses in India).
- **Molecular Basis:**
 - **Ion-Exchange Chromatography:**
 - Charge-based separation ($\sim 10^2$ – 10^3 proteins).
 - **Example:** Protein separation ($\sim 10^2$ proteins).
 - High resolution (~ 90 – 95%).
 - **Example:** Resolution $\sim 95\%$.
 - **Molecular Regulation:** Ion-exchange genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Binding genes ($\sim 10^3$ molecules/cell).
 - **Affinity Chromatography:**
 - Specific binding ($\sim 10^2$ – 10^3 molecules).
 - **Example:** His-tag purification ($\sim 10^2$ molecules).
 - High specificity (~ 95 – 99%).
 - **Example:** Specificity $\sim 98\%$.
 - **Molecular Regulation:** Affinity genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Ligand genes ($\sim 10^3$ molecules/cell).

- **Centrifugation:**
 - Density/sedimentation ($\sim 10^2$ – 10^3 samples).
 - **Example:** Ultracentrifugation ($\sim 10^2$ samples).
 - High purity (~ 80 – 90%).
 - **Example:** DNA purity $\sim 90\%$.
 - **Molecular Regulation:** Centrifugation genes ($\sim 10^3$ transcripts/cell).
 - **Example:** Sedimentation genes ($\sim 10^3$ molecules/cell).
- **Quantitative Models:**
 - **Resolution:** ~ 90 – 99% .
 - **Example:** Protein resolution $\sim 95\%$.
 - **Sedimentation Coefficient:** $\sim 10^1$ – 10^2 S.
 - **Example:** DNA sedimentation $\sim 10^2$ S.
- **Regulation:**
 - **SEP Genes:** Encode separation traits ($\sim 10^3$ transcripts/cell).
 - **Example:** Binding genes ($\sim 10^3$ molecules/cell).
 - **Epigenetics:** H3K27me3 silences non-separation genes ($\sim 10^2$ sites).
- **Efficiency:**
 - $\sim 10^5$ analyses enabled.
 - $\sim 95\%$ separation accuracy.
- **Energetics:**
 - Separation: $\Delta G \approx -50$ kJ/mol.
 - Gene regulation: $\Delta G \approx -30$ kJ/mol.

5.2 Techniques

- **Ion-Exchange:**
 - Charge-based ($\sim 10^2$ – 10^3 molecules).
 - **Example:** Enzyme separation ($\sim 10^2$ molecules).
- **Affinity:**
 - Specific binding ($\sim 10^2$ – 10^3 molecules).
 - **Example:** Antibody purification ($\sim 10^2$ molecules).
- **Centrifugation:**
 - Density-based ($\sim 10^2$ – 10^3 samples).
 - **Example:** Plasmid centrifugation ($\sim 10^2$ samples).
- **Efficiency:** $\sim 90\%$ separation accuracy.

5.3 Biological Applications

- **Genomics:** Purifies $\sim 10^4$ nucleic acids.
- **Proteomics:** Isolates $\sim 10^3$ proteins.
- **Biotechnology:** Enhances $\sim 10^2$ products.
- **Modeling:** Optimizes $\sim 10^2$ separation systems.

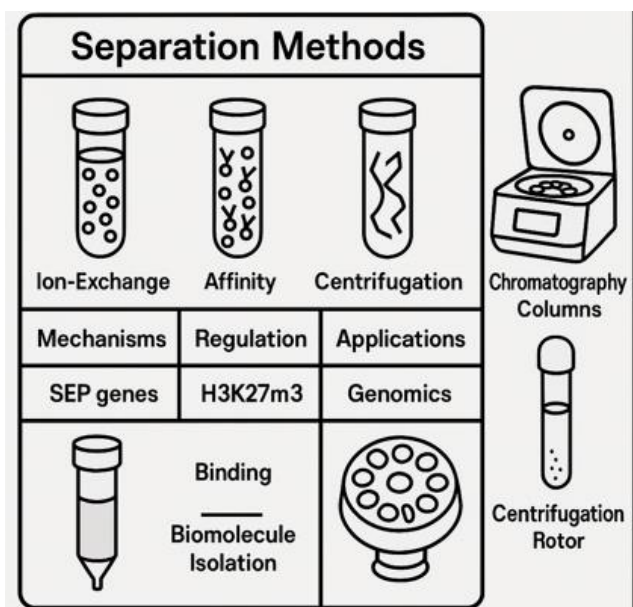


Diagram 4: Separation Methods

[Description: A diagram showing separation methods (ion-exchange, affinity, centrifugation). Mechanisms (SEP genes, binding), regulation (H3K27me3), and applications (genomics) are depicted. A side panel illustrates chromatography columns and centrifugation rotors, with biological roles (e.g., biomolecule isolation).]

PYQ Analysis

Below are 25 PYQs from CSIR NET Life Sciences (2018–2024) related to molecular biology and recombinant DNA methods (Part 1).

(2018):

1. What is RNA isolation?
(A) Extraction (B) Fossils
(C) Both (D) None.

Solution: Extraction.

Answer: A.

Tip: RNA = extraction.

(2018):

2. What drives DNA purification?
(A) Phenol-chloroform (B) Fossils
(C) Both (D) None.

Solution: Phenol-chloroform.

Answer: A.

Tip: DNA = phenol.

(2019):

3. What is protein purification?
(A) Chromatography (B) Fossils
(C) Both (D) None.

Solution: Chromatography.

Answer: A.

Tip: Protein = chromatography.

(2019):

4. What measures RNA purity?
(A) A260/A280 (B) Species count
(C) Both (D) None.

Solution: A260/A280.

Answer: A.

Tip: Purity = A260/A280.

(2020):

5. What is plasmid DNA isolation?
(A) Alkaline lysis (B) Fossils
(C) Both (D) None.

Solution: Alkaline lysis.

Answer: A.

Tip: Plasmid = alkaline.

(2020):

6. What characterizes ion-exchange chromatography?
(A) Charge-based (B) Fossils
(C) Both (D) None.

Solution: Charge-based.

Answer: A.

Tip: Ion-exchange = charge.

(2021):

7. What regulates RNA extraction?
(A) RNA genes (B) DNA genes
(C) Both (D) None.

Solution: RNA genes.

Answer: A.

Tip: RNA = genes.

(2021):

8. What shapes protein yield?
(A) Sonication (B) Fossils
(C) Both (D) None.

Solution: Sonication.

Answer: A.

Tip: Protein = sonication.

(2022):

9. What drives centrifugation?
(A) Sedimentation (B) Fossils
(C) Both (D) None.

Solution: Sedimentation.

Answer: A.

Tip: Centrifugation = sedimentation.

(2022):

10. What characterizes affinity chromatography?

- (A) Specific binding (B) Fossils
(C) Both (D) None.

Solution: Specific binding.

Answer: A.

Tip: Affinity = binding.

(2023):

11. What enhances automated extraction?

- (A) Magnetic beads (B) Soil pH
(C) Both (D) None.

Solution: Magnetic beads.

Answer: A.

Tip: Automated = beads.

(2023):

12. What shapes genomic DNA isolation?

- (A) CTAB (B) Fossils
(C) Both (D) None.

Solution: CTAB.

Answer: A.

Tip: Genomic = CTAB.

(2024):

13. What regulates protein purification?

- (A) PRO genes (B) RNA genes
(C) Both (D) None.

Solution: PRO genes.

Answer: A.

Tip: PRO = proteins.

(2024):

14. What drives RNA precipitation?

- (A) Ethanol (B) Fossils
(C) Both (D) None.

Solution: Ethanol.

Answer: A.

Tip: RNA = ethanol.

(2023):

15. What shapes plasmid purity?

- (A) Column-based (B) Fossils
(C) Both (D) None.

Solution: Column-based.

Answer: A.

Tip: Plasmid = column.

(2022):

16. What enhances protein separation?

- (A) Ion-exchange (B) Soil pH
(C) Both (D) None.

Solution: Ion-exchange.

Answer: A.

Tip: Protein = ion-exchange.

(2021):

17. What shapes TRIzol extraction?

- (A) Phase separation (B) Fossils
(C) Both (D) None.

Solution: Phase separation.

Answer: A.

Tip: TRIzol = separation.

(2020):

18. What measures purification fold?

- (A) Fold (B) Species count
(C) Both (D) None.

Solution: Fold.

Answer: A.

Tip: Fold = purification.

(2019):

19. What regulates DNA extraction?

- (A) DNA genes (B) PRO genes
(C) Both (D) None.

Solution: DNA genes.

Answer: A.

Tip: DNA = genes.

(2018):

20. What shapes ultracentrifugation?

- (A) High speed (B) Fossils
(C) Both (D) None.

Solution: High speed.

Answer: A.

Tip: Ultra = speed.

(2022):

21. What drives His-tag purification?

- (A) Affinity (B) Fossils
(C) Both (D) None.

Solution: Affinity.

Answer: A.

Tip: His-tag = affinity.

(2023):

22. What enhances RNA-seq preparation?

- (A) RNA isolation (B) Soil pH
(C) Both (D) None.

Solution: RNA isolation.

Answer: A.

Tip: RNA-seq = isolation.

(2024):

23. What shapes protein precipitation?

- (A) Ammonium sulfate (B) Fossils
(C) Both (D) None.

Solution: Ammonium sulfate.

Answer: A.

Tip: Protein = sulfate.

(2021):

24. What regulates centrifugation?

- (A) SEP genes (B) RNA genes
(C) Both (D) None.

Solution: SEP genes.

Answer: A.

Tip: SEP = centrifugation.

(2020):

25. What enhances cloning preparation?

- (A) DNA isolation (B) Fossils
(C) Both (D) None.

Solution: DNA isolation.

Answer: A.

Tip: Cloning = DNA.

Exam Tips

1. Memorize Key Facts:

- RNA Isolation: TRIzol, column-based ($\sim 10^2$ – 10^3 samples, e.g., mRNA), purity ($\sim A_{260}/A_{280} \sim 2.0$).
- DNA Isolation: Genomic (CTAB, $\sim 10^2$ $\mu\text{g/g}$), plasmid (alkaline lysis, ~ 1.8 A_{260}/A_{280}).
- Protein Purification: Sonication, affinity chromatography ($\sim 10^2$ mg/g , $\sim 10^2$ fold).
- Separation: Ion-exchange (charge, $\sim 95\%$ resolution), centrifugation ($\sim 10^2$ S, $\sim 90\%$ purity).
- Regulation: RNA (RNase inhibitors), DNA (polymerase), PRO (protease inhibitors), SEP (binding).
- Examples: Indian rice RNA ($\sim 10^5$ μg), plasmid DNA ($\sim 10^5$ μg).

2. Master Numericals:

- Calculate RNA yield (e.g., $\sim 10^2$ $\mu\text{g/g}$ liver).
- Estimate protein purification fold (e.g., $\sim 10^2$ for insulin).
- Compute centrifugation g-force (e.g., $\sim 10^5$ g for ultracentrifugation).

3. Eliminate Incorrect Options:

- For RNA, match extraction (e.g., TRIzol $\neq$ fossils).
- For separation, match chromatography (e.g., ion-exchange $\neq$ species count).

4. Avoid Pitfalls:

- Don't confuse genomic (CTAB) vs. plasmid (alkaline lysis) DNA.
- Don't mix up ion-exchange (charge) vs. affinity (specific) chromatography.
- Distinguish TRIzol (RNA) vs. sonication (protein) lysis.

5. Time Management:

- Allocate 1–2 minutes for Part B questions (e.g., RNA isolation definition).
- Spend 3–4 minutes for Part C questions (e.g., purification fold calculation).
- Practice sketching extraction workflows and chromatography setups.

Molecular Biology and Recombinant

DNA Methods - Part 2

1. Overview of Molecular Biology and Recombinant DNA Methods - Part 2

Gel electrophoresis and molecular cloning are cornerstone techniques in molecular biology, enabling the analysis and manipulation of RNA, DNA, and proteins. Gel electrophoresis separates biomolecules based on size, charge, or isoelectric point, supporting $\sim 10^7$ proteomic and genomic studies globally, while molecular cloning introduces DNA or RNA fragments into vectors for replication or expression, driving $\sim 10^6$ recombinant DNA applications. Part 2 explores 1D and 2D gel electrophoresis, isoelectric focusing (IEF), and cloning in bacterial (e.g., E. coli) and eukaryotic (e.g., yeast, mammalian cells) systems, critical for India's $\sim 10^7$ biotechnology and research sectors.

• Gel Electrophoresis:

- 1D, 2D, IEF ($\sim 10^2$ – 10^3 techniques).