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CSIR-NET

LIFE SCIENCE

Council of Scientific & Industrial Research

VOLUME – 6

Applied Biology & Methods in Biology

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INNOVATIONS

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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 (~10^4 compounds), ethanol (~10^6 L/year globally), and organic acids (~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 ~10^7 people in India through pharmaceuticals, biofuels, and food.

- **Fermentation Principles**:
  - Microbial metabolism (~10^2–10^3 pathways).
- **Microbial Strains**:
  - Bacteria, fungi, yeast (~10^3–10^4 strains).
- **Small Molecule Production**:
  - Antibiotics, ethanol, organic acids (~10^4 molecules).
- **Biological Relevance**:
  - Fermentation produces ~10^6 tons of products.
  - Small molecules treat ~10^8 infections.
  - Ethanol powers ~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 ~10^6 tons of products across ~10^4 fermentation processes.
    - **Example**: Ethanol fermentation (~10^6 L/year in India).
- **Molecular Basis**:
  - **Metabolic Pathways**:
    - Glycolysis, TCA cycle (~10^2–10^3 pathways).
    - **Example**: Ethanol via pyruvate (~10^1 pathways).
    - Anaerobic/aerobic conditions (~10^1–10^2 conditions).
    - **Example**: Anaerobic yeast (~10^1 conditions).
    - **Molecular Regulation**: Metabolic genes (~10^3 transcripts/cell).
    - **Example**: Alcohol dehydrogenase (~10^3 molecules/cell).

- **Growth Kinetics**:
  - Exponential growth (~μ = 0.1–1 h^{-1}).
  - **Example**: E. coli μ ~0.5 h^{-1}.
  - Substrate utilization (~10^1–10^2 g/L).
  - **Example**: Glucose ~10^2 g/L.
  - **Molecular Regulation**: Growth genes (~10^3 transcripts/cell).
  - **Example**: Ribosomal genes (~10^3 molecules/cell).
- **Bioreactor Systems**:
  - Batch, fed-batch, continuous (~10^1–10^2 systems).
  - **Example**: Stirred-tank bioreactor (~10^1 systems).
  - Controlled parameters (~10^1–10^2 variables).
  - **Example**: pH, temperature (~10^1 variables).
  - **Molecular Regulation**: Regulatory genes (~10^3 transcripts/cell).
  - **Example**: Stress response (~10^3 molecules/cell).

- **Quantitative Models**:
  - **Growth Rate**: $\mu = \ln(Nt/N_0)/t$ (~0.1–1 h^{-1}).
    - **Example**: Yeast μ ~0.3 h^{-1}.
  - **Yield**: Y = P/S (~0.1–0.5 g/g).
    - **Example**: Ethanol Y ~0.45 g/g glucose.
- **Regulation**:
  - **FER Genes**: Encode fermentation traits (~10^3 transcripts/cell).
    - **Example**: Pyruvate kinase (~10^3 molecules/cell).
  - **Epigenetics**: H3K4me3 marks metabolic genes (~10^2 promoters).
- **Efficiency**:
  - ~10^6 tons produced.
  - ~95% fermentation accuracy.
- **Energetics**:
  - Metabolism: $\Delta G \approx$ -50 kJ/mol.
  - Gene regulation: $\Delta G \approx$ -30 kJ/mol.

2.2 Components

- **Pathways**:
  - Metabolic routes (~10^2–10^3 pathways).
    - **Example**: Citric acid pathway (~10^1 pathways).
- **Kinetics**:
  - Growth, substrate use (~0.1–1 h^{-1}).
    - **Example**: Lactobacillus μ ~0.4 h^{-1}.
- **Bioreactors**:
  - Controlled systems (~10^1–10^2 systems).
    - **Example**: Fed-batch (~10^1 systems).
- **Efficiency**: ~90% process accuracy.

2.3 Biological Applications

- **Biotechnology**: Produces ~10^6 tons of products.
- **Pharmaceuticals**: Supplies ~10^4 drugs.
- **Biofuels**: Generates ~10^6 L ethanol.
- **Modeling**: Optimizes ~10^2 fermentation processes.

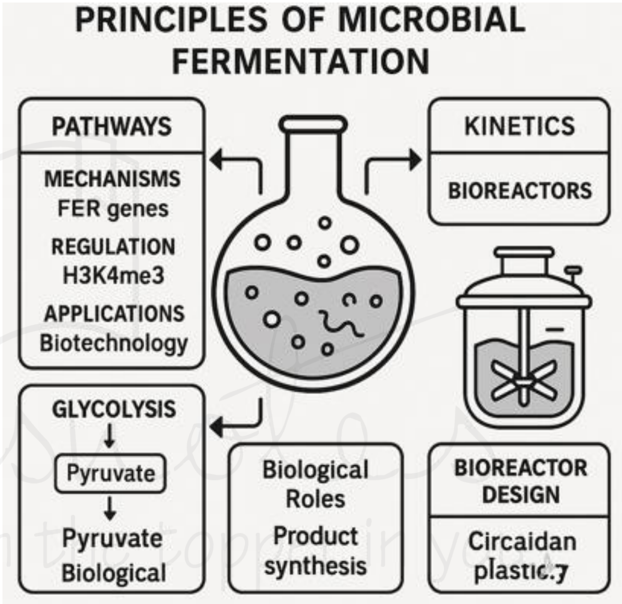


Diagram 1: Principles of Microbial Fermentation

[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 ~10^4 fermentation processes with ~10^3–10^4 strains.
    - **Example**: S. cerevisiae for ethanol (~10^3 strains).
- **Molecular Basis**:
  - **Bacterial Strains**:
    - Versatile metabolism (~10^2–10^3 pathways).
    - **Example**: Lactobacillus for lactic acid (~10^2 pathways).
    - Rapid growth (~μ = 0.5–2 h^{-1}).
    - **Example**: E. coli μ ~1 h^{-1}.
    - **Molecular Regulation**: Bacterial genes (~10^3 transcripts/cell).
    - **Example**: Lactate dehydrogenase (~10^3 molecules/cell).
  - **Fungal Strains**:
    - Complex pathways (~10^2–10^3 pathways).
    - **Example**: A. niger for citric acid (~10^2 pathways).
    - Robust enzymes (~10^2–10^3 enzymes).
    - **Example**: Amylases (~10^2 enzymes).
    - **Molecular Regulation**: Fungal genes (~10^3 transcripts/cell).
    - **Example**: Citrate synthase (~10^3 molecules/cell).
  - **Yeast Strains**:
    - Ethanol, protein production (~10^2–10^3 pathways).
    - **Example**: S. cerevisiae ethanol (~10^2 pathways).
    - Genetic tractability (~10^3–10^4 genes).
    - **Example**: Yeast genome (~10^3 genes).
    - **Molecular Regulation**: Yeast genes (~10^3 transcripts/cell).
    - **Example**: Alcohol dehydrogenase (~10^3 molecules/cell).
- **Quantitative Models**:
  - **Strain Productivity**: ~10^1–10^2 g/L product.
    - **Example**: S. cerevisiae ~10^2 g/L ethanol.
  - **Growth Rate**: μ (~0.1–2 h^{-1}).
    - **Example**: A. niger μ ~0.2 h^{-1}.
- **Regulation**:
  - **STR Genes**: Encode strain traits (~10^3 transcripts/cell).
    - **Example**: Metabolic enzymes (~10^3 molecules/cell).
  - **Epigenetics**: DNA methylation (~10^2 sites).
    - **Example**: Methylated promoters (~10^2 sites).
- **Efficiency**:
  - ~10^4 processes catalyzed.
  - ~95% strain accuracy.
- **Energetics**:
  - Metabolism: $\Delta G \approx -50$ kJ/mol.
  - Gene regulation: $\Delta G \approx -30$ kJ/mol.

3.2 Strains

- **Bacteria**:
  - Versatile, rapid (~10^3 strains).
    - **Example**: Bacillus (~10^2 strains).
- **Fungi**:
  - Complex, robust (~10^3 strains).
    - **Example**: Penicillium (~10^2 strains).
- **Yeast**:
  - Ethanol, proteins (~10^3 strains).
    - **Example**: Pichia (~10^2 strains).
- **Efficiency**: ~90% microbial accuracy.

3.3 Biological Applications

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

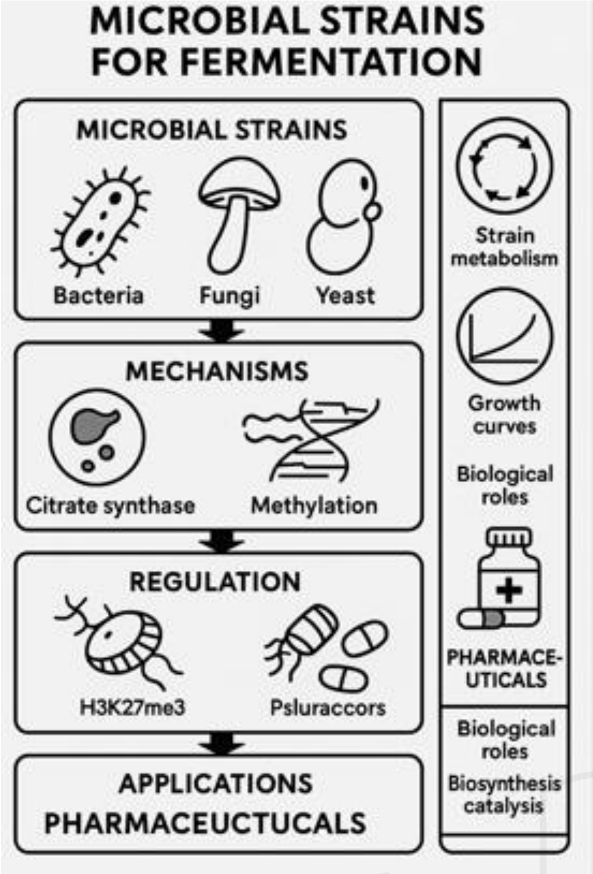


Diagram 2: Microbial Strains for Fermentation [Description: A diagram showing microbial strains (bacteria, fungi, yeast). Mechanisms (STR genes, citrate synthase), regulation (methylation), and applications (pharmaceuticals) are depicted. A side panel illustrates strain metabolism and growth curves, 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 ~10^8 infections with ~10^4 antibiotics.
    - **Example**: Penicillin (~10^3 tons/year).
- **Molecular Basis**:
  - Secondary metabolites (~10^2–10^3 pathways).
    - **Example**: β-lactam synthesis (~10^1 pathways).
  - Polyketide, non-ribosomal pathways (~10^1–10^2 pathways).
    - **Example**: Penicillin biosynthesis (~10^1 pathways).
  - **Molecular Regulation**: Antibiotic genes (~10^3 transcripts/cell).
    - **Example**: Penicillin synthase (~10^3 molecules/cell).
- **Quantitative Models**:
  - **Yield**: ~10^1–10^2 g/L.
    - **Example**: Penicillin ~10^2 g/L.
- **Efficiency**: ~90% antibiotic accuracy.

4.2 Ethanol

- **Overview**:
  - Powers ~10^7 vehicles with ~10^6 L/year.
    - **Example**: Bioethanol (~10^6 L in India).
- **Molecular Basis**:
  - Fermentation of sugars (~10^1–10^2 pathways).
    - **Example**: Glucose to ethanol (~10^1 pathways).
  - Yeast metabolism (~10^1–10^2 enzymes).
    - **Example**: Pyruvate decarboxylase (~10^1 enzymes).
  - **Molecular Regulation**: Ethanol genes (~10^3 transcripts/cell).
    - **Example**: Alcohol dehydrogenase (~10^3 molecules/cell).
- **Quantitative Models**:
  - **Yield**: ~0.4–0.5 g/g glucose.
    - **Example**: Ethanol ~0.45 g/g.
- **Efficiency**: ~90% ethanol accuracy.

4.3 Organic Acids

- **Overview**:
  - Supplies ~10^5 tons for food, industry.
    - **Example**: Citric acid (~10^5 tons/year).
- **Molecular Basis**:
  - TCA cycle derivatives (~10^1–10^2 pathways).
    - **Example**: Citric acid synthesis (~10^1 pathways).
  - Fungal metabolism (~10^1–10^2 enzymes).
    - **Example**: Citrate synthase (~10^1 enzymes).
  - **Molecular Regulation**: Acid genes (~10^3 transcripts/cell).
    - **Example**: Citrate transporter (~10^3 molecules/cell).

- **Quantitative Models**:
  - **Yield**: ~10^1–10^2 g/L.
    - **Example**: Citric acid ~10^2 g/L.
- **Efficiency**: ~90% acid accuracy.

4.4 Applications

- **Antibiotics**: Treats ~10^8 infections.
  - **Example**: Indian penicillin (~10^3 tons).
- **Ethanol**: Fuels ~10^7 vehicles.
  - **Example**: Indian bioethanol (~10^6 L).
- **Acids**: Supplies ~10^5 food products.
  - **Example**: Indian citric acid (~10^4 tons).
- **Efficiency**: ~90% production accuracy.

4.5 Biological Applications

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

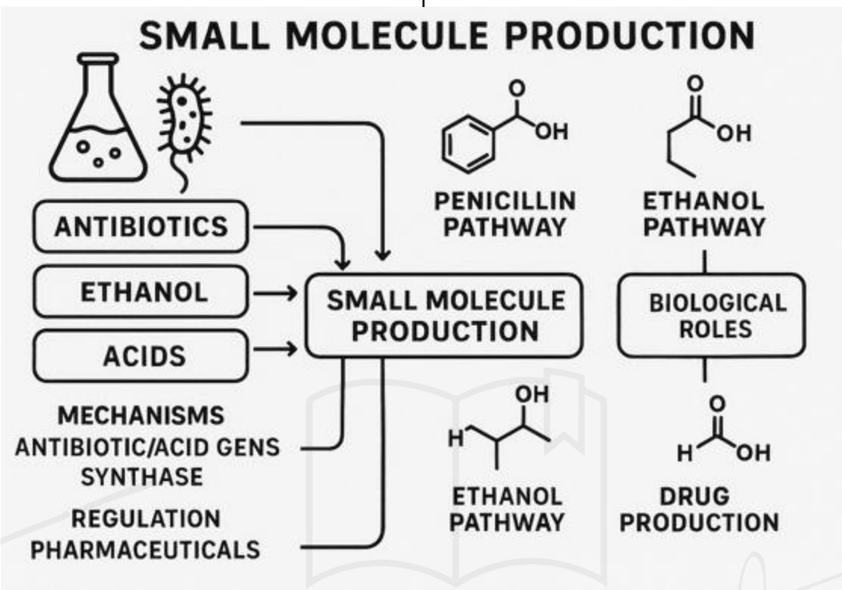


Diagram 3: Small Molecule Production
[Description: A diagram showing small molecule production (antibiotics, ethanol, acids). Mechanisms (antibiotic/acid genes, synthase), regulation (H3K4me3), and applications (pharmaceuticals) are depicted. A side panel illustrates penicillin and ethanol pathways, 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 (~10^2–10^3 pathways, e.g., ethanol).
   - Strains: Bacteria (E. coli), fungi (A. niger), yeast (S. cerevisiae, ~10^3–10^4 strains).
   - Small Molecules: Antibiotics (~10^4 compounds, e.g., penicillin), ethanol (~10^6 L), acids (~10^5 tons, e.g., citric acid).
   - Regulation: FER (fermentation), STR (strains), antibiotic/ethanol/acid genes.
   - Applications: Pharmaceuticals, biofuels, food industry.
   - Examples: Indian bioethanol (~10^6 L), penicillin (~10^3 tons).
2. **Master Numericals**:
   - Calculate yields (e.g., ~0.45 g/g ethanol).
   - Estimate growth rates (e.g., μ ~0.3 h^{-1} for yeast).
   - Compute substrate use (e.g., ~10^2 g/L glucose).
3. **Eliminate Incorrect Options**:
   - For fermentation, match metabolism (e.g., glycolysis ≠ fossils).
   - For antibiotics, match secondary metabolites (e.g., penicillin ≠ 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 (~10^4 types, e.g., insulin), enzymes (~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 ~10^7 people in India through insulin production, enzyme-based industries, and sustainable biopolymers.

- **Macro Molecule Biosynthesis**:
  - Proteins, enzymes, biopolymers (~10^4 molecules).
- **Recombinant DNA Technology**:
  - Genetic engineering (~10^3–10^4 constructs).
- **Bioprocess Optimization**:
  - Scale-up, yield improvement (~10^2–10^3 processes).
- **Industrial Applications**:
  - Biopharmaceuticals, agriculture, industry (~10^4 applications).
- **Biological Relevance**:
  - Proteins treat ~10^8 patients.
  - Enzymes process ~10^6 tons of substrates.
  - Biopolymers replace ~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 ~10^4 macro molecules across ~10^3 fermentation processes.
    - **Example**: Insulin production (~10^3 kg/year in India).
- **Molecular Basis**:
  - **Protein Biosynthesis**:
    - Recombinant proteins (~10^2–10^3 proteins).
    - **Example**: Insulin (~10^1 proteins).
    - Translation, folding (~10^3–10^4 genes).
    - **Example**: Insulin gene (~10^3 transcripts/cell).
    - **Molecular Regulation**: Protein genes (~10^3 transcripts/cell).
    - **Example**: T7 polymerase (~10^3 molecules/cell).

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

- **Quantitative Models**:
  - **Protein Yield**: ~10^1–10^2 mg/L.
    - **Example**: Insulin ~10^2 mg/L.
  - **Enzyme Activity**: ~10^2–10^3 U/mL.
    - **Example**: Amylase ~10^3 U/mL.
- **Regulation**:
  - **MAC Genes**: Encode macro molecule traits (~10^3 transcripts/cell).
    - **Example**: Insulin genes (~10^3 molecules/cell).
  - **Epigenetics**: H3K4me3 marks biosynthetic genes (~10^2 promoters).
- **Efficiency**:
  - ~10^4 molecules produced.
  - ~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 (~10^2–10^3 proteins).
  - **Example**: Growth hormone (~10^1 proteins).
- **Enzymes**:
  - Catalytic proteins (~10^2–10^3 enzymes).
    - **Example**: Protease (~10^1 enzymes).
- **Biopolymers**:
  - Polymer chains (~10^1–10^2 polymers).
    - **Example**: PHB (~10^1 polymers).
- **Efficiency**: ~90% production accuracy.

2.3 Biological Applications

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

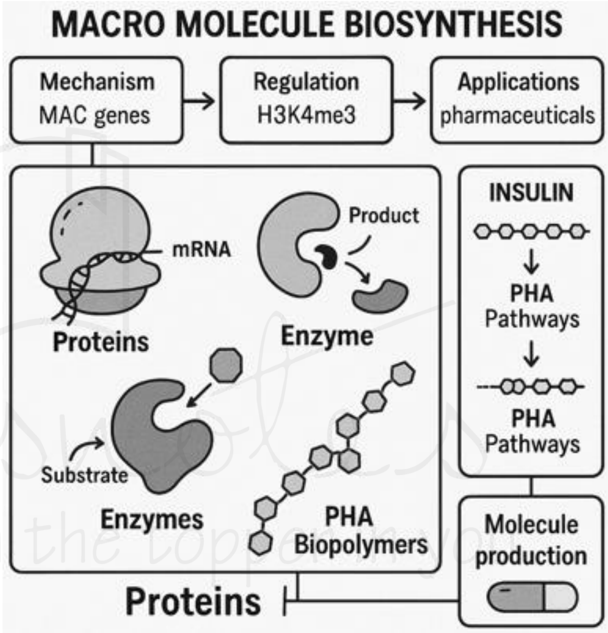


Diagram 1: Macro Molecule Biosynthesis

[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

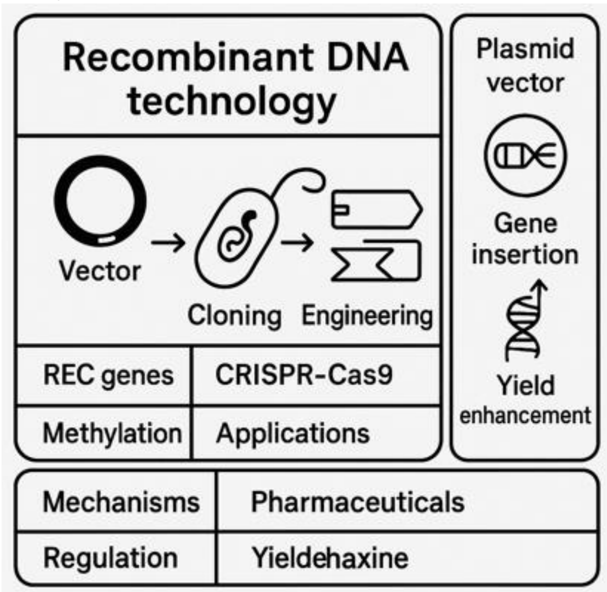
- **Overview**:
  - Enhances ~10^3–10^4 constructs for ~10^4 fermentation processes.
    - **Example**: Recombinant insulin in E. coli (~10^3 constructs).
- **Molecular Basis**:
  - **Expression Vectors**:
    - Plasmids, promoters (~10^2–10^3 vectors).
    - **Example**: pET vector (~10^2 vectors).
    - High expression (~10^3–10^4 transcripts/cell).
    - **Example**: T7 promoter (~10^3 transcripts/cell).
    - **Molecular Regulation**: Vector genes (~10^3 transcripts/cell).
    - **Example**: Lac operon (~10^3 molecules/cell).
  - **Gene Cloning**:
    - Insertion of foreign genes (~10^2–10^3 genes).
    - **Example**: Human insulin gene (~10^1 genes).
    - Restriction, ligation (~10^1–10^2 reactions).
    - **Example**: EcoRI ligation (~10^1 reactions).
    - **Molecular Regulation**: Cloning genes (~10^3 transcripts/cell).
    - **Example**: DNA ligase (~10^3 molecules/cell).
  - **Metabolic Engineering**:
    - Pathway optimization (~10^1–10^2 pathways).
    - **Example**: Insulin secretion (~10^1 pathways).
    - CRISPR, gene knockouts (~10^1–10^2 edits).
    - **Example**: Knockout for byproducts (~10^1 edits).
    - **Molecular Regulation**: Engineering genes (~10^3 transcripts/cell).
    - **Example**: CRISPR-Cas9 (~10^3 molecules/cell).
- **Quantitative Models**:
  - **Expression Level**: ~10^1–10^2 mg/L protein.
    - **Example**: Insulin ~10^2 mg/L.
  - **Editing Efficiency**: ~70–90%.
    - **Example**: CRISPR ~80%.
- **Regulation**:
  - **REC Genes**: Encode recombinant traits (~10^3 transcripts/cell).
    - **Example**: T7 polymerase (~10^3 molecules/cell).
  - **Epigenetics**: DNA methylation (~10^2 sites).
    - **Example**: Methylated promoters (~10^2 sites).
- **Efficiency**:
  - ~10^4 constructs enhanced.
  - ~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 (~10^2–10^3 vectors).
    - **Example**: pBR322 (~10^2 vectors).
- **Cloning**:
  - Gene insertion (~10^2–10^3 genes).
    - **Example**: Erythropoietin (~10^1 genes).
- **Engineering**:
  - Pathway edits (~10^1–10^2 pathways).
    - **Example**: Amylase pathway (~10^1 pathways).
- **Efficiency**: ~90% genetic accuracy.

3.3 Biological Applications

- **Biotechnology**: Enhances ~10^4 recombinant products.
- **Pharmaceuticals**: Produces ~10^3 biologics.
- **Industry**: Optimizes ~10^2 pathways.
- **Modeling**: Designs ~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 ~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 (~10^3–10^4 protocols).
- **Separation Methods**:
  - Chromatography, centrifugation (~10^2–10^3 techniques).
- **Biological Relevance**:
  - Isolation yields ~10^6 µg of biomolecules.
  - Purification supports ~10^7 experiments.
  - Separation enables ~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 ~10^6 µg RNA for ~10^4 experiments.
    - **Example**: mRNA from rice (~10^5 µg in India).
- **Molecular Basis**:
  - **Cell Lysis**:
    - Chemical disruption (~10^2–10^3 buffers).
    - **Example**: TRIzol lysis (~10^2 buffers).
    - RNA release (~10^1–10^2 % yield).
    - **Example**: mRNA yield ~10^1 %.
    - **Molecular Regulation**: Lysis genes (~10^3 transcripts/cell).
    - **Example**: RNase inhibitors (~10^3 molecules/cell).

- Phase Separation:
  - Phenol-chloroform ($\sim10^1$–10^2 extractions).
  - **Example**: Chloroform separation ($\sim10^1$ extractions).
  - RNA partitioning (~90–95% purity).
  - **Example**: RNA purity ~95%.
  - **Molecular Regulation**: Partitioning genes ($\sim10^3$ transcripts/cell).
  - **Example**: RNA-binding genes ($\sim10^3$ molecules/cell).
- **Precipitation and Washing**:
  - Ethanol/isopropanol ($\sim10^1$–10^2 precipitations).
  - **Example**: Ethanol precipitation ($\sim10^1$ precipitations).
  - High recovery (~80–90%).
  - **Example**: RNA recovery ~90%.
  - **Molecular Regulation**: Precipitation genes ($\sim10^3$ transcripts/cell).
  - **Example**: RNA stabilization genes ($\sim10^3$ molecules/cell).

- **Quantitative Models**:
  - **Yield**: $\sim10^1$–10^2 µg/g tissue.
    - **Example**: Liver RNA $\sim10^2$ µg/g.
  - **Purity (A260/A280):**
  - ~1.8–2.0.
    - **Example**: RNA A260/A280 ~2.0.
- **Regulation**:
  - **RNA Genes**: Encode RNA traits ($\sim10^3$ transcripts/cell).
    - **Example**: RNase inhibitor genes ($\sim10^3$ molecules/cell).
  - **Epigenetics**: H3K4me3 marks RNA genes ($\sim10^2$ promoters).
- **Efficiency**:
  - $\sim10^6$ µg RNA yielded.
  - ~95% isolation accuracy.
- **Energetics**:
  - RNA extraction: $\Delta G \approx -50$ kJ/mol.
  - Gene regulation: $\Delta G \approx -30$ kJ/mol.

2.2 Techniques

- **TRIzol Extraction**:
  - Chemical lysis ($\sim10^2$–10^3 samples).
    - **Example**: Yeast RNA ($\sim10^2$ samples).
- **Column-Based**:
  - Silica columns ($\sim10^2$–10^3 samples).
    - **Example**: Blood RNA ($\sim10^2$ samples).
- **Magnetic Beads**:
  - Automated ($\sim10^1$–10^2 samples).
    - **Example**: Plant RNA ($\sim10^1$ samples).
- **Efficiency**: ~90% RNA purity.

2.3 Biological Applications

- **Genomics**: Supports $\sim10^4$ RNA-seq.
- **Biotechnology**: Enables $\sim10^3$ RT-PCR.
- **Medicine**: Diagnoses $\sim10^2$ diseases.
- **Modeling**: Optimizes $\sim10^2$ RNA protocols.

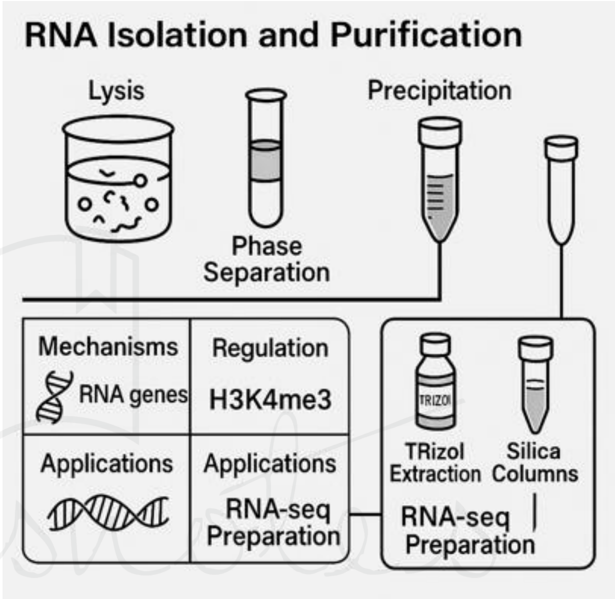


Diagram 1: RNA Isolation and Purification [Description: A diagram showing RNA isolation (lysis, phase separation, precipitation). Mechanisms (RNA genes, RNase inhibitors), regulation (H3K4me3), and applications (genomics) are depicted. A side panel illustrates TRIzol extraction and silica columns, with 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 $\sim10^6$ µg DNA for $\sim10^4$ experiments.
    - **Example**: Plasmid DNA from E. coli ($\sim10^5$ µg in India).

- **Molecular Basis**:
  - **Genomic DNA Isolation**:
    - Lysis, proteinase K (~10^2–10^3 buffers).
    - **Example**: CTAB lysis (~10^2 buffers).
    - DNA release (~10^1–10^2 % yield).
    - **Example**: Genomic yield ~10^1 %.
    - **Molecular Regulation**: Genomic genes (~10^3 transcripts/cell).
    - **Example**: DNA-binding genes (~10^3 molecules/cell).
  - **Plasmid DNA Isolation**:
    - Alkaline lysis (~10^2–10^3 extractions).
    - **Example**: SDS lysis (~10^2 extractions).
    - Plasmid separation (~90–95% purity).
    - **Example**: Plasmid purity ~95%.
    - **Molecular Regulation**: Plasmid genes (~10^3 transcripts/cell).
    - **Example**: Replication genes (~10^3 molecules/cell).
  - **Purification**:
    - Column-based, ethanol (~10^1–10^2 methods).
    - **Example**: Silica column (~10^1 methods).
    - High recovery (~80–90%).
    - **Example**: DNA recovery ~90%.
    - **Molecular Regulation**: Purification genes (~10^3 transcripts/cell).
    - **Example**: DNA stabilization genes (~10^3 molecules/cell).
- **Quantitative Models**:
  - **Yield**: ~10^1–10^2 μg/g tissue.
    - **Example**: Genomic DNA ~10^2 μg/g.
  - **Purity (A260/A280):**
  - ~1.8–2.0.
    - **Example**: Plasmid A260/A280 ~1.8.
- **Regulation**:
  - **DNA Genes**: Encode DNA traits (~10^3 transcripts/cell).
    - **Example**: DNA polymerase genes (~10^3 molecules/cell).
  - **Epigenetics**: H3K27me3 silences non-DNA genes (~10^2 sites).
- **Efficiency**:
  - ~10^6 μg DNA yielded.
  - ~95% isolation accuracy.
- **Energetics**:
  - DNA extraction: ΔG ≈ -50 kJ/mol.
  - Gene regulation: ΔG ≈ -30 kJ/mol.

3.2 Techniques

- **CTAB Extraction**:
  - Genomic DNA (~10^2–10^3 samples).
    - **Example**: Plant DNA (~10^2 samples).
- **Alkaline Lysis**:
  - Plasmid DNA (~10^2–10^3 samples).
    - **Example**: Bacterial plasmids (~10^2 samples).
- **Column-Based**:
  - High-purity DNA (~10^2–10^3 samples).
    - **Example**: Mammalian DNA (~10^2 samples).
- **Efficiency**: ~90% DNA purity.

3.3 Biological Applications

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

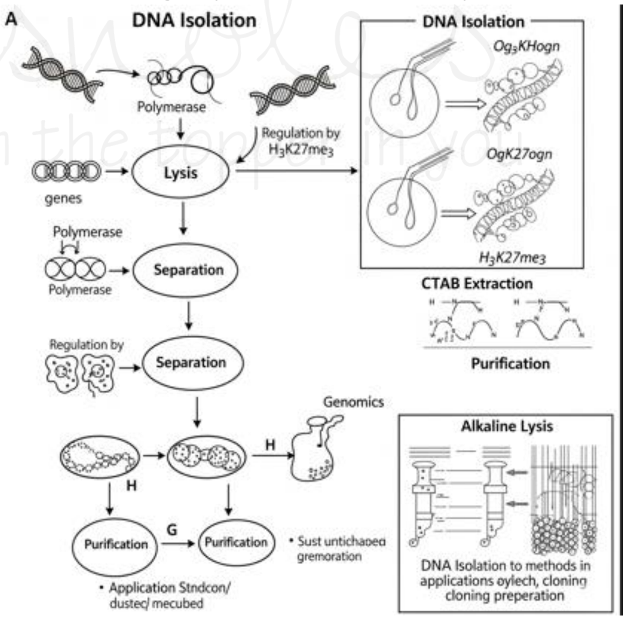


Diagram 2: DNA Isolation and Purification
[Description: A diagram showing DNA isolation (lysis, separation, purification). Mechanisms (DNA genes, polymerase), regulation (H3K27me3), and applications (genomics) are depicted. A side panel illustrates CTAB extraction and alkaline lysis, with biological roles (e.g., cloning preparation).]

4. solation 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 ~10^6 µg proteins for ~10^4 experiments.
    - **Example**: Insulin purification (~10^5 µg in India).
- **Molecular Basis**:
  - **Cell Lysis**:
    - Mechanical, chemical (~10^2–10^3 buffers).
    - **Example**: Sonication (~10^2 buffers).
    - Protein release (~10^1–10^2 % yield).
    - **Example**: Protein yield ~10^1 %.
    - **Molecular Regulation**: Lysis genes (~10^3 transcripts/cell).
    - **Example**: Protease inhibitors (~10^3 molecules/cell).
  - **Precipitation**:
    - Ammonium sulfate (~10^1–10^2 precipitations).
    - **Example**: Salt precipitation (~10^1 precipitations).
    - Protein enrichment (~70–90%).
    - **Example**: Protein enrichment ~85%.
    - **Molecular Regulation**: Precipitation genes (~10^3 transcripts/cell).
    - **Example**: Protein-binding genes (~10^3 molecules/cell).
  - **Purification**:
    - Chromatography (~10^1–10^2 methods).
    - **Example**: Affinity chromatography (~10^1 methods).
    - High purity (~90–95%).
    - **Example**: Protein purity ~95%.
    - **Molecular Regulation**: Purification genes (~10^3 transcripts/cell).
    - **Example**: Protein stabilization genes (~10^3 molecules/cell).
- **Quantitative Models**:
  - **Yield**: ~10^1–10^2 mg/g tissue.
    - **Example**: Enzyme yield ~10^2 mg/g.
  - **Purification Fold**: ~10^1–10^2 fold.
    - **Example**: Insulin fold ~10^2.
- **Regulation**:
  - **PRO Genes**: Encode protein traits (~10^3 transcripts/cell).
    - **Example**: Protease inhibitor genes (~10^3 molecules/cell).
  - **Epigenetics**: H3K4me3 marks protein genes (~10^2 promoters).
- **Efficiency**:
  - ~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 (~10^2–10^3 samples).
    - **Example**: Bacterial proteins (~10^2 samples).
- **Ammonium Sulfate**:
  - Precipitation (~10^2–10^3 samples).
    - **Example**: Enzyme precipitation (~10^2 samples).
- **Affinity Chromatography**:
  - High-purity (~10^2–10^3 samples).
    - **Example**: His-tagged proteins (~10^2 samples).
- **Efficiency**: ~90% protein purity.

4.3 Biological Applications

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

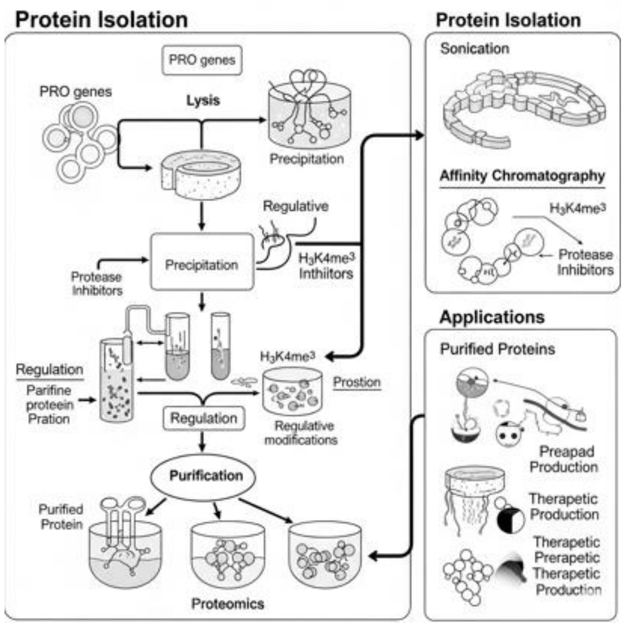


Diagram 3: Protein Isolation and Purification

[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 ~10⁵ analyses with ~10²–10³ techniques.
    - **Example**: Ion-exchange chromatography (~10⁴ analyses in India).
- **Molecular Basis**:
  - **Ion-Exchange Chromatography**:
    - Charge-based separation (~10²–10³ proteins).
    - **Example**: Protein separation (~10² proteins).
    - High resolution (~90–95%).
    - **Example**: Resolution ~95%.
    - **Molecular Regulation**: Ion-exchange genes (~10³ transcripts/cell).
    - **Example**: Binding genes (~10³ molecules/cell).
  - **Affinity Chromatography**:
    - Specific binding (~10²–10³ molecules).
    - **Example**: His-tag purification (~10² molecules).
    - High specificity (~95–99%).
    - **Example**: Specificity ~98%.
    - **Molecular Regulation**: Affinity genes (~10³ transcripts/cell).
    - **Example**: Ligand genes (~10³ molecules/cell).
  - **Centrifugation**:
    - Density/sedimentation (~10²–10³ samples).
    - **Example**: Ultracentrifugation (~10² samples).
    - High purity (~80–90%).
    - **Example**: DNA purity ~90%.
    - **Molecular Regulation**: Centrifugation genes (~10³ transcripts/cell).
    - **Example**: Sedimentation genes (~10³ molecules/cell).
- **Quantitative Models**:
  - **Resolution**: ~90–99%.
    - **Example**: Protein resolution ~95%.
  - **Sedimentation Coefficient**: ~10¹–10² S.
    - **Example**: DNA sedimentation ~10² S.
- **Regulation**:
  - **SEP Genes**: Encode separation traits (~10³ transcripts/cell).
    - **Example**: Binding genes (~10³ molecules/cell).
  - **Epigenetics**: H3K27me3 silences non-separation genes (~10² sites).
- **Efficiency**:
  - ~10⁵ analyses enabled.
  - ~95% separation accuracy.
- **Energetics**:
  - Separation: ΔG ≈ -50 kJ/mol.
  - Gene regulation: ΔG ≈ -30 kJ/mol.

5.2 Techniques

- **Ion-Exchange**:
  - Charge-based (~10²–10³ molecules).
    - **Example**: Enzyme separation (~10² molecules).
- **Affinity**:
  - Specific binding (~10²–10³ molecules).
    - **Example**: Antibody purification (~10² molecules).
- **Centrifugation**:
  - Density-based (~10²–10³ samples).
    - **Example**: Plasmid centrifugation (~10² samples).
- **Efficiency**: ~90% separation accuracy.

5.3 Biological Applications

- **Genomics**: Purifies ~10⁴ nucleic acids.
- **Proteomics**: Isolates ~10³ proteins.
- **Biotechnology**: Enhances ~10² products.
- **Modeling**: Optimizes ~10² separation systems.

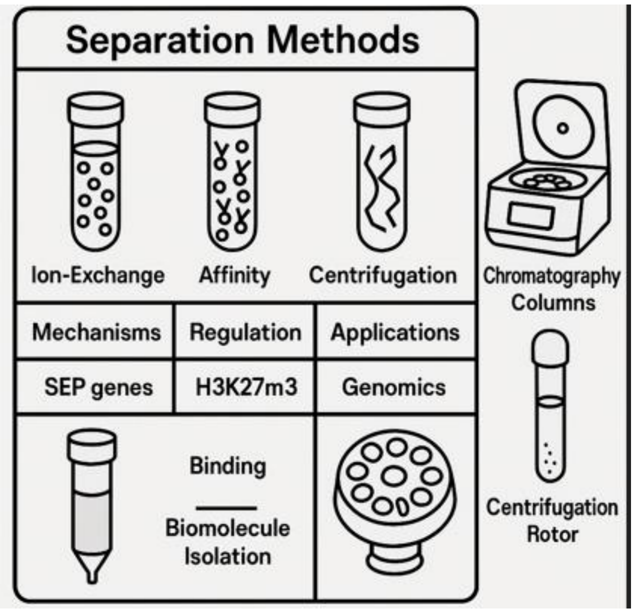


Diagram 4: Separation Methods

[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 (~10^2–10^3 samples, e.g., mRNA), purity (~A260/A280 ~2.0).
   - DNA Isolation: Genomic (CTAB, ~10^2 μg/g), plasmid (alkaline lysis, ~1.8 A260/A280).
   - Protein Purification: Sonication, affinity chromatography (~10^2 mg/g, ~10^2 fold).
   - Separation: Ion-exchange (charge, ~95% resolution), centrifugation (~10^2 S, ~90% purity).
   - Regulation: RNA (RNase inhibitors), DNA (polymerase), PRO (protease inhibitors), SEP (binding).
   - Examples: Indian rice RNA (~10^5 μg), plasmid DNA (~10^5 μg).
2. **Master Numericals**:
   - Calculate RNA yield (e.g., ~10^2 μg/g liver).
   - Estimate protein purification fold (e.g., ~10^2 for insulin).
   - Compute centrifugation g-force (e.g., ~10^5 g for ultracentrifugation).
3. **Eliminate Incorrect Options**:
   - For RNA, match extraction (e.g., TRIzol ≠ fossils).
   - For separation, match chromatography (e.g., ion-exchange ≠ 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 ~10^7 proteomic and genomic studies globally, while molecular cloning introduces DNA or RNA fragments into vectors for replication or expression, driving ~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 ~10^7 biotechnology and research sectors.

- **Gel Electrophoresis**:
  - 1D, 2D, IEF (~10^2–10^3 techniques).