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كلية العلوم

قـســــــــــم التقنيات الاحيائية الطبية

INDUSTRIAL MICROBIOLOGY

Lec. 3

Microbial Production of Antibiotics

by

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1. Introduction

Definition

Antibiotics are **bioactive secondary metabolites** produced by microorganisms that inhibit or destroy the growth of other microbes, usually at very low concentrations.

Secondary metabolites are compounds not essential for normal growth or reproduction of the organism but provide ecological advantages, such as defense or competition.

Key Features

- Produced during the **stationary phase** of growth.
- Usually synthesized via **specific metabolic pathways** (e.g., polyketide or non-ribosomal peptide synthesis).
- Have **selective toxicity** – harmful to microbes but relatively safe for host cells.

Applications

- **Medical:** Treatment of bacterial, fungal, and parasitic infections.
- **Veterinary:** Control of animal diseases and as growth promoters.
- **Agriculture:** Control of plant pathogens.
- **Biotechnology:** Selective agents in genetic engineering.

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2. Historical Background

Year	Discovery / Event	Scientist
1928	Discovery of **Penicillin**	Alexander Fleming
1940	Isolation of **Gramicidin**	René Dubos
1944	**Streptomycin** discovered	Selman Waksman
1950s–1970s	“Golden Age” of antibiotic discovery	Multiple researchers

Year	Discovery / Event	Scientist
1980s onward	Genetic engineering of antibiotic biosynthesis	Modern biotechnology

Impact: Revolutionized medicine, drastically reducing mortality from bacterial infections.

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3. Microorganisms Producing Antibiotics

A. Actinomycetes

- Gram-positive, filamentous bacteria resembling fungi.
- Found mainly in soil.
- Responsible for ~70% of all known antibiotics.

Examples:

- *Streptomyces griseus* → Streptomycin
- *S. venezuelae* → Chloramphenicol
- *S. aureofaciens* → Tetracycline

B. Bacteria

- Especially *Bacillus* species (spore-forming rods).
- Produce peptide antibiotics.

Examples:

- *Bacillus subtilis* → Bacitracin
- *B. polymyxa* → Polymyxins

C. Fungi

- Filamentous molds produce β-lactam antibiotics.

Examples:

- *Penicillium chrysogenum* → Penicillin
- *Acremonium chrysogenum* (formerly *Cephalosporium*) → Cephalosporins

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4. Characteristics of Antibiotic Biosynthesis

- **Secondary metabolism:** Occurs after the exponential growth phase.
- **Complex regulation:** Controlled by nutrient limitation, pH, oxygen, and signal molecules.
- **Non-essential:** Removal of antibiotic biosynthetic genes often doesn't affect growth.

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5. Steps in Industrial Production

Step 1: Strain Selection and Improvement

- **Screening:** Soil samples are plated to isolate antibiotic producers (e.g., cross-streak method).
- **Primary screening:** Detect inhibition zones on test organisms (e.g., *E. coli*, *S. aureus*).
- **Secondary screening:** Quantitative assays to estimate yield and spectrum of activity.

Strain improvement methods:

1. **Mutagenesis:** UV light, chemical mutagens (NTG, EMS).
2. **Recombination:** Hybridization between high-yield mutants.
3. **Genetic engineering:** Insertion of overexpressed pathway genes.
4. **Protoplast fusion:** Combining desirable traits from two strains.

Step 2: Fermentation Process

A. Inoculum Development

- Sequence: **Stock culture → Seed culture → Production fermenter.**
- Ensures uniform and active cells for large-scale fermentation.

B. Types of Fermentation

1. **Submerged Fermentation (SmF):**
    - Microbes grow in a liquid nutrient medium.
    - Used for *Penicillium*, *Streptomyces*.
    - Easier oxygen control and scaling.
2. **Solid-State Fermentation (SSF):**
    - Growth on moist solid substrates (e.g., bran, bagasse).
    - Suitable for fungi or *Streptomyces* with low water requirement.

Step 3: Composition of Production Medium

Component	Function	Common Sources
Carbon source	Energy and carbon skeletons	Glucose, molasses, starch
Nitrogen source	Protein synthesis	Soy meal, yeast extract, ammonium salts
Precursors	Direct building blocks	Phenylacetic acid (Penicillin G)
Minerals	Enzyme cofactors	Mg^{2+}, Fe^{2+}, Zn^{2+}
Antifoams	Prevent foaming	Vegetable oils, silicone compounds

Step 4: Fermentation Conditions

Parameter	Control Range	Effect
pH	6.5 – 7.5	Optimal enzyme activity
Temperature	25–30°C	Balanced growth and yield
Aeration rate	0.5–1 vvm	Oxygen supply for aerobic microbes
Agitation	200–400 rpm	Homogeneous mixing
Duration	3–10 days	Depends on antibiotic type

Step 5: Product Recovery (Downstream Processing)

1. **Broth clarification:** Filtration or centrifugation to remove biomass.
2. **Extraction:** Solvent extraction (ethyl acetate, butanol) or resin adsorption.
3. **Concentration:** Evaporation or precipitation.
4. **Purification:** Crystallization, chromatography.
5. **Formulation:** Addition of stabilizers, drying, and packaging.

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6. Industrial Examples

Antibiotic	Producer	Process Notes
Penicillin G	*Penicillium chrysogenum*	Fed-batch SmF with lactose & phenylacetic acid
Streptomycin	*Streptomyces griseus*	Aerobic SmF, soybean meal medium

Antibiotic	Producer	Process Notes
Erythromycin	*Saccharopolyspora erythraea*	Complex carbon sources, pH 7.0
Tetracycline	*Streptomyces aureofaciens*	pH 6.8–7.2, long fermentation (7–10 days)
Cephalosporin C	*Acremonium chrysogenum*	Multistage aeration, submerged process

7. Regulation of Antibiotic Biosynthesis

- **Carbon catabolite repression:** High glucose inhibits secondary metabolism.
- **Nitrogen regulation:** Excess nitrogen suppresses antibiotic synthesis.
- **Phosphate repression:** High phosphate concentration reduces yield.
- **Autoregulators (γ-butyrolactones):** Small molecules that trigger antibiotic gene expression in *Streptomyces*.
- **Feedback control:** End-product inhibition of pathway enzymes.

8. Genetic Engineering in Antibiotic Production

Techniques:

1. **Gene cloning** – transferring biosynthetic genes into high-yield hosts.
2. **Pathway engineering** – overexpressing key enzymes, removing repressors.
3. **CRISPR/Cas9 editing** – precise modification of regulatory elements.
4. **Combinatorial biosynthesis** – mixing genes from different pathways to create new analogs ("unnatural natural products").

Example:
Engineered *Streptomyces coelicolor* strains producing hybrid macrolides.

9. Quality Control and Standardization

- **Potency testing:** Bioassays against standard strains.
- **Purity testing:** HPLC, TLC, or spectrophotometry.
- **Sterility checks:** Absence of microbial contaminants.
- **Stability studies:** Shelf-life determination under various conditions.

10. Challenges in Antibiotic Production

- Antibiotic resistance spreading globally.
- Diminishing returns from soil screening.
- High cost of R&D and regulatory hurdles.
- Environmental issues with waste broth disposal.