Mahidol University
Faculty of Science

SCBT431

ANTIBIOTICS PRODUCTION IN INDUSTRIES

Pharmaceutical Products 1

Asst.Prof. Dr.Adisak Romsang

Dept. Biotechnology, Fac. Science, Mahidol University
Room K610 Tel. 02201-5962 Email: adisak.rom@mahidol.ac.th

PHARMACEUTICAL PRODUCTS

- **Pharmaceutical products** - also known as **medicines or drugs** - are special preparations used in modern and traditional medicine essentially for the prevention and treatment of diseases, and protection of public health.
- Pharmaceutical products consist of **active ingredients**, which are combined with additional materials (excipients) selected to control dosage delivery, enhance performance and facilitate manufacture.
- Many pharmaceutical products are **proteins or polypeptides**. They can be obtained from nature by extraction or produced by microorganisms genetically modified, namely **recombinant proteins**. Separation and purification steps are usually the most difficult and may account for up to 60% of total cost (Lienqueo and Asenjo, 2000).

The Government Pharmaceutical Organization (GPO)

PHARMACEUTICAL PRODUCTS

Developed from herbs, food supplements, cosmetics and extracts

Herbal products

Tested active ingredients (food/abuse/pest)

Chemical products

Medical devices

plasters, gloves, elastic bandages and disinfectants

Biological products

Medicines

drugs for specific diseases

Equipment and medical tools

Medicine sets

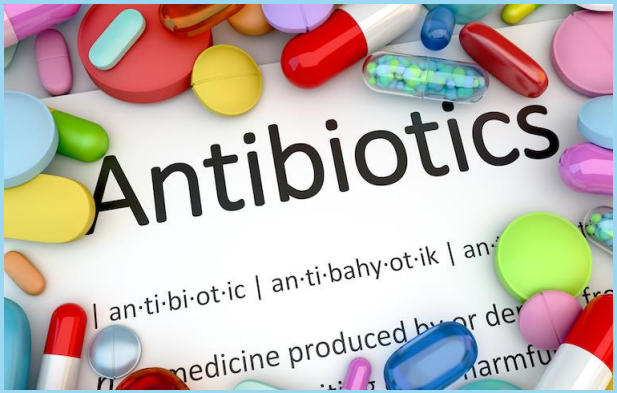
products convenient to purchase and carry

vaccines, serums and antitoxins

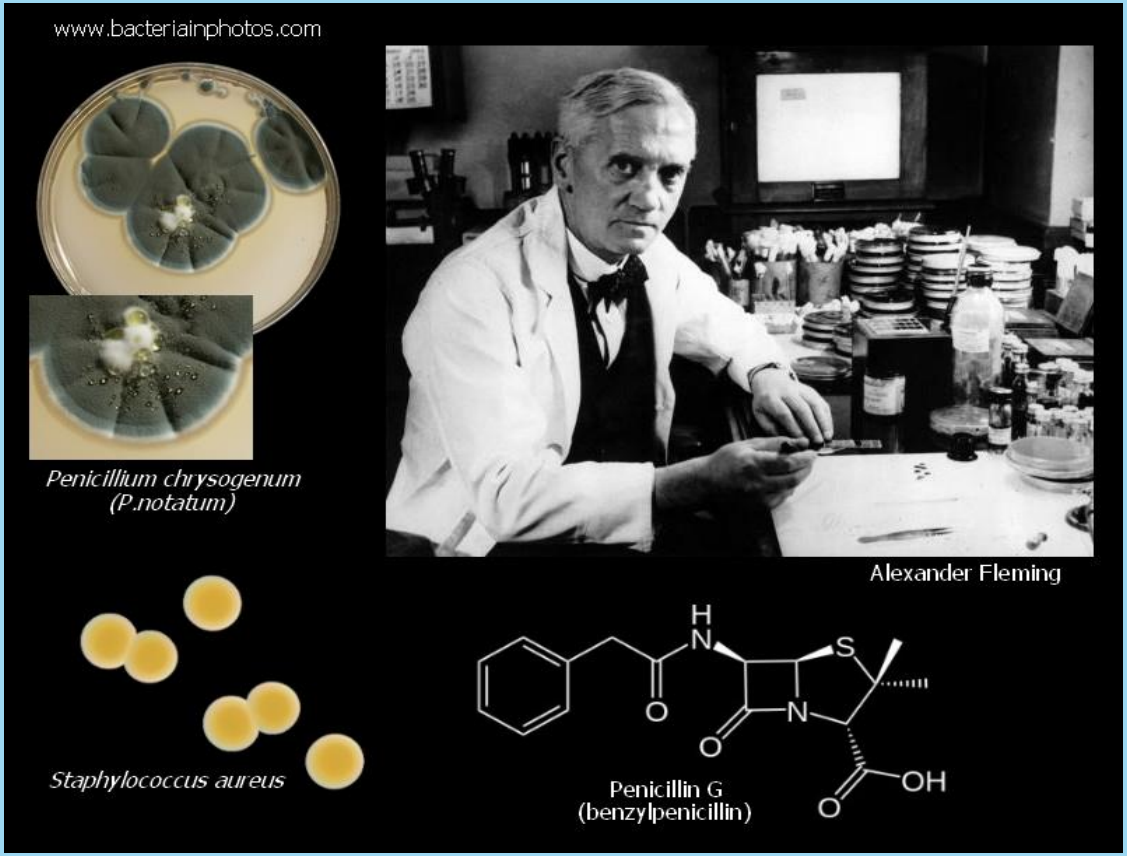
stents, knee prosthesis, blood pressure or blood glucose monitors

ANTIBIOTICS

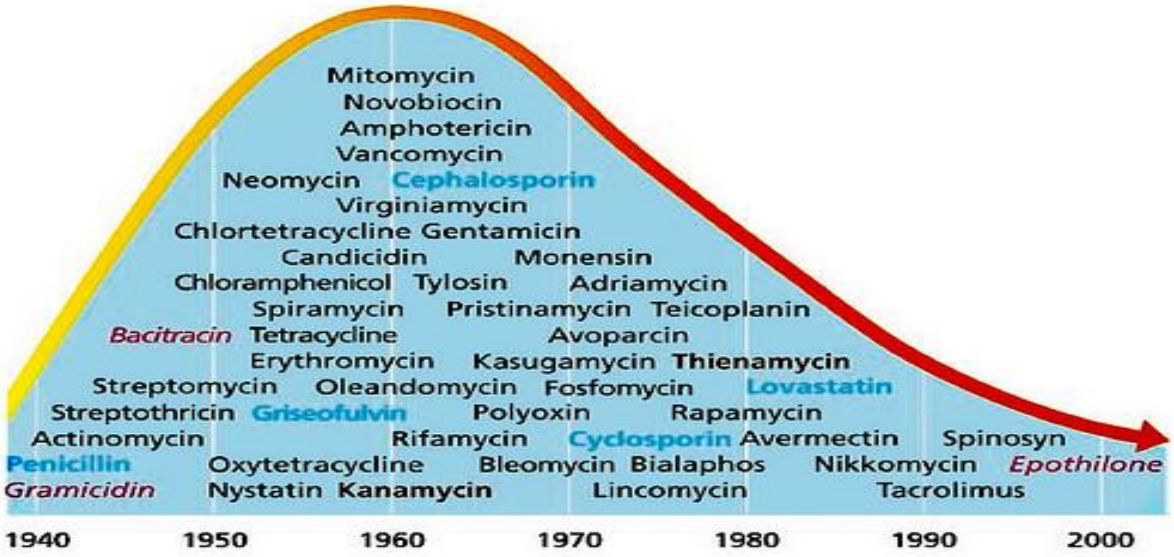
- An antibiotic is a drug that kills or slows the growth of bacteria, mostly is a secondary metabolites can produce during either tropophase or idiophase.
- Antibiotics are chemicals produced by or derived from microorganisms (i.e. bugs or germs such as bacteria and fungi).

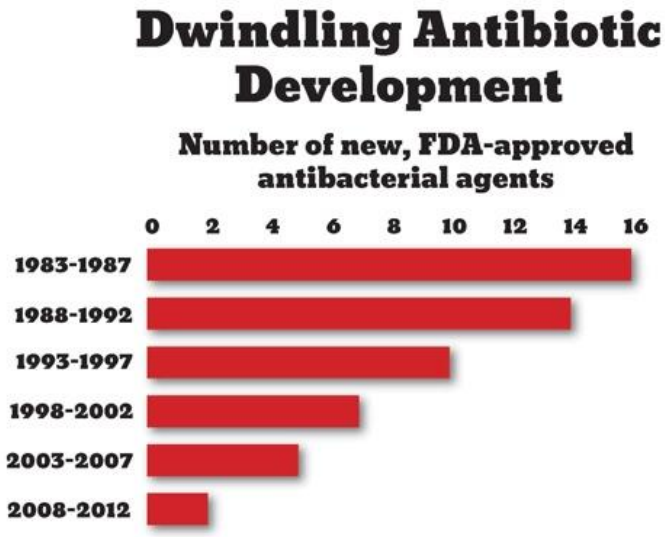


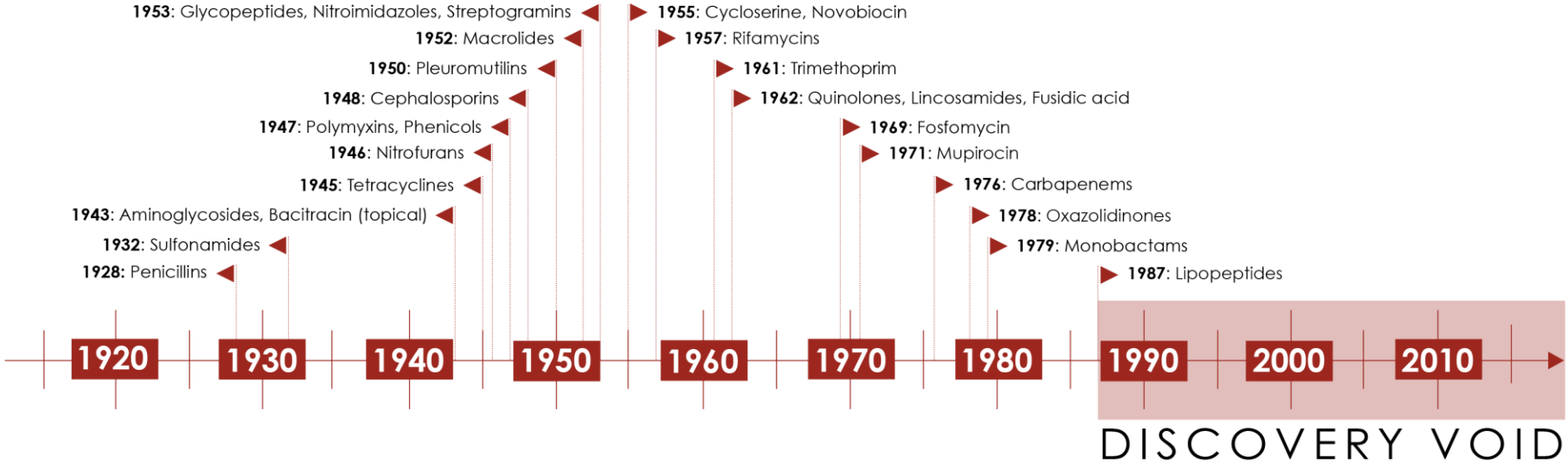
The first antibiotic was discovered by Alexander Fleming (Nobel lecture, December 11, 1945) in 1929 in a significant breakthrough for medical science.
Staphylococcus aureus played an important role in discovery of **penicillin G** (benzylpenicillin) produced by *Penicillium chrysogenum* (*P. notatum*)



ANTIBIOTICS DISCOVERY



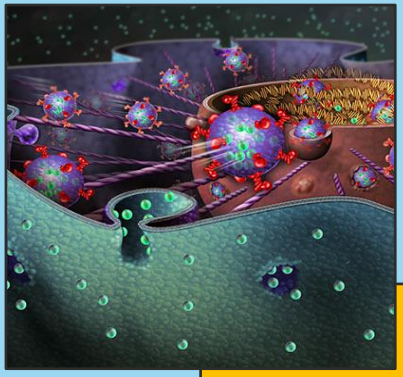




ROLES OF ANTIBIOTICS

Exogenous roles

- Protect against other competing organisms
- Regulation of commensalism or cohabitation
- Protection of physiochemical factors
- Detoxification of trace elements



Endogenous roles

- Signals for morphogenesis
- Signals for mating
- Detoxification of metabolism
- Supply the building materials of cell wall
- Reserve materials that not access in other organisms

NOTE: Antibiotics do not kill viruses -not effective in treating viral infections

MODE OF ACTION OF ANTIBIOTICS

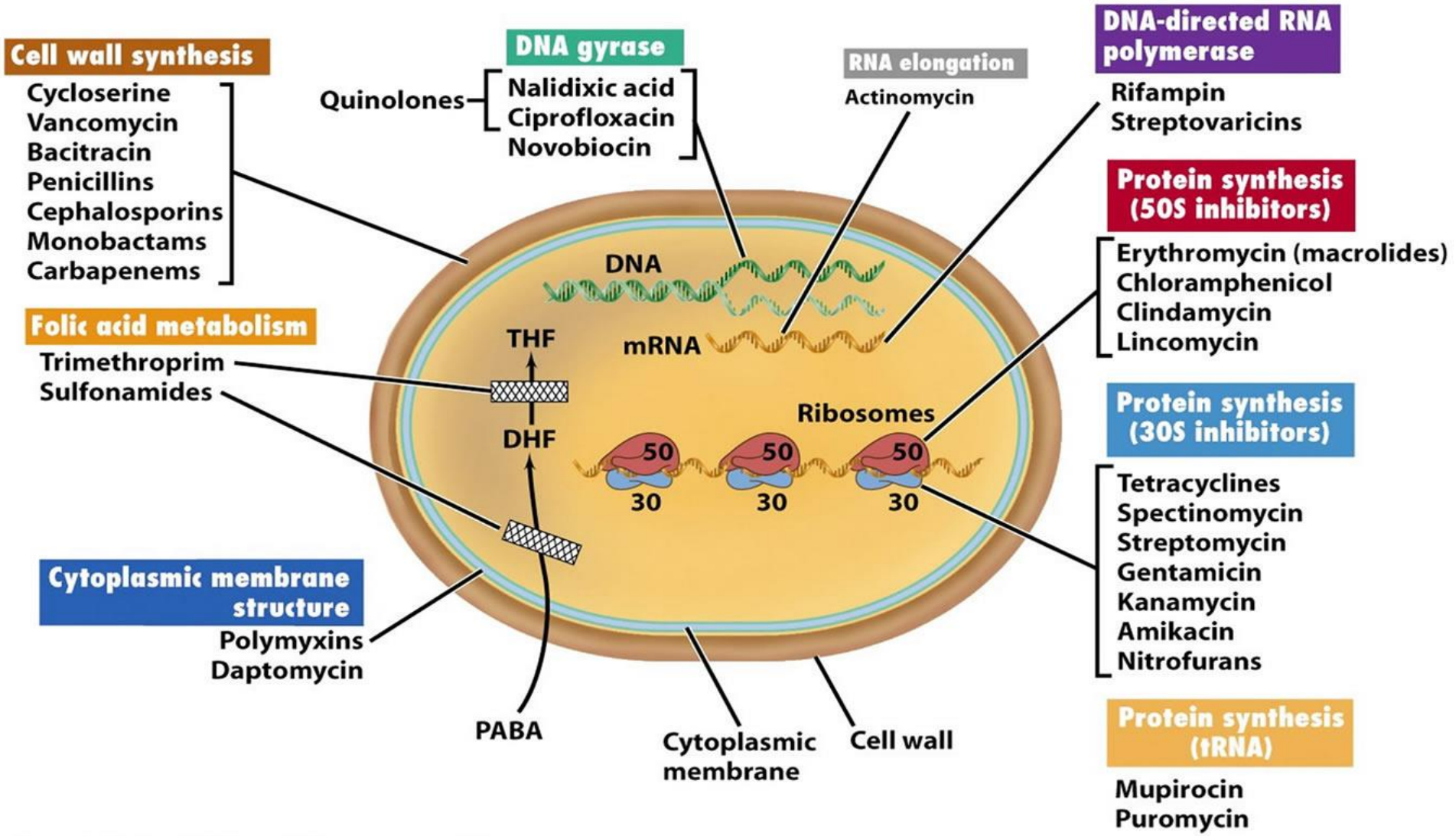


Figure 20-14 Brock Biology of Microorganisms 11/e

ANTIBIOTICS STRUCTURES

Chemical Classification (Public Loves GOOD Quality BATSMAN)

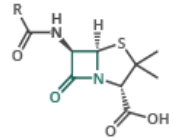
- **P**olypeptides- Polymyxin, Colistin, Bacitracin
- **P**oyene antibiotics- Nystatin, Amphotericin-B, Hamycin
- **L**incosamide- Lincomycin, Clindamycin
- **G**lycopeptides- Vancomycin, Teicoplanin
- **O**xazolidinone- Linezolid
- **O**thers----------Riampicin, Griseofulvin, etc
- **D**iaminopyrimidines- Trimethoprim, Pyrimethamine
- **Q**uinolones- Nalidixic acid, ciprofloxacin
- **B**eta-lactam- Penicillins, Cephalosporins, Monobactams, Carbapenems
- **A**minoglycosides- Streptomycin, Gentamycin
- **T**etracyclines- Oxytetracycline, Doxycycline
- **S**ulphonamides- Sulfadiazine, Sulfamethoxazole,
- **M**acrolides- Erythromycin, Clarithromycin
- **A**zoles- Fluconazole, Clotrimazole
- **N**itroimidazoles- Metronidazole, Tinidazole
- **N**icotinic acid derivatives- Isoniazide, Pyrizinamide, Ethionamide
- **N**itrobenzene derivaties- Chloramphenicol
- **N**itrofuran derivatives- Nitrofurantoin, Furazolidone

DIFFERENT CLASSES OF ANTIBIOTICS - AN OVERVIEW

Key: COMMONLY ACT AS BACTERIOSTATIC AGENTS, RESTRICTING GROWTH & REPRODUCTION — COMMONLY ACT AS BACTERICIDAL AGENTS, CAUSING BACTERIAL CELL DEATH

ß-LACTAMS

MOST WIDELY USED ANTIBIOTICS IN THE NHS



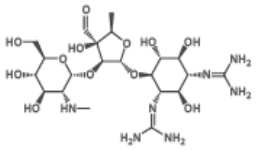
All contain a beta-lactam ring

EXAMPLES
Penicillins (shown) such as amoxicillin and flucloxacillin; *Cephalosporins* such as cefalexin.

MODE OF ACTION
Inhibit bacteria cell wall biosynthesis.

AMINOGLYCOSIDES

FAMILY OF OVER 20 ANTIBIOTICS



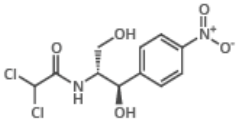
All contain aminosugar substructures

EXAMPLES
Streptomycin (shown), neomycin, kanamycin, paromomycin.

MODE OF ACTION
Inhibit the synthesis of proteins by bacteria, leading to cell death.

CHLORAMPHENICOL

COMMONLY USED IN LOW INCOME COUNTRIES



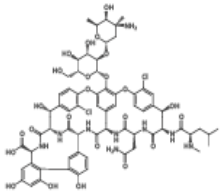
Distinct individual compound

MODE OF ACTION
Inhibits synthesis of proteins, preventing growth.

No longer a first line drug in any developed nation (except for conjunctivitis) due to increased resistance and worries about safety.

GLYCOPEPTIDES

COMMON 'DRUGS OF LAST RESORT'



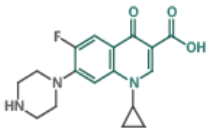
Consist of carbohydrate linked to a peptide formed of amino acids

EXAMPLES
Vancomycin (shown), teicoplanin.

MODE OF ACTION
Inhibit bacteria cell wall biosynthesis.

QUINOLONES

RESISTANCE EVOLVES RAPIDLY



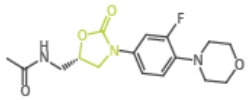
All contain fused aromatic rings with a carboxylic acid group attached

EXAMPLES
Ciprofloxacin (shown), levofloxacin, trovafloxacin.

MODE OF ACTION
Interfere with bacteria DNA replication and transcription.

OXAZOLIDINONES

POTENT ANTIBIOTICS COMMONLY USED AS 'DRUGS OF LAST RESORT'



All contain 2-oxazolidone somewhere in their structure

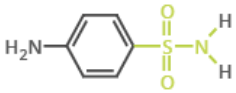
EXAMPLES
Linezolid (shown), posizolid, tedizolid, cycloserine.

MODE OF ACTION
Inhibit synthesis of proteins by bacteria, preventing growth.

DISCOVERY — 1930 — 1940 — 1950 — 1960 — 1970 — 1980

SULFONAMIDES

FIRST COMMERCIAL ANTIBIOTICS WERE SULFONAMIDES



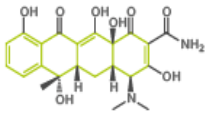
All contain the sulfonamide group

EXAMPLES
Prontosil, sulfanilamide (shown), sulfadiazine, sulfisoxazole.

MODE OF ACTION
Do not kill bacteria but prevent their growth and multiplication. Cause allergic reactions in some patients.

TETRACYCLINES

BECOMING LESS POPULAR DUE TO DEVELOPMENT OF RESISTANCE



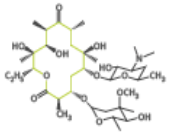
All contain 4 adjacent cyclic hydrocarbon rings

EXAMPLES
Tetracycline (shown), doxycycline, limecycline, oxytetracycline.

MODE OF ACTION
Inhibit synthesis of proteins by bacteria, preventing growth.

MACROLIDES

SECOND MOST PRESCRIBED ANTIBIOTICS IN THE NHS



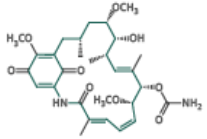
All contain a 14-, 15-, or 16-membered macrolide ring

EXAMPLES
Erythromycin (shown), clarithromycin, azithromycin.

MODE OF ACTION
Inhibit protein synthesis by bacteria, occasionally leading to cell death.

ANSAMYCINS

CAN ALSO DEMONSTRATE ANTIVIRAL ACTIVITY



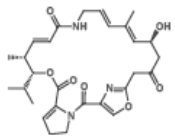
All contain an aromatic ring bridged by an aliphatic chain.

EXAMPLES
Geldanamycin (shown), rifamycin, naphthomycin.

MODE OF ACTION
Inhibit the synthesis of RNA by bacteria, leading to cell death.

STREPTOGRAMINS

TWO GROUPS OF ANTIBIOTICS THAT ACT SYNERGISTICALLY



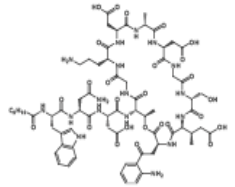
Combination of two structurally differing compounds, from groups denoted A & B

EXAMPLES
Pristinamycin IIA (shown), Pristinamycin IA.

MODE OF ACTION
Inhibit the synthesis of proteins by bacteria, leading to cell death.

LIPOPEPTIDES

INSTANCES OF RESISTANCE RARE



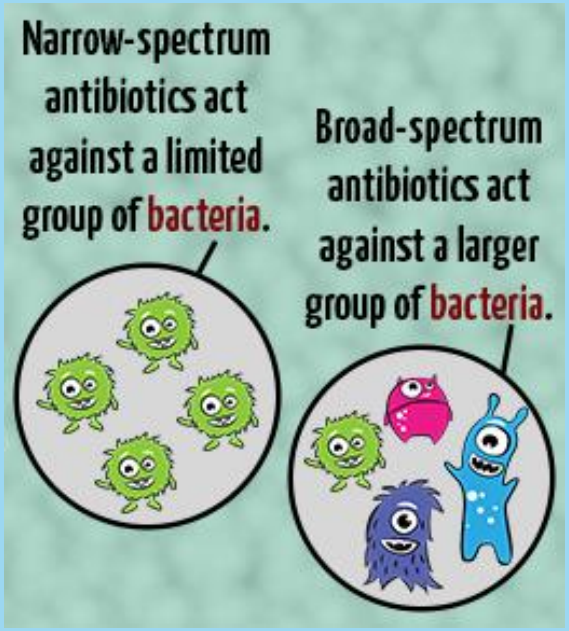
All contain a lipid bonded to a peptide

EXAMPLES
Daptomycin (shown), surfactin.

MODE OF ACTION
Disrupt multiple cell membrane functions, leading to cell death.

CHEMOTHERAPEUTIC SPECTRA

- **Narrow-spectrum Antibiotics**: Act on a single / limited group of micro-organisms; e.g., isoniazid given for mycobacterium
- **Extended-spectrum Antibiotics**: Effective against Gram-positive organisms and a significant number of Gram-negative organisms; e.g., ampicillin
- **Broad-spectrum Antibiotics**: Effective against a wide variety of microbial species; e.g., tetracycline & chloramphenicol.
- Can alter the nature of intestinal flora = super infection



Broad spectrum antibiotics :

1. Amoxicillin
2. Tetracycline
3. Cephalosporin
4. Chloramphenicol
5. Erythromycin

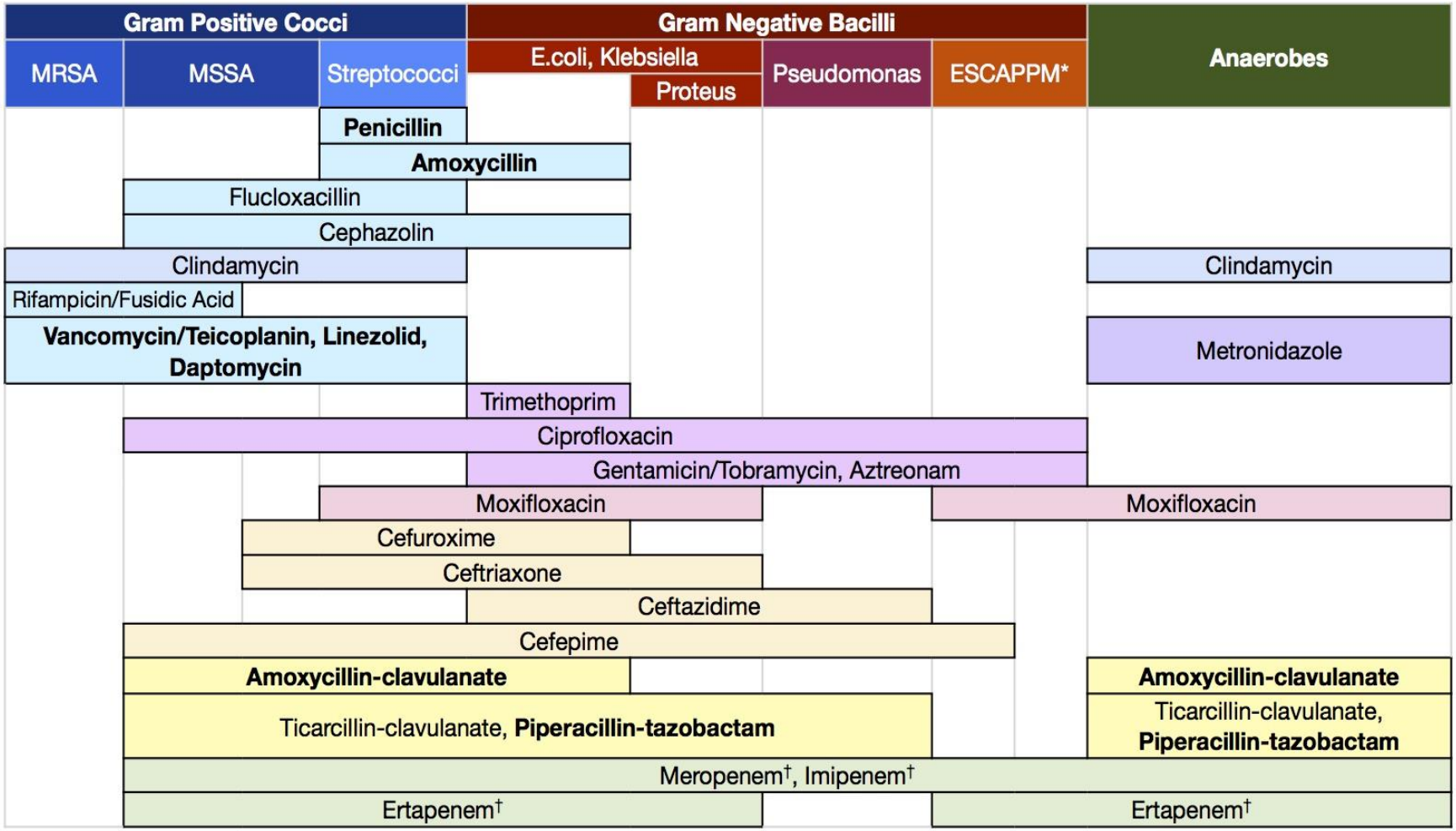
Short spectrum antibiotics:

1. Penicillin –G
2. Cloxacillin
3. Vancomycin
4. Bacitracin
5. Fluxacillin

piperacillin+tazobactam **BROAD**	metronidazole **NARROW**
cephazolin **NARROW**	moxifloxacin **BROAD**
benzylpenicillin **NARROW**	meropenem **BROAD**
ceftriaxone **BROAD**	trimethoprim **NARROW**

Antibiotic Sensitivity Overview

(taken from the wellingtonicu.com drug manual)



Antibiotics in **bold** also cover Enterococcus Faecalis. For simplicity, atypical organisms are not shown.

ESBL-producing organisms are **not** susceptible to most antibiotics containing a beta-lactam ring; carbapenems† are the usual agent of choice.

*ESCAPPM organisms are Enterobacter spp., Serratia spp., Citrobacter freundii, Aeromonas spp., Proteus spp., Providencia spp. & Morganella morganii.

COMBINATIONS OF DRUGS

Advantages

- Synergism; the combination is more effective than either drug used separately; β-lactams and aminoglycosides Infections of unknown origin

Disadvantages

- Bacteriostatic (tetracycline) drugs may interfere with bactericidal (penicillin and cephalosporin) drugs

Selection of Antibacterial Agent

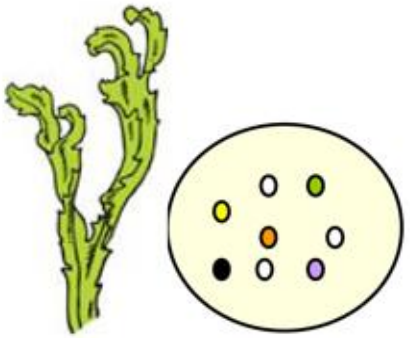
- Empiric therapy - prior to identification of organism – critically ill patients
- Organism's susceptibility to the antibiotic
- Patient factors - immune system, renal/hepatic function
- Effect of site of infection on therapy –blood brain barrier
- Safety of the agent
- Cost of therapy

Mahidol University
Faculty of Science

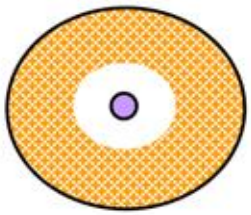
ANTIBIOTICS PRODUCTION

…Discovery and Development…

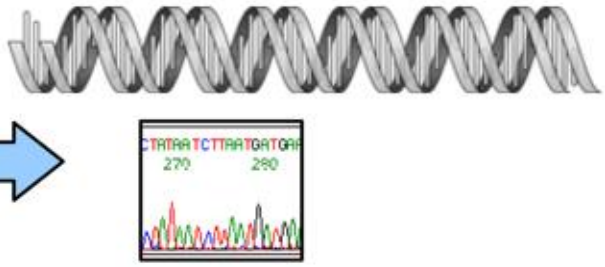
a long, risky road



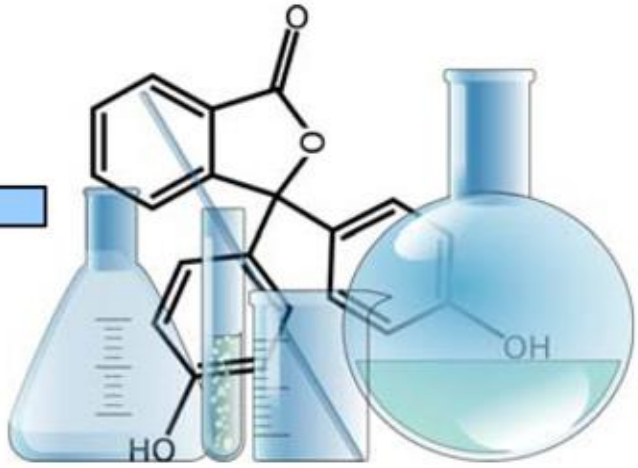
Isolation of microorganisms from the environment



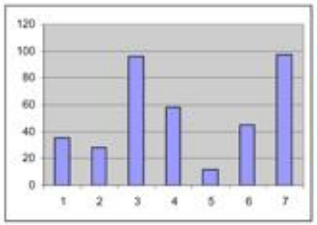
Assessment of the isolates for AM activity



Identification of isolates/De-replication at the producer level

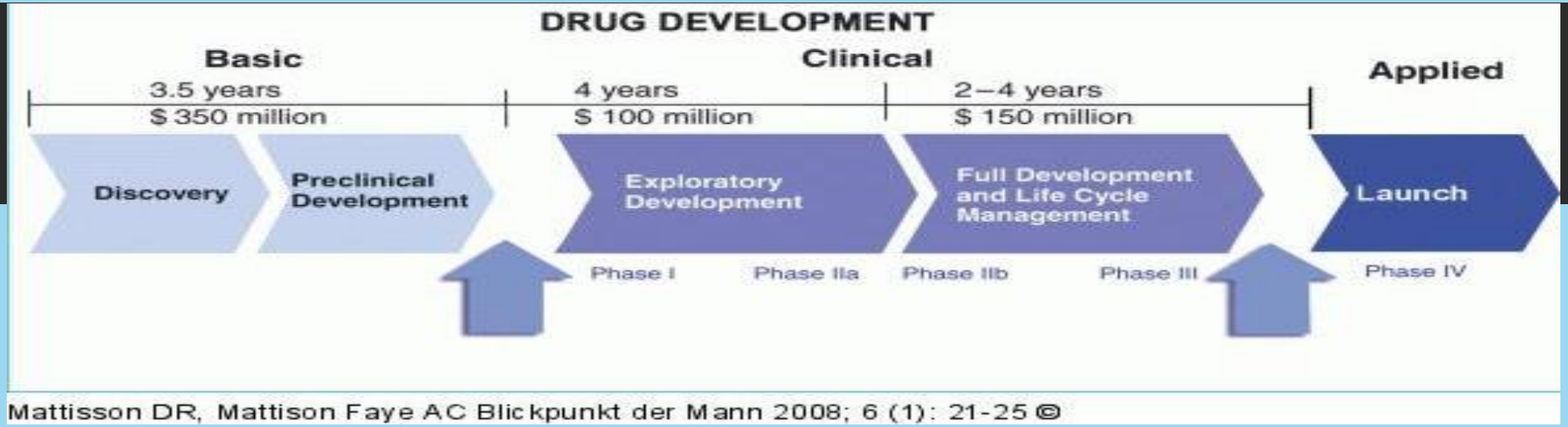


Extraction and identification of bioactive compounds/ De-replication at the compound level

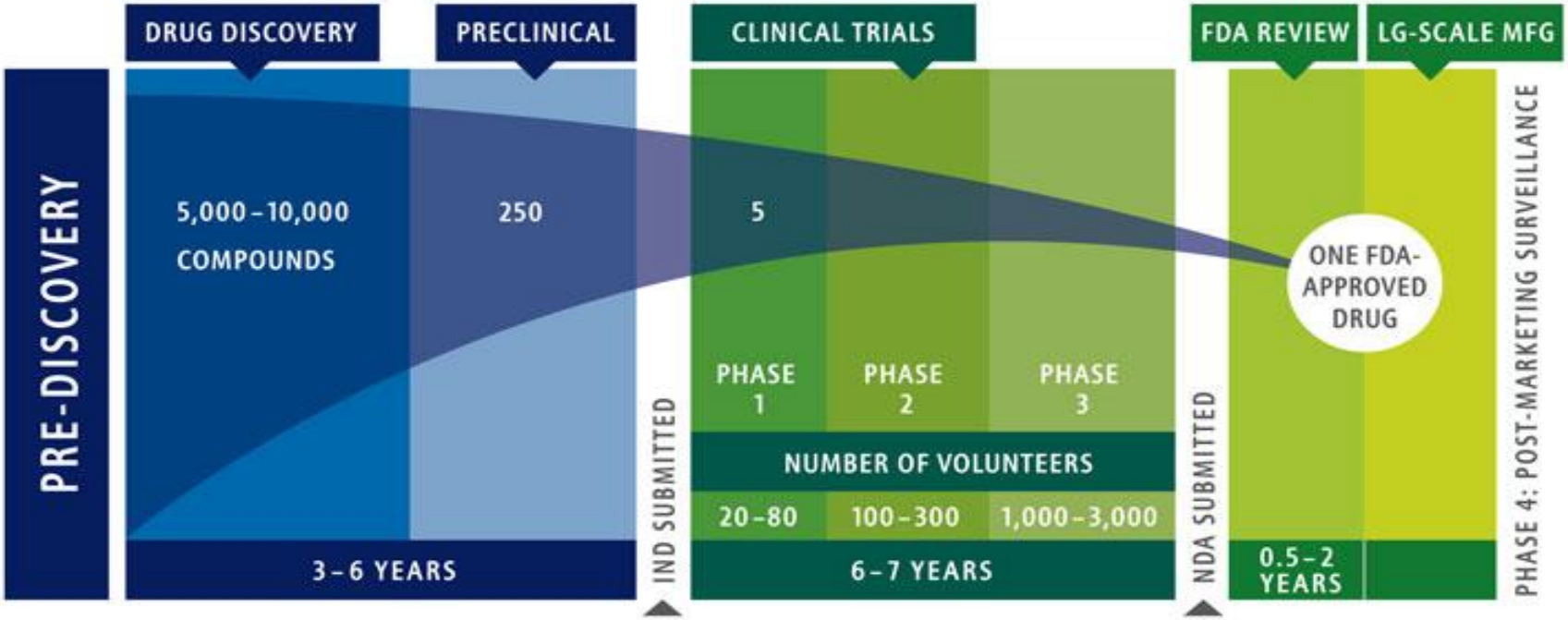


Production optimization

In vivo evaluation, clinical trials and commercialization



Mattisson DR, Mattison Faye AC Blickpunkt der Mann 2008; 6 (1): 21-25 ©



Source: Pharmaceutical Research and Manufacturers of America

ANTIBIOTICS

- Currently 8000 antibiotics are known
- Each year around 300 new antibiotically active compounds are detected, of which 30-35% are antibiotics
- Only 123 antibiotics of bacterial origin are produced by fermentation
- Only chloramphenicol, phosphonomycin and pyrrolnitrin are produced synthetically
- Significance for the strain is unclear

USES OF ANTIBIOTICS

- Antitumor antibiotics
- Antibiotics for plant pathology
- Antibiotics as food preservatives
- Antibiotics used as animal growth promoters and in veterinary medicine
- Antibiotics as tools in biochemistry and molecular biology

ANTIBIOTIC PRODUCERS

- Over 5000 antibiotics have been identified from the culture of Gram positive, Gram negative organisms and filamentous fungi, but only 100 antibiotics have been commercially used to treat human, animal and plant disease.
- The genus ***Streptomycete*** is responsible for more than 60% of known antibiotics. While further 15% are made by number of related Actinomycete, Micromonospora, Actinomadura, Streptoverticillium and Thermoactinomycetes.

1. *Streptomyces* spp.: produce chloramphenicol, erythromycin, kanamycin, neomycin, nystatin, rifampin, streptomycin, tetracyclines, vancomycin
2. *Micromonospora* spp.: produce gentamicin
3. *Bacillus* spp.: produce bacitracin, polymxins
4. Fungi
   - ❑ *Penicillium griseofulvum*: produce griseofulvin
   - ❑ *Cephalosporium* spp.: produce cephalosporins

ANTIBIOTIC PRODUCERS

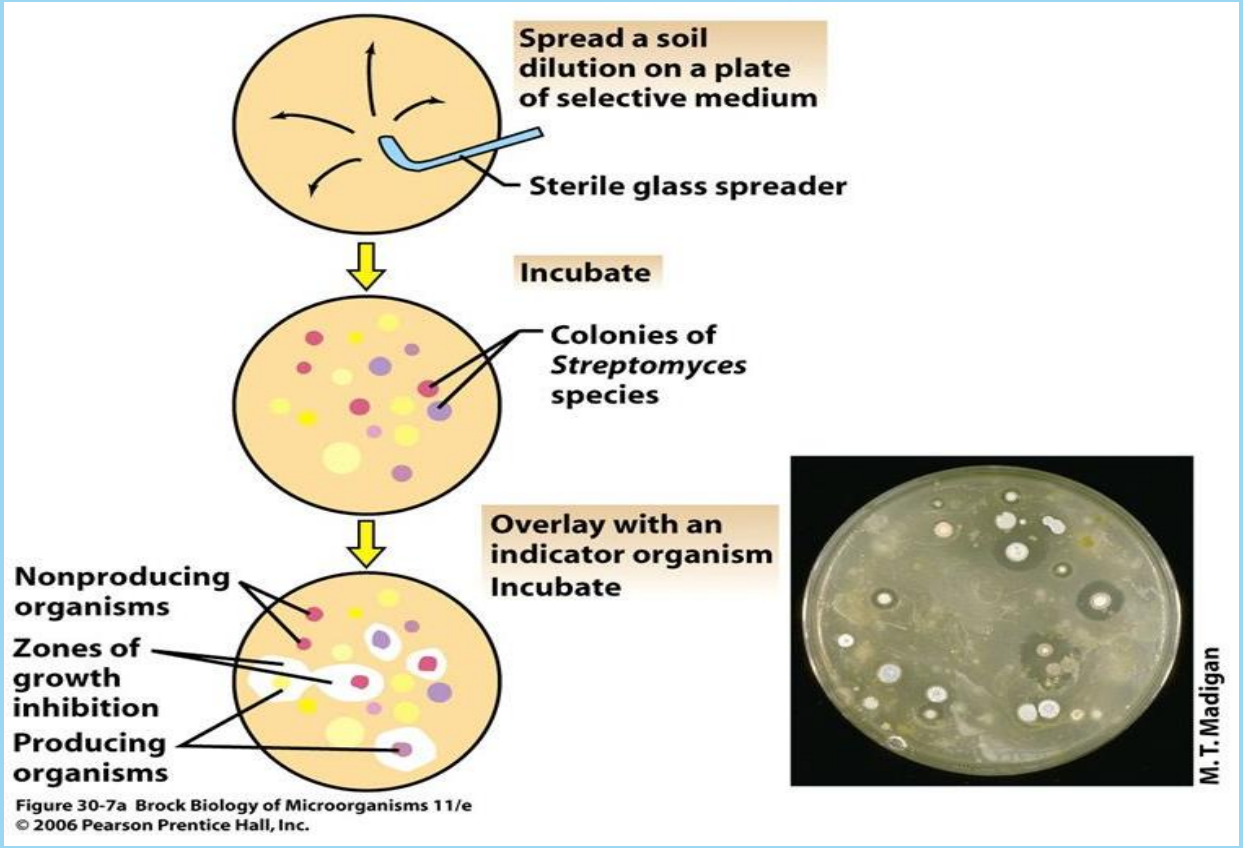
Antibiotic	Producer organism	Activity	Site or mode of action
Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria	Wall synthesis
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum	Wall synthesis
Griseofulvin	*Penicillium griseofulvum*	Dermatophytic fungi	Microtubules
Bacitracin	*Bacillus subtilis*	Gram-positive bacteria	Wall synthesis
Polymyxin B	*Bacillus polymyxa*	Gram-negative bacteria	Cell membrane
Amphotericin B	*Streptomyces nodosus*	Fungi	Cell membrane
Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria	Protein synthesis
Neomycin	*Streptomyces fradiae*	Broad spectrum	Protein synthesis
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria	Protein synthesis
Tetracycline	*Streptomyces rimosus*	Broad spectrum	Protein synthesis
Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria	Protein synthesis
Gentamicin	*Micromonospora purpurea*	Broad spectrum	Protein synthesis
Rifamycin	*Streptomyces mediterranei*	Tuberculosis	Protein synthesis

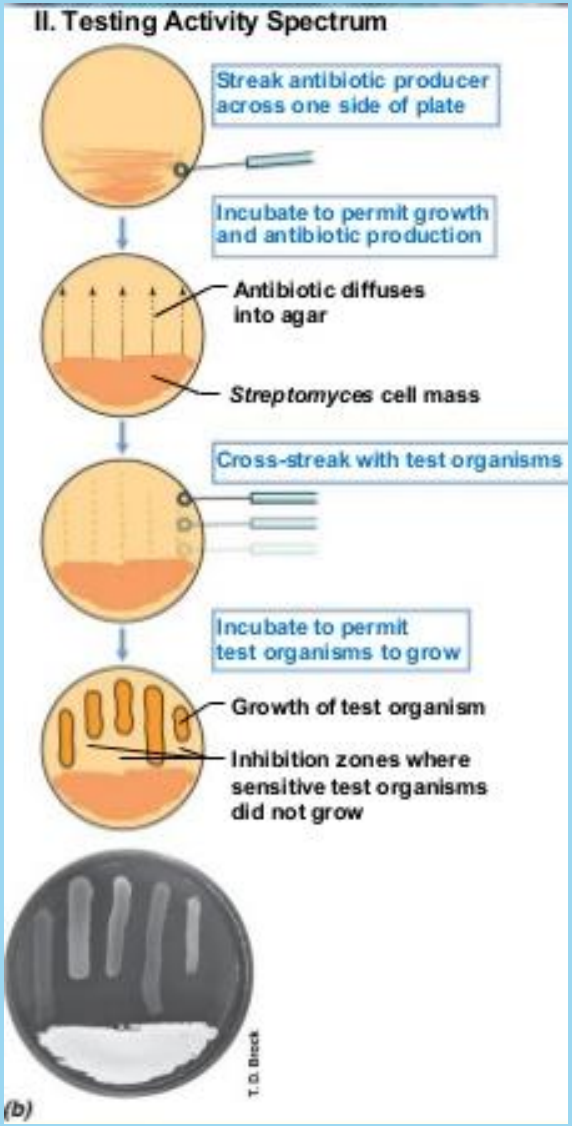
STRAIN SELECTION

- **Screening and selection method**
  - Sample preparation → serial dilution → extraction & isolation
  - Sterile reagents and materials, selective dissolve i.e. Tween
  - Specific medium for selecting a certain microorganisms
- **Antimicrobial sensitivity testing**
  - Zone of inhibition method
  - Disc diffusion method
  - Plate sensitivity assay
  - MIC and MBC determination
- **Test for specific biological activity**
  - Physiological function test
  - Mode and mechanism of inhibition
  - Molecular identification and analysis

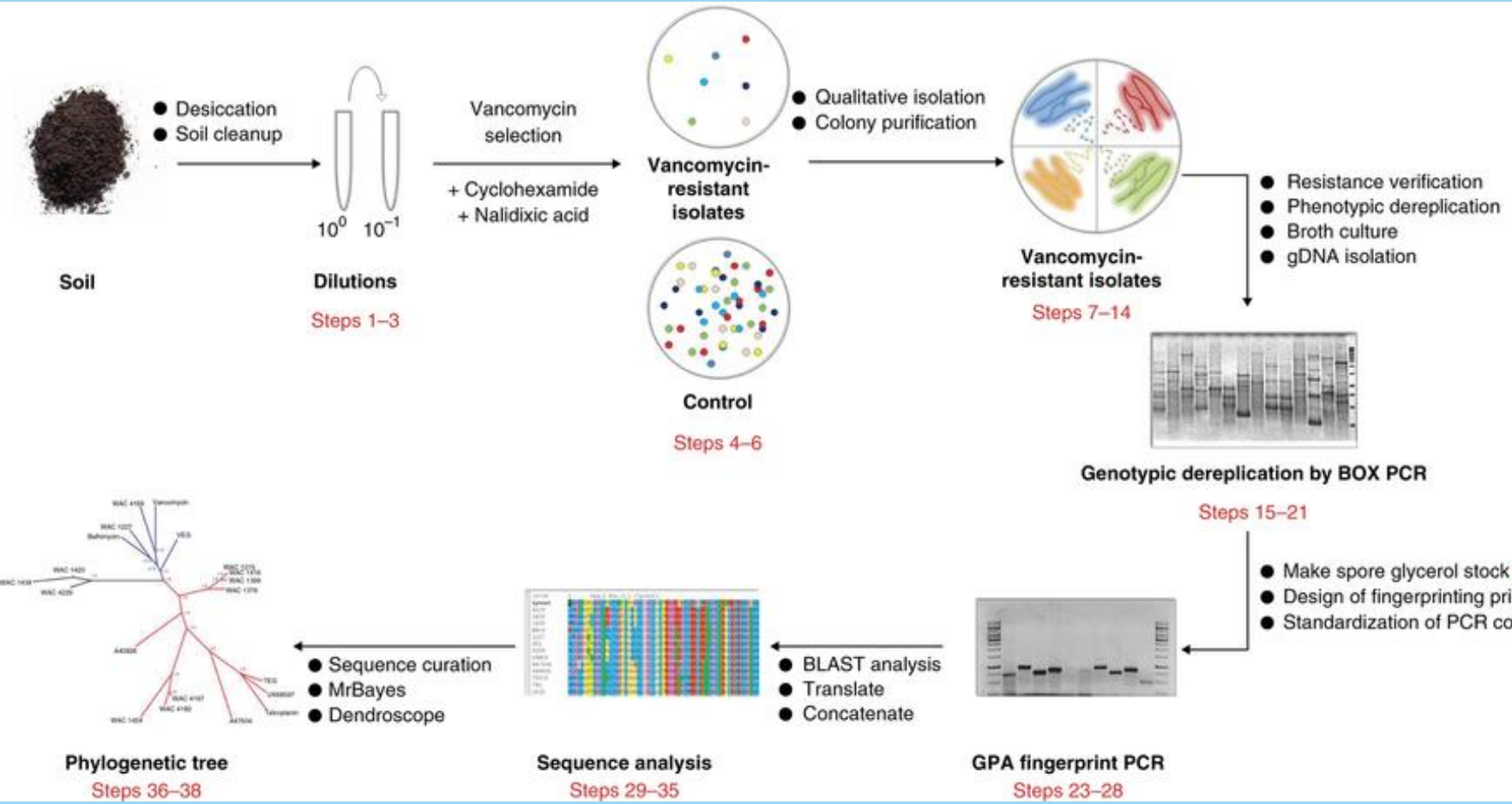
SCREENING AND SELECTION

- **Screening and selection method**
  - Sample preparation → serial dilution → extraction & isolation
  - Sterile reagents and materials, selective dissolve i.e. Tween
  - Specific medium for selecting a certain microorganisms

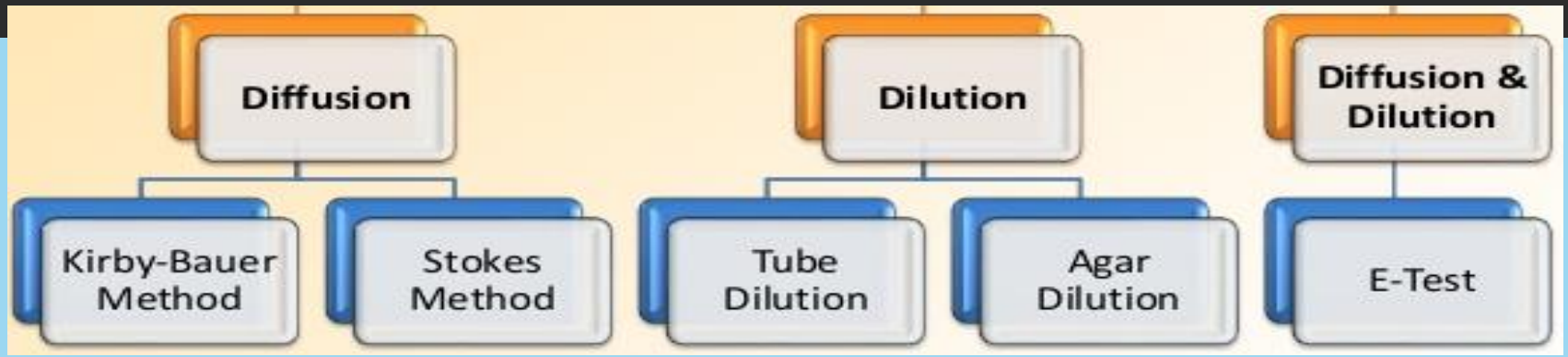




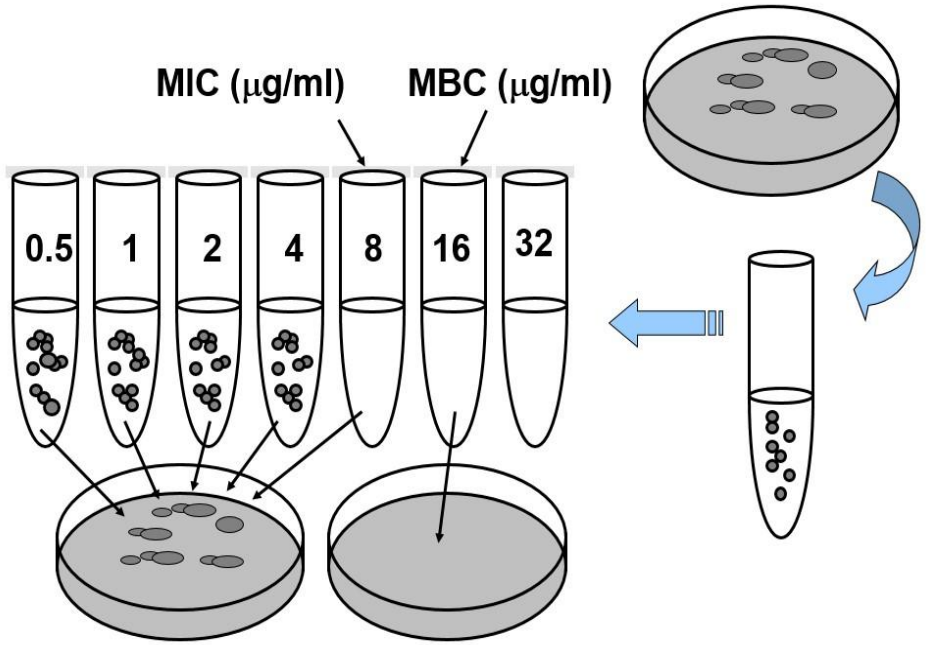
SCREENING AND SELECTION



ANTIMICROBIAL SENSITIVITY ASSAY



Tube broth dilution method



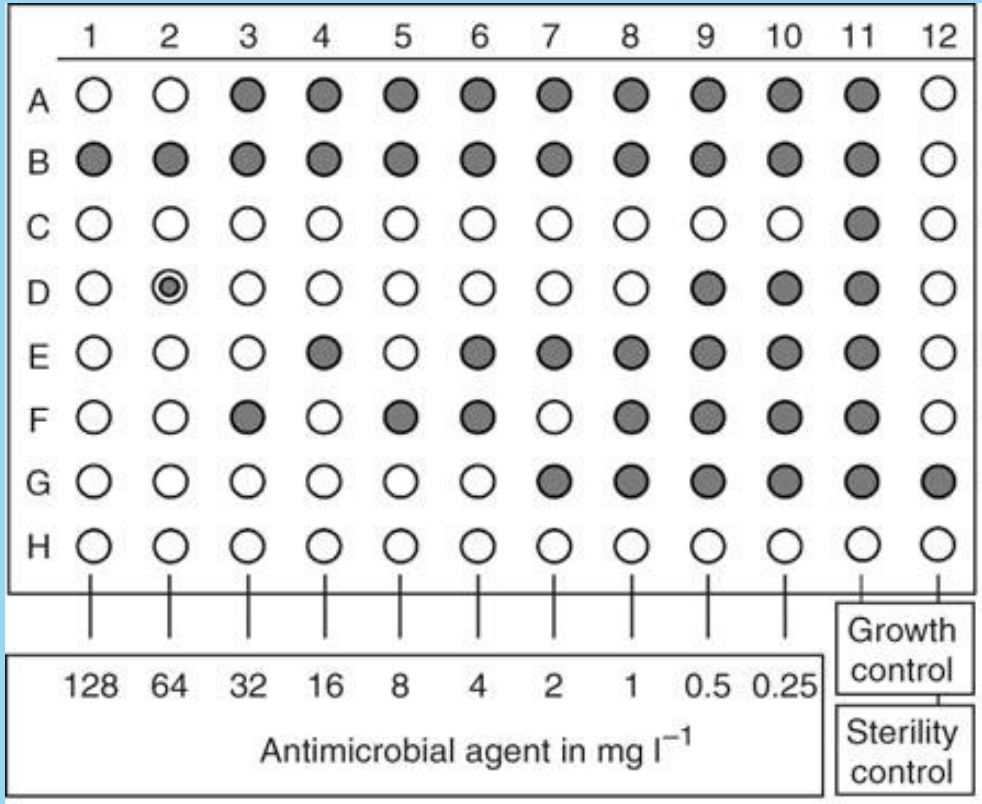
MIC:

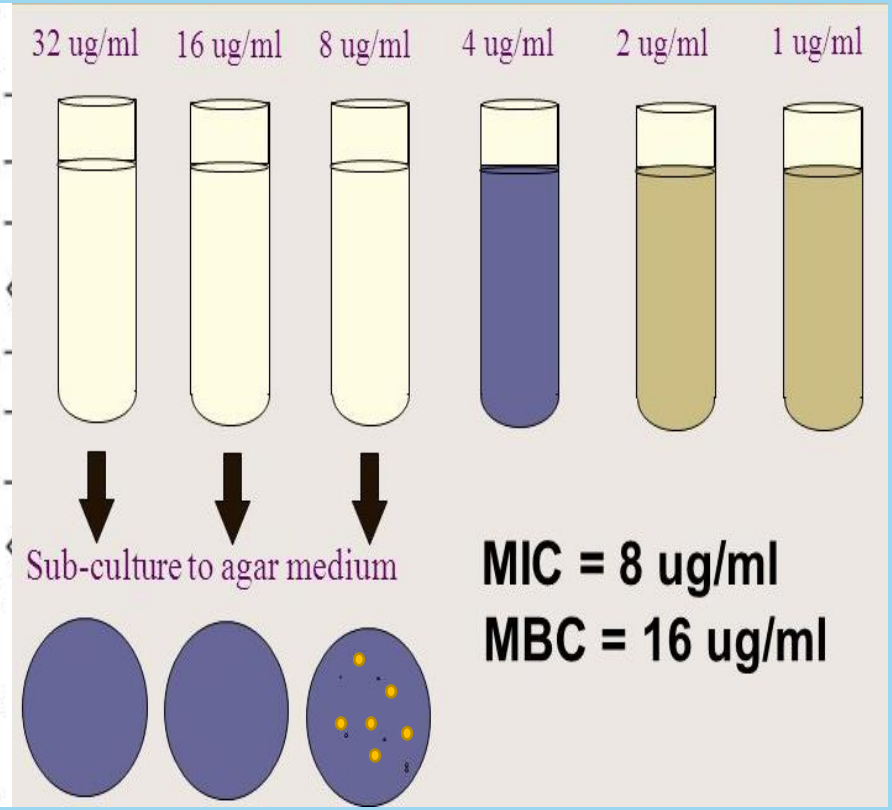
It is the lowest concentration of the antimicrobial agent that inhibits the growth of the test organism but not necessarily kills it.

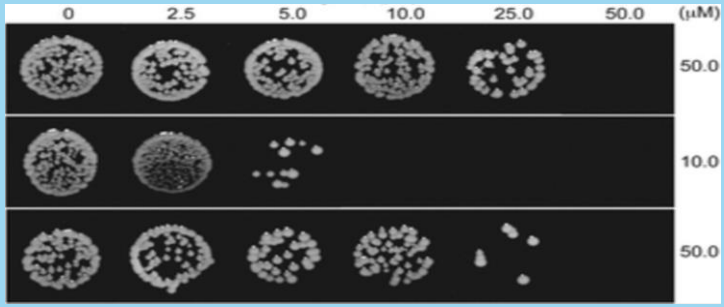
MBC (minimum bactericidal conc.):

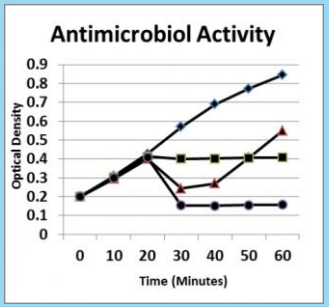
It is the lowest concentration of the antimicrobial agent that kills the test organism.

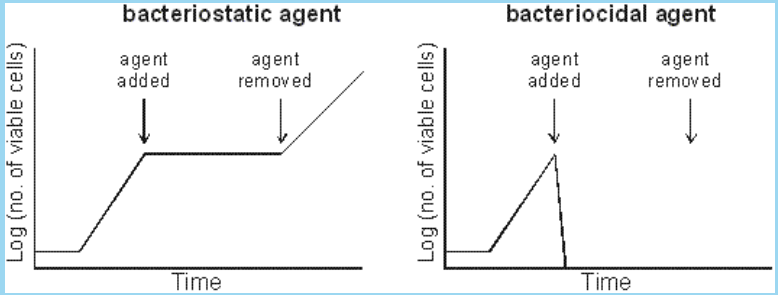
DETERMINATION OF MIC AND MBC





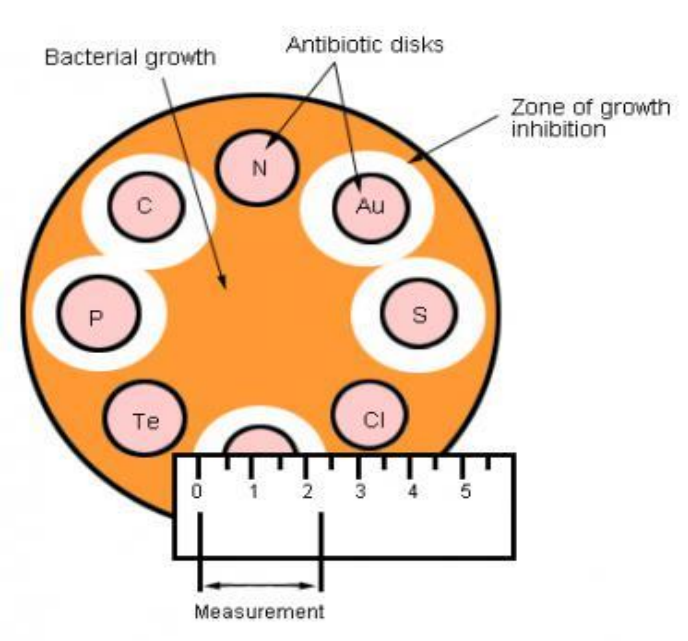


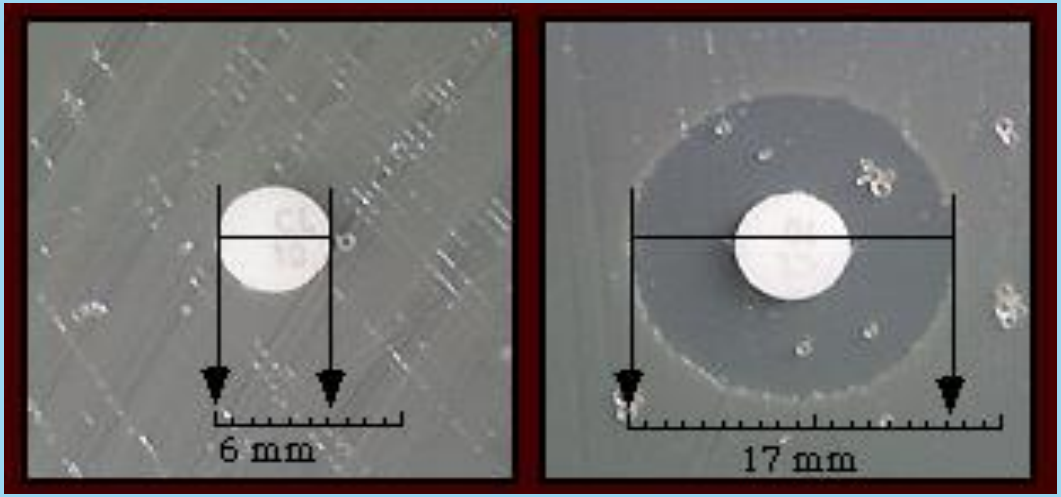


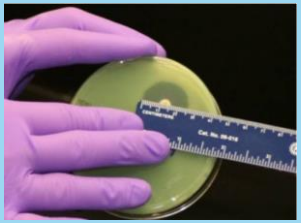


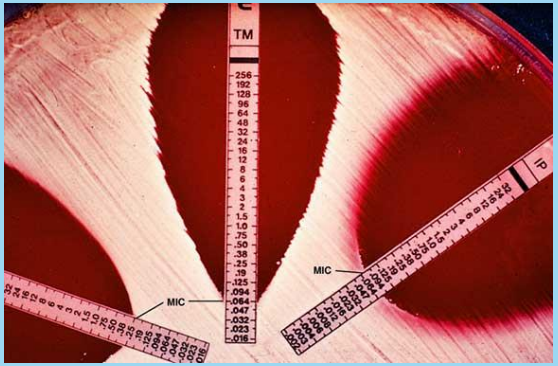
DISC DIFFUSION & E-TEST

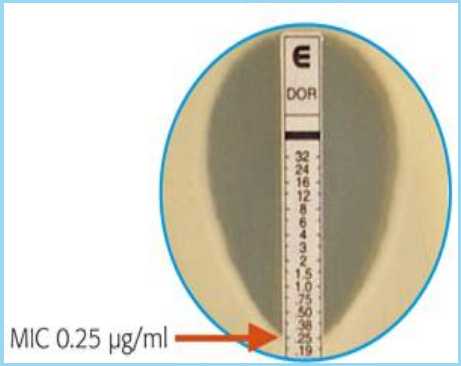
Disc diffusion method











STRAIN IMPROVEMENT

Strain improvement: Science and technology of manipulating and improving microbial strains, in order to enhance their metabolic capacities for biotechnological applications

- Rapid growth
- Genetic stability
- Non-toxicity to humans
- Large cell size, for easy removal from the culture fluid
- Ability to use cheaper substrates
- Elimination of the compounds that interfere with downstream process
- Increase productivity
- To improve the use of carbon and nitrogen sources
- Reduction of cultivation cost
  - lower price in nutrition
  - lower requirement for oxygen
- Production of additional compounds to inhibit contaminant microbes

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 591349, 16 pages
http://dx.doi.org/10.1155/2015/591349

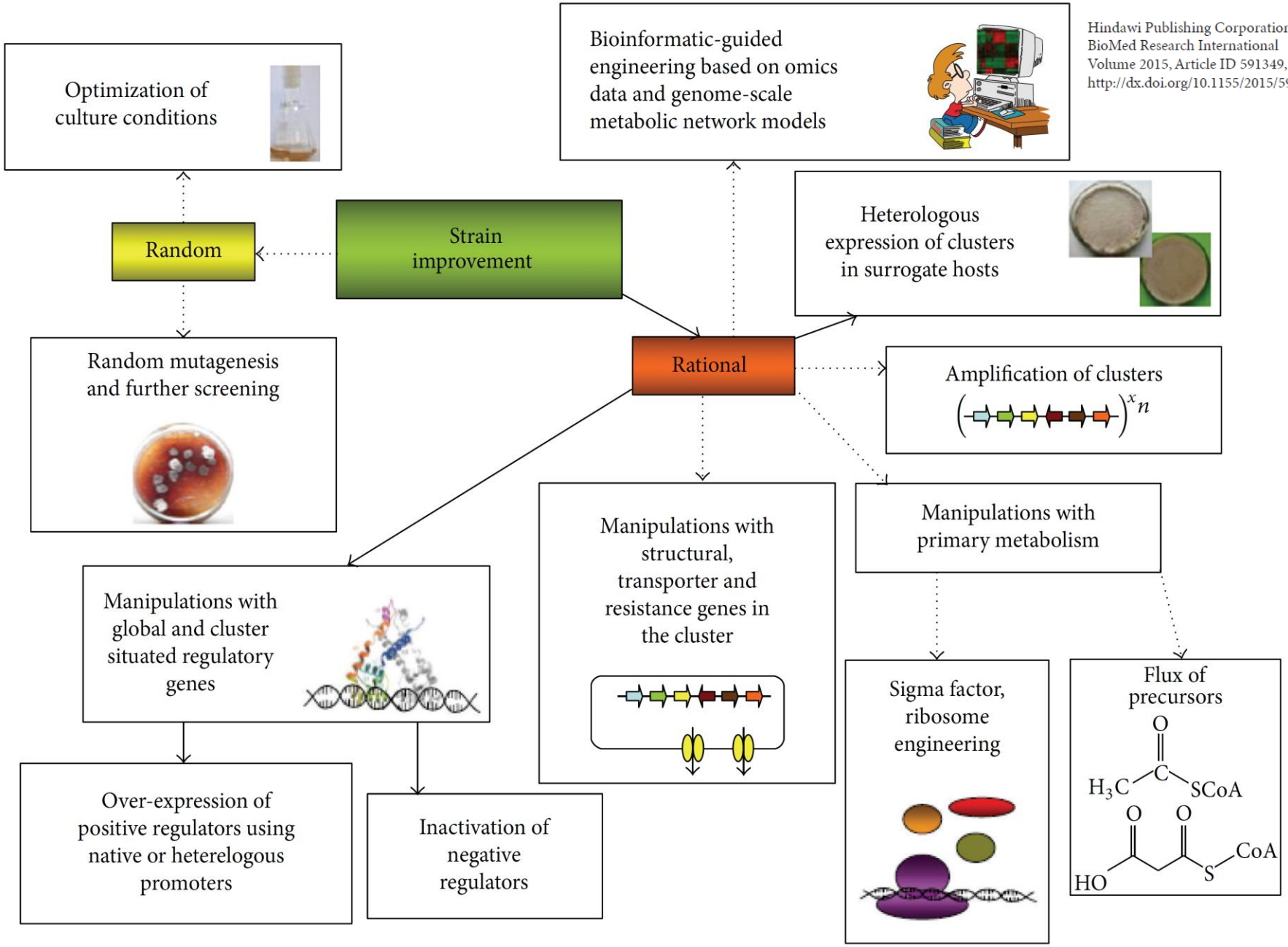


FIGURE 3: Approaches used for improving secondary metabolite production in actinobacteria. Solid arrows indicate strategies described in this review; dash-dotted arrows denote other strategies that are used.

STRAIN IMPROVEMENT

Regulation of antibiotics production

- **Feedback control**: primary metabolite inhibition or repression i.e. leucine as precursor/correlated to penicillin production blocking of leucine = inhibiting production of penicillin
- **Production of secondary metabolites**: controlled by structural gene, regulatory gene, resistance gene, permeability gene
  - Induction of enzyme i.e. 1. Factor A induces streptomycin production 2. methionine induces cephalosporin production
  - End-product regulation i.e. penicillin, chloramphenicol, streptomycin
  - Catabolite regulation i.e. Glucose (C-source), Ammonia (N-source)
  - Phosphate regulation: require for growth but inhibit 2^{nd} metabolites delay idiophase → repress phosphatase → shift in carbohydrate metabolism → limit in $NADPH_2$ as e^- carrier for antibiotic production
  - Autoregulation: mostly controlled by low MW compounds or signaling molecules i.e. Factor A → high expression of gene cluster for streptomycin

STRAIN IMPROVEMENT

Genetic Manipulation

- **Mutation**
  - Random selection: depend on strain, mutagenesis, biosynthesis pathway, regulation
  - Desirable mutants: increase gene copy and induction, decrease repression, tolerate to feedback inhibition and catabolite repression
- **Recombinant DNA technology**:
  - Fungi: 1. sexual/perfect/meiosis
    2. parasexual/imperfect/mitosis
  - Bacteria: transformation, conjugation, transduction
    - Actinomycetes: natural via conjugation, protoplast fusion (DNA exchange induced by PEG)

RECOMBINANT STRAINS

- The biology of gene manipulation is concerned with the selection and use of a suitable carrier molecule and a living system

Major group	Prokaryotic/ Eukaryotic	Type	Examples
Bacteria	Prokaryotic	Gram negative Gram positive	*Escherichia coli* *Bacillus subtilis* *Streptomyces* spp.
Fungi	Eukaryotic	Microbial Filamentous	*Saccharomyces cerevisiae* *Aspergillus nidulans*
Plants	Eukaryotic	Protoplast Intact cells Whole organism	Various types
Animals	Eukaryotic	Insect cells Mammalian cells Oocytes Whole organism	*Drosophila melanogaster* Various types

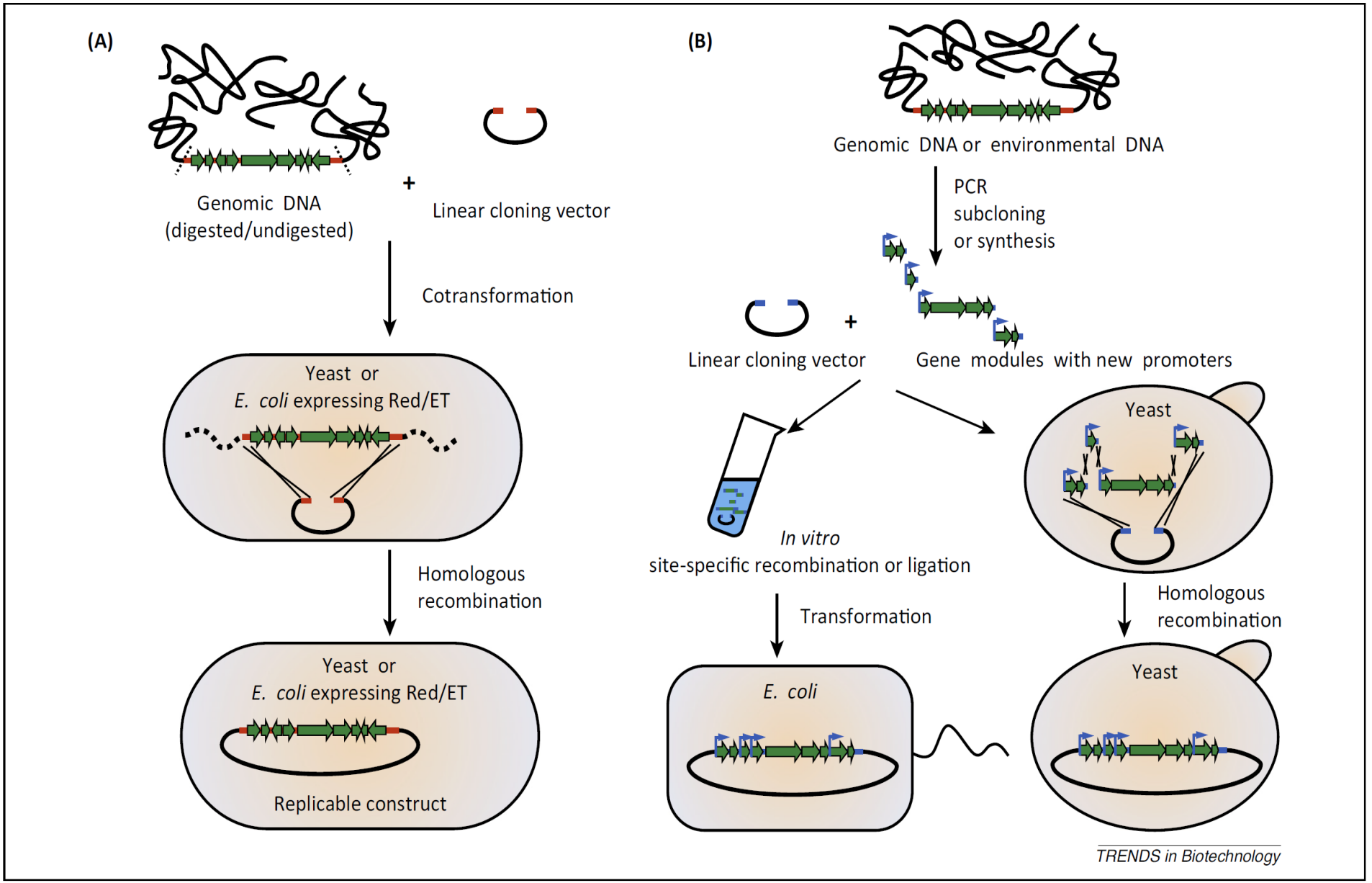


Figure 2. Direct cloning and reassembly of secondary metabolite biosynthetic gene clusters. **(A)** To capture a long cluster, a linear cloning vector flanked with homology arms is prepared and then co-transferred with the cluster-harboring genomic DNA into yeast (TAR) or engineered *Escherichia coli* (LLHR). Catalyzed by the native recombination mechanism in yeast or expressed Red/ET in *E. coli*, the homologous recombination between the cluster ends and the vector homology arms yields a circular replicable construct. **(B)** For reassembly of a cluster, genes are isolated individually by PCR, subcloning, or chemical synthesis, and are modified by the addition of homology ends, ends with restriction sites, or specific recombination sites. Strong or inducible promoters can be introduced in front of genes. Next, the gene fragments are assembled in a vector by *in vivo* homologous recombination in yeast, or by *in vitro* site-specific recombination or ligation. Assembled products can be further amplified in *E. coli*.

Mahidol University
Faculty of Science

IDEAL CHARACTERISTICS FOR ENGINEERED STRAINS

"Discussion and Suggestion"

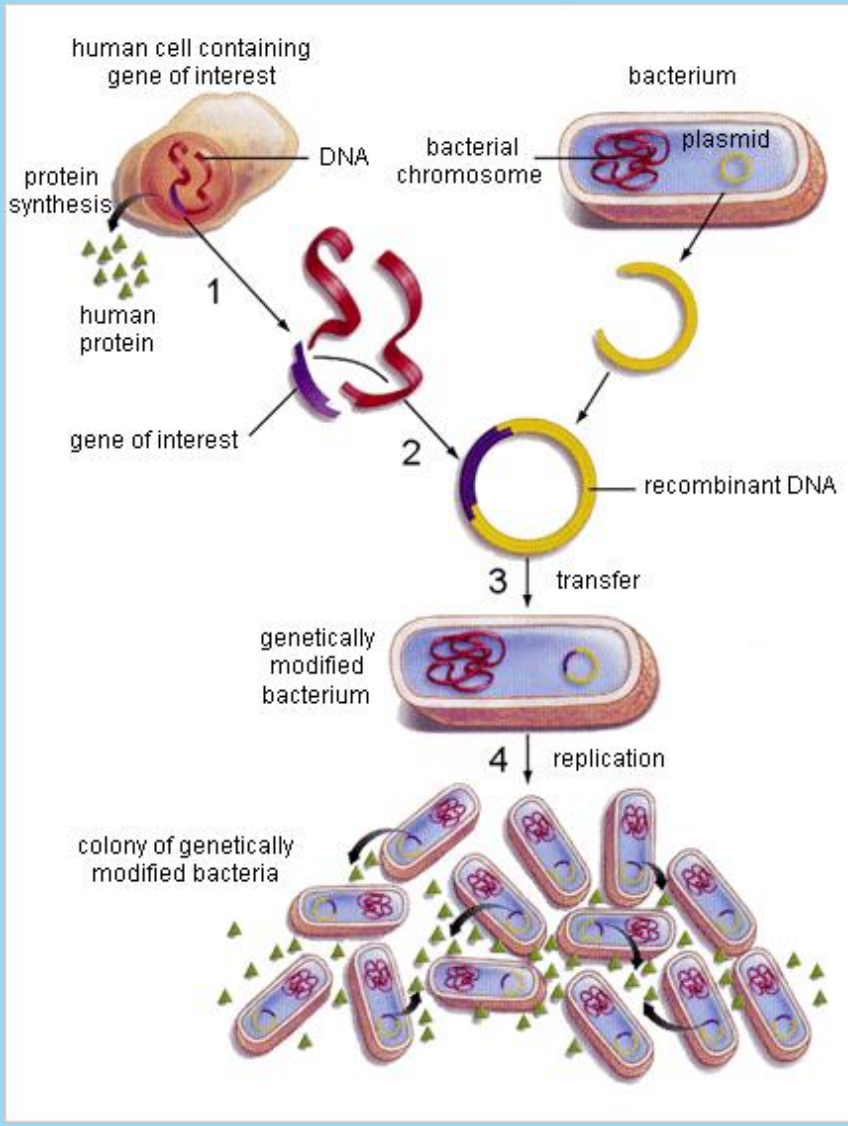
Facilitated by Adisak Romsang, Ph.D.

PRODUCTION METHOD

- Upstream processing
- Bioreactor design and preferable conditions
- Downstream processing
- **Optimization of microbial activity by**

1. Optimizing environmental conditions
   - Modification of physical parameter(temperature, agitation, etc)
   - Modification of chemical parameter (pH,O_2 concentration)
   - Modification of biological parameter (enzymes)
2. Optimizing nutrition of microorganisms
   - Carbon sources
   - Nitrogen sources
   - Mineral sources and other sources
   - Precursor
   - Enzymes

GENETIC ENGINEERING



OPEN ACCESS Freely available online

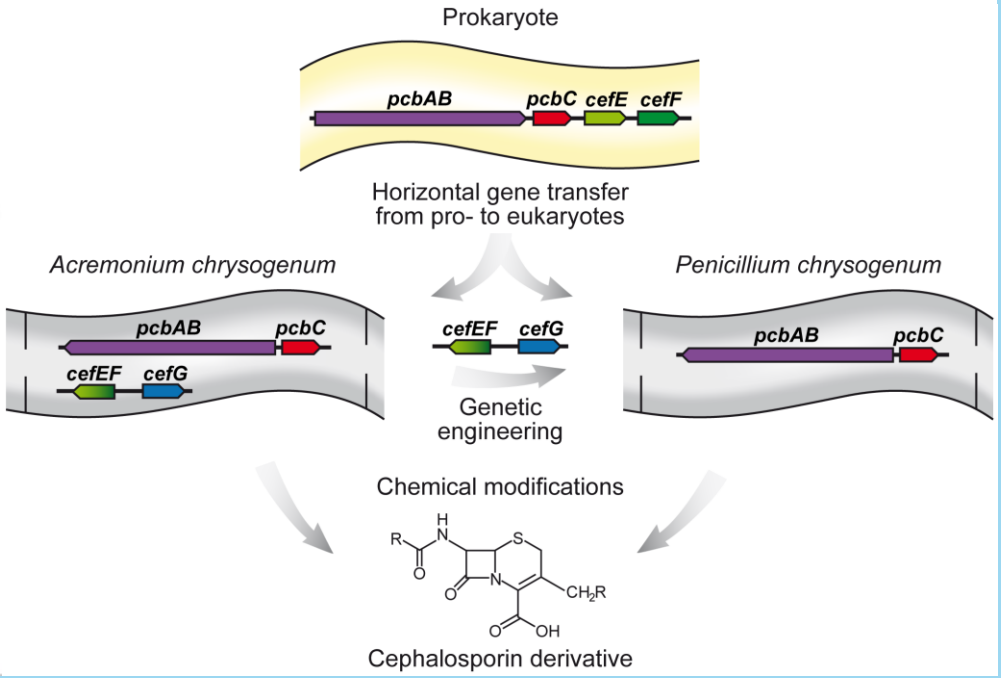
PLOS | PATHOGENS

Pearls

Putting Fungi to Work: Harvesting a Cornucopia of Drugs, Toxins, and Antibiotics

Ulrich Kück[1,2]*, Sandra Bloemendal[1,2], Ines Teichert[2]

1 Christian Doppler Laboratory for Fungal Biotechnology, Ruhr-Universität Bochum, Bochum, Germany, 2 Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Bochum, Germany



Protein Engineering

Protein engineering involves the use of genetic manipulations to **alter the coding sequence** of a (cloned) gene and thus the properties of the protein encoded by that gene.

RATIONAL DESIGN

1. Computer aided design

2. Site-directed mutagenesis

Individual mutated gene

3. Transformation

4. Protein expression

5. Protein purification

6. *not applied*

Constructed mutant enzyme

DIRECTED EVOLUTION

1. *not applied*

2. Random mutagenesis

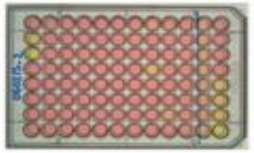
Library of mutated genes
(>10,000 clones)

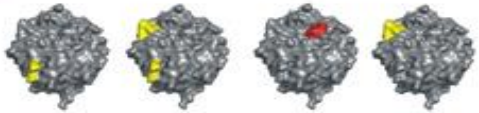
3. Transformation

4. Protein expression

5. *not applied*

6. Screening and selection
- stability
- selectivity
- affinity
- activity





Selected mutant enzymes

Improve protein **stability**
Increase protein **purity** during
Increase protein **expression**
Modify **cofactor** requirement
Increase enzyme **activity**
Modify enzyme **specificity**
Study the **function** of a protein

7. Biochemical testing

Protein Engineer

http://dx.doi.org/10.1016/j.tibtech.2014.10.009

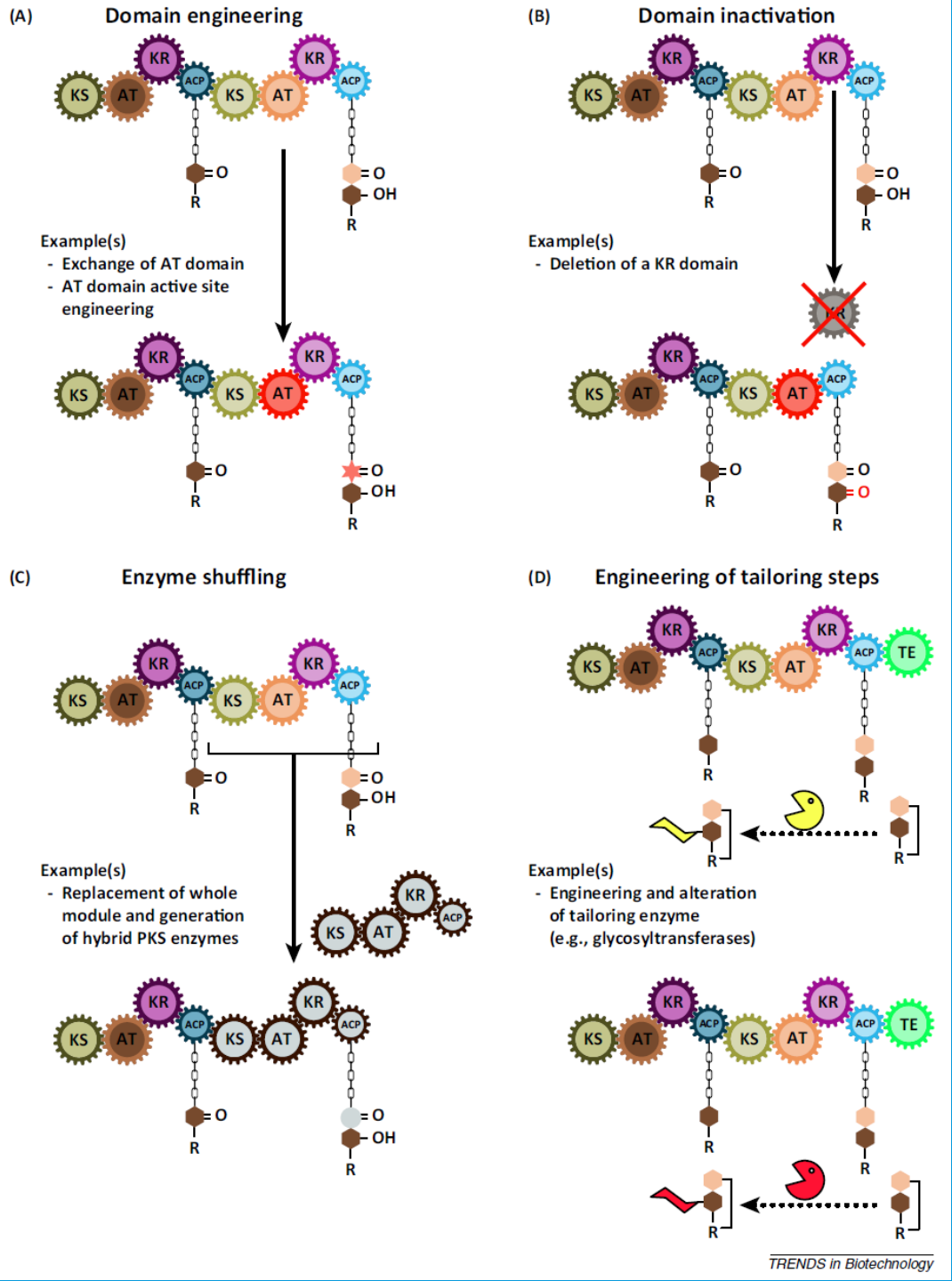
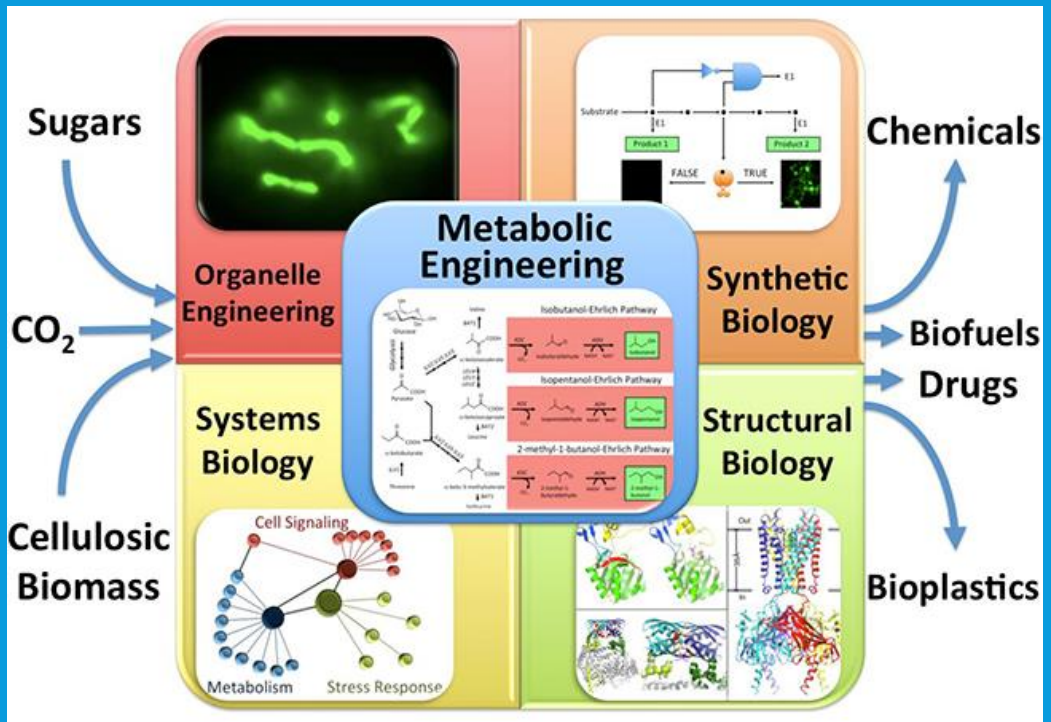


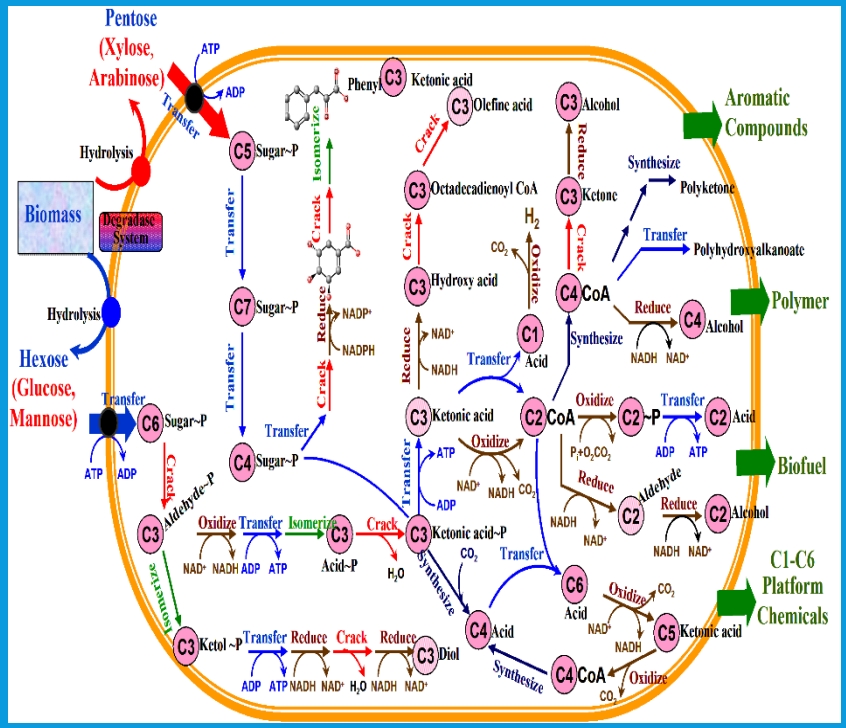
Figure 3. Strategies to engineer type I polyketide biosynthetic pathways. (A) Generation of novel compounds by AT domain engineering. (B) Interfering with b-carbon reduction by domain inactivation. (C) Generation of novel compounds by the replacement of whole modules. (D) Generation of novel compounds by engineering of tailoring modifications. Abbreviations: ACP, acyl carrier protein; AT, acyltransferase; ER, enoylreductase; KR, ketoreductase; KS, ketosynthase; TE, thioesterase.

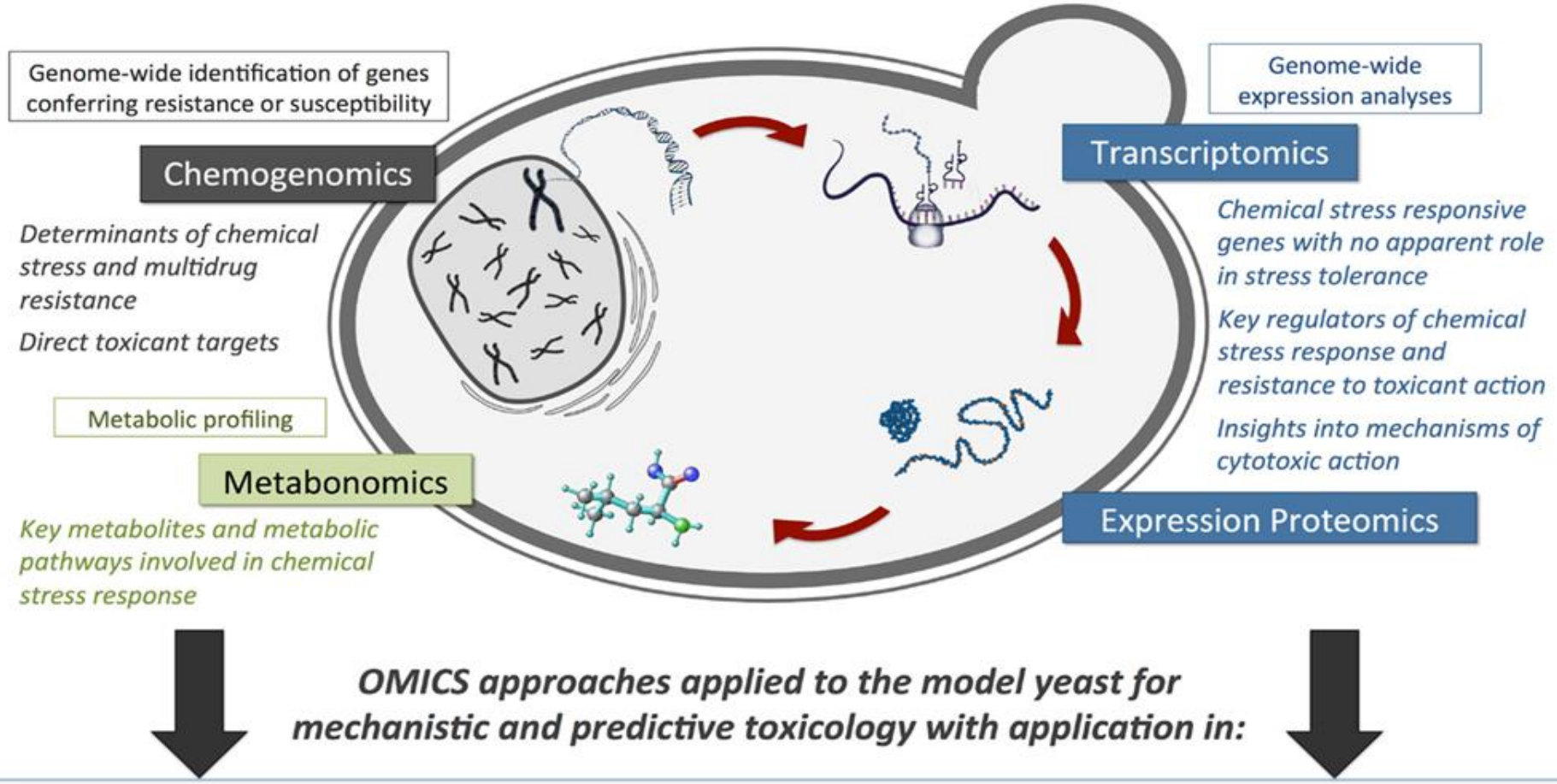
Metabolic Engineering

The practice of optimizing genetic and regulatory processes within cells to increase the cells' production of a certain substance.

Genetic engineering techniques can then be used to modify the network in order to relieve these constraints.







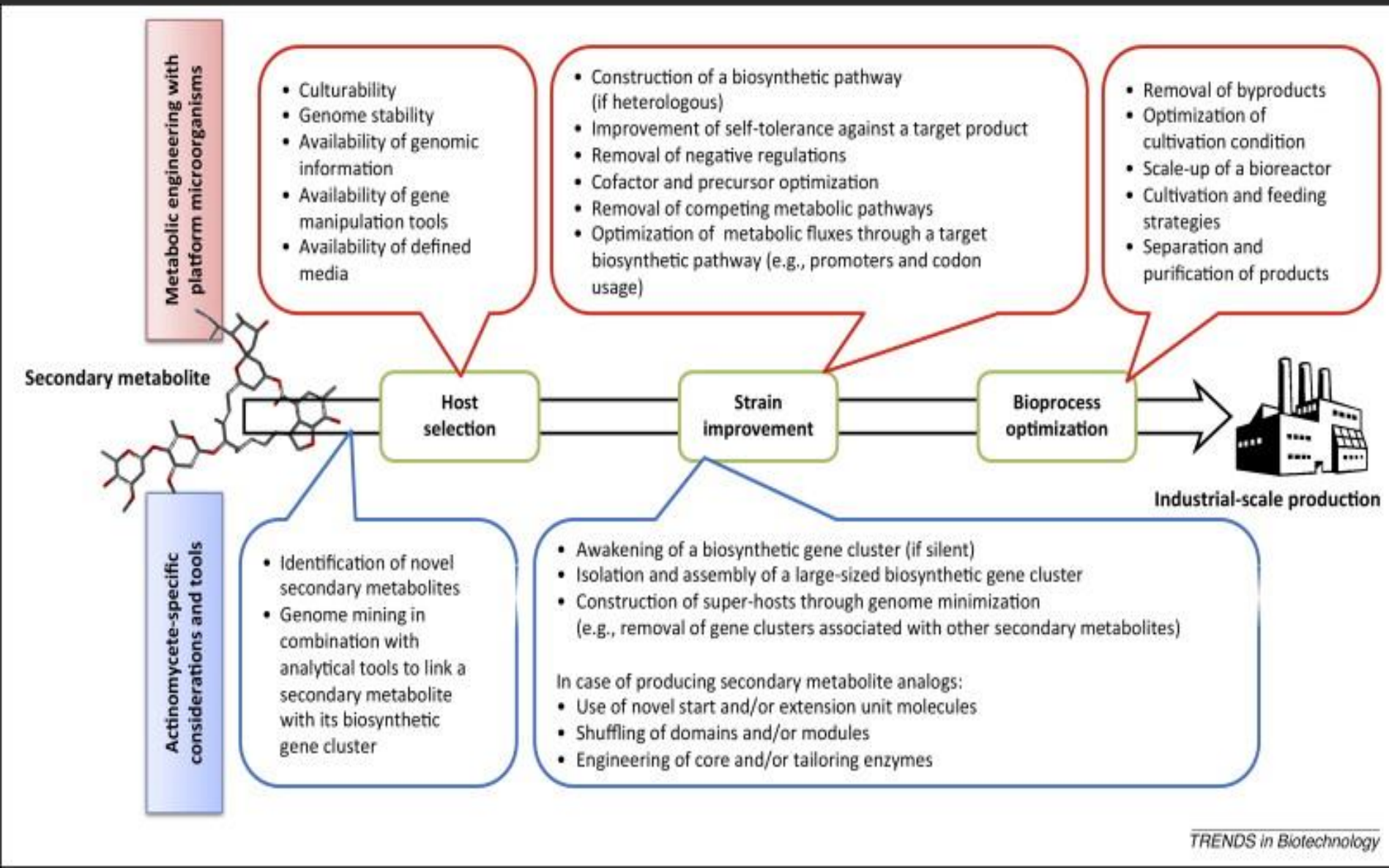
OMICS approaches applied to the model yeast for mechanistic and predictive toxicology with application in:

- ***Environmental Health***
- ***Medicinal research and drug development***
- ***Biotechnology***

- Identify predictive biomarkers of chemical toxicity
- Predict toxicological outcomes of exposure to environmental pollutants/pesticides and drugs
- Identify predictive biomarkers of fermentation impairment
- Elucidate mechanisms of drug action and identify new candidates for drug development
- Identify Pollutants/pesticides off-targets and the relationship with human diseases
- Find targets for genetic manipulation to increase crop or industrial microbial strain robustness
- Identify direct and off-target effects of drugs to predict adverse side-effects and repurposing of existing drugs

ANTIBIOTICS PRODUCTION & METABOLIC ENGINEERING

HTTP://DX.DOI.ORG/10.1016/J.TIBTECH.2014.10.009

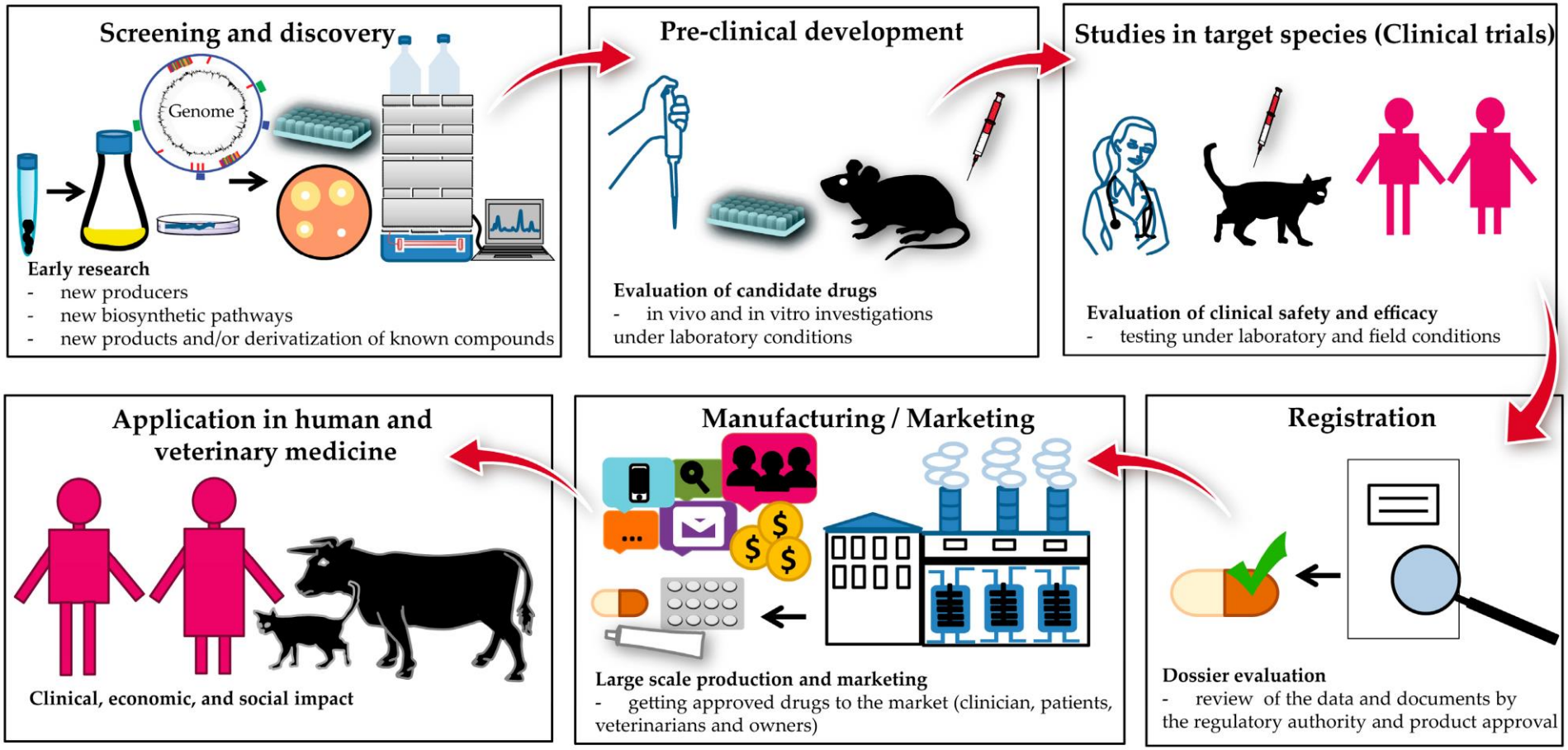


CONSIDERATION

- Isolation or collection of cultures
- Screening of cultures to detect those with antimicrobial activity
- Development of methods for submerged-culture production
- Development of methods for isolation and purification of antibiotic
- Determination of antibiotic properties (physical: adsorption and absorption, chemical: reactions, solubility in solvents, stability to acids, alkalis, heat etc.)
- Evaluation of antibiotic
  - Pharmacological tests
  - Antimicrobial activity
  - Comparison with existing antibiotic
- Development of pilot plant production methods
- Submission of licence for clinical trials
- Testing of purified antibiotic
- Development of plant scale production methods
- Obtaining a product licence for clinical use
- Other considerations:
  - Development of methods to control production of antibiotic
  - Development of new applications
  - Development of marketing and distribution system
  - Financing of business

ACTINOMYCETE-DERIVED POLYKETIDES AS A SOURCE OF ANTIBIOTICS AND LEAD STRUCTURES FOR THE DEVELOPMENT OF NEW ANTIMICROBIAL DRUGS

- *Antibiotics* **2019**, *8*(4), 157; https://doi.org/10.3390/antibiotics8040157



Mahidol University
Faculty of Science

SCBT431

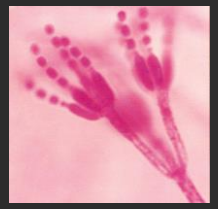
ANTIBIOTICS PRODUCTION IN INDUSTRIES

Pharmaceutical Products 1

Asst.Prof. Dr.Adisak Romsang

Dept. Biotechnology, Fac. Science, Mahidol University
Room K610 Tel. 02201-5962 Email: adisak.rom@mahidol.ac.th

PENICILLIN



- Penicillins are a group of β-lactam containing bactericidal antibiotics. **Natural penicillins (penicillins V and G) are effective against several Gram-positive bacteria.** They inhibit the bacterial cell wall synthesis and cause cell death. Some persons (approximately 0.5-5% of population) are allergic to penicillin.
- The basic structure of all the penicillin consists of a lactam ring and a thizolidine ring fused together to form 6-aminopenicillanic acid.
- From the huge quantities of penicillins produced by fermentation, about 40% are used for human healthcare, 15% for animal healthcare and 45% for the preparation of semi-synthetic penicillins.
- *Penlcillium notatum* was used for the large-scale production of penicillins. Currently, ***Penicillium chrysogenum*** and its improved mutant strains are preferred. With new strains, production runs into several thousands of units/ml. High yield strains is Q176.
- Genetic engineering for improved penicillin production: extra genes coding for the enzymes cyclase and acyltransferase inserted into *P. chrysogenum*.

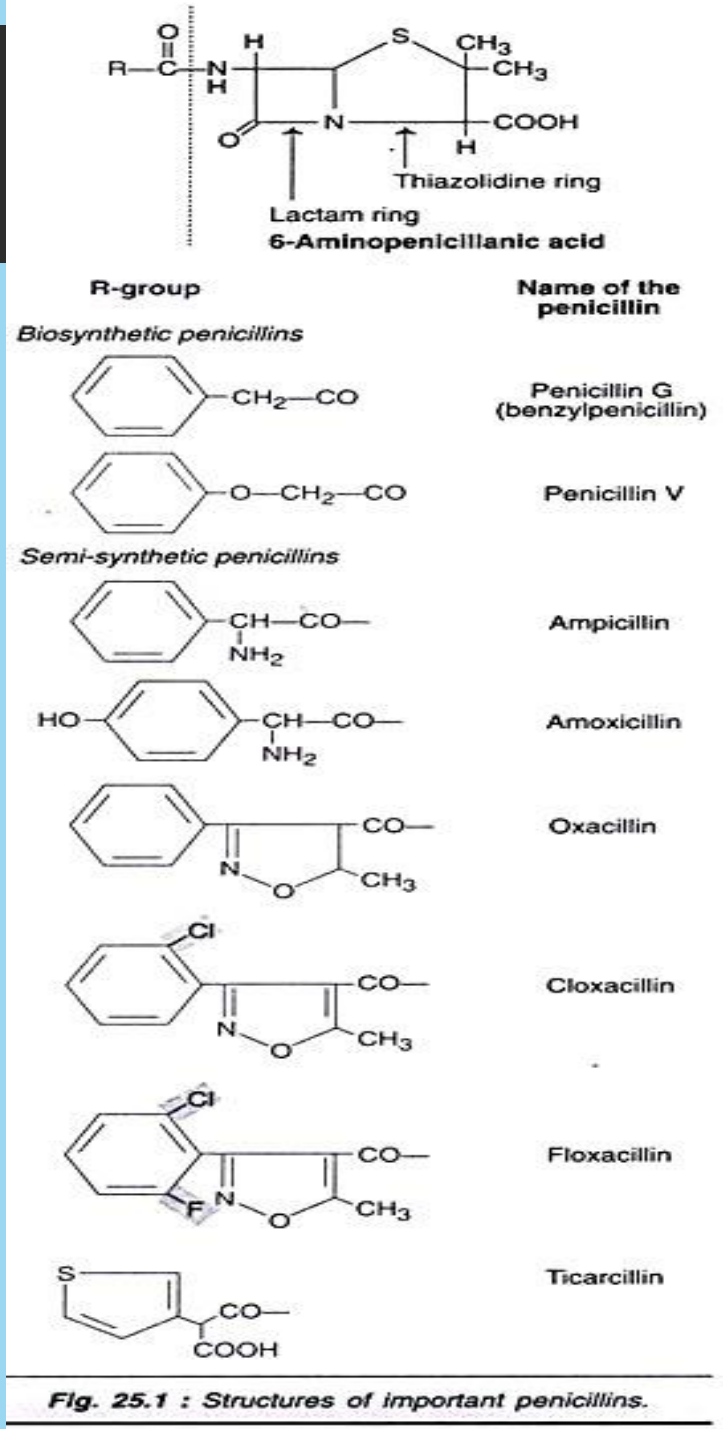
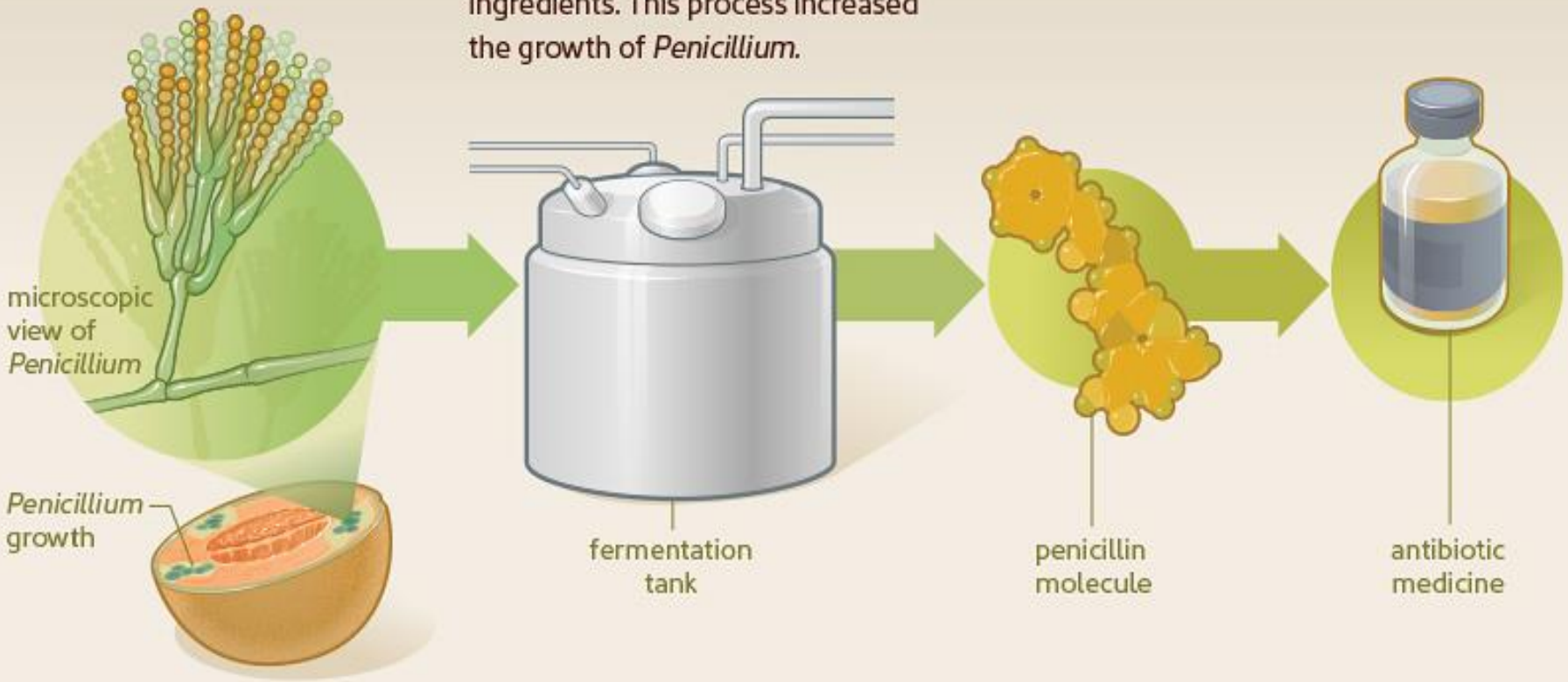


Fig. 25.1 : Structures of important penicillins.

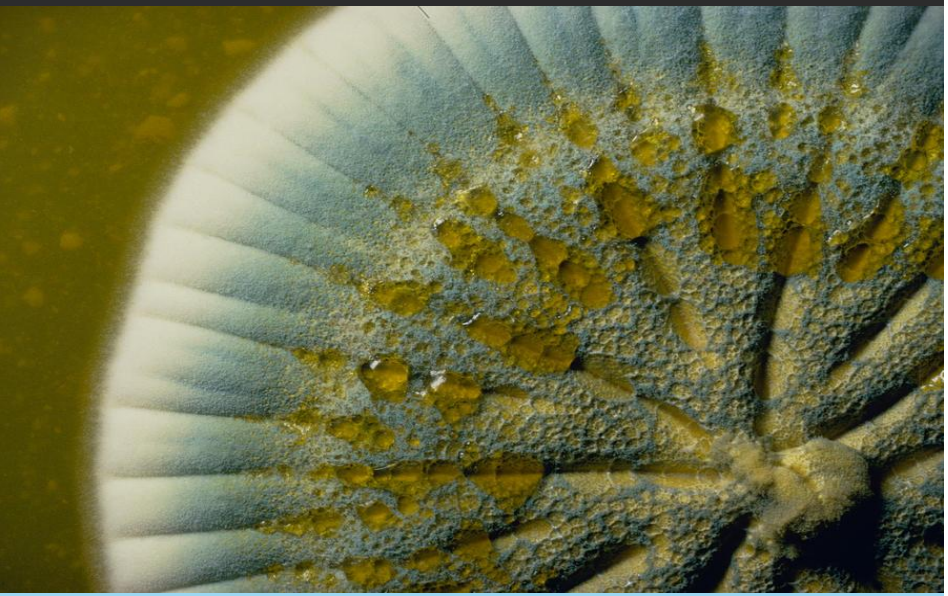
HOW DID THEY MAKE PENICILLIN?

FOR MANY YEARS, scientists knew that certain molds killed some bacteria. However, researchers needed to understand how to harness this antibacterial microbe and to manufacture enough of the substance before they could make a useful medicine.

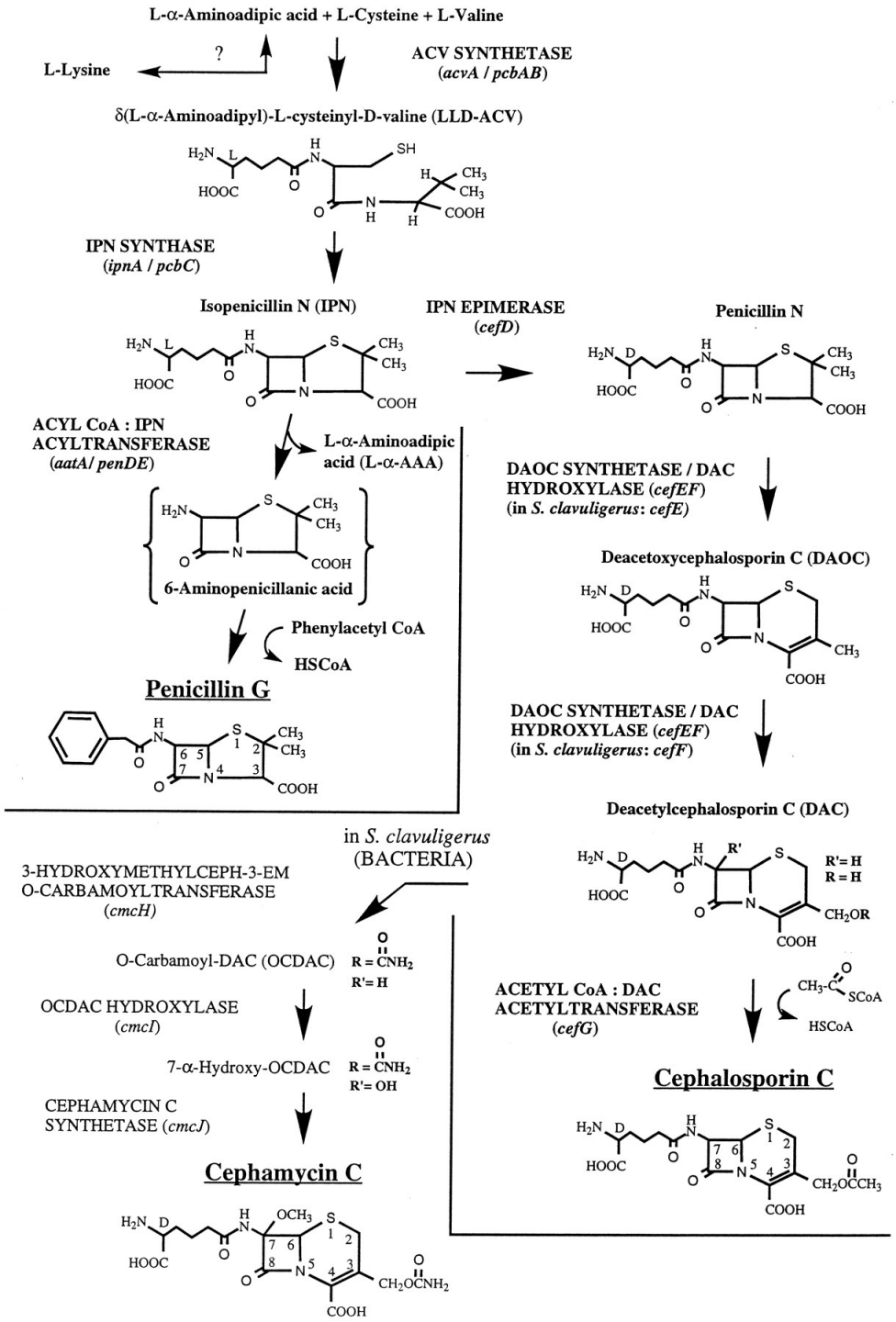
1. *Penicillium* mold naturally produces the antibiotic penicillin
2. Scientists learned to grow *Penicillium* mold in deep fermentation tanks by adding a kind of sugar and other ingredients. This process increased the growth of *Penicillium*.
3. Then, scientists separated the penicillin product from the mold.
4. Finally, penicillin is purified for use as an antibiotic medicine.



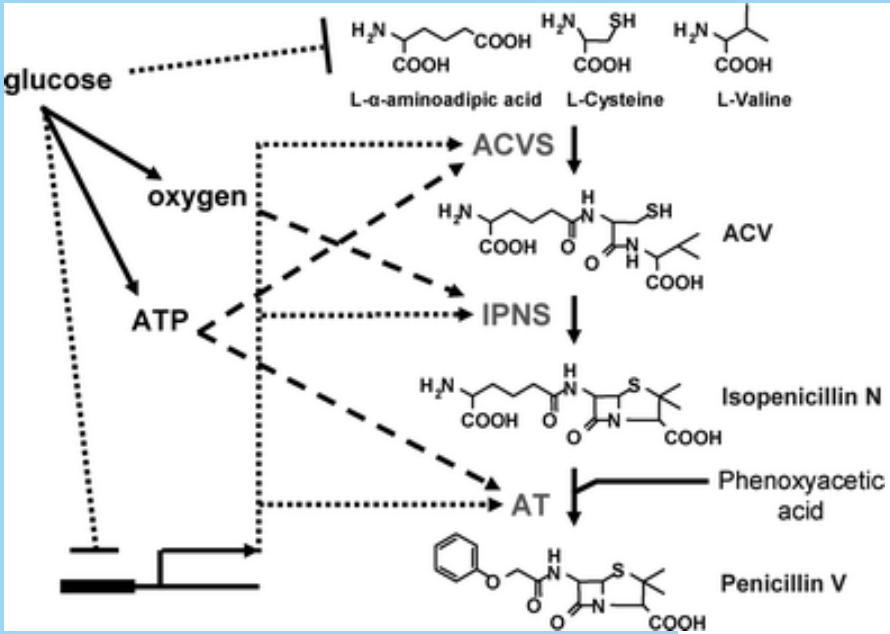
PENICILLIN



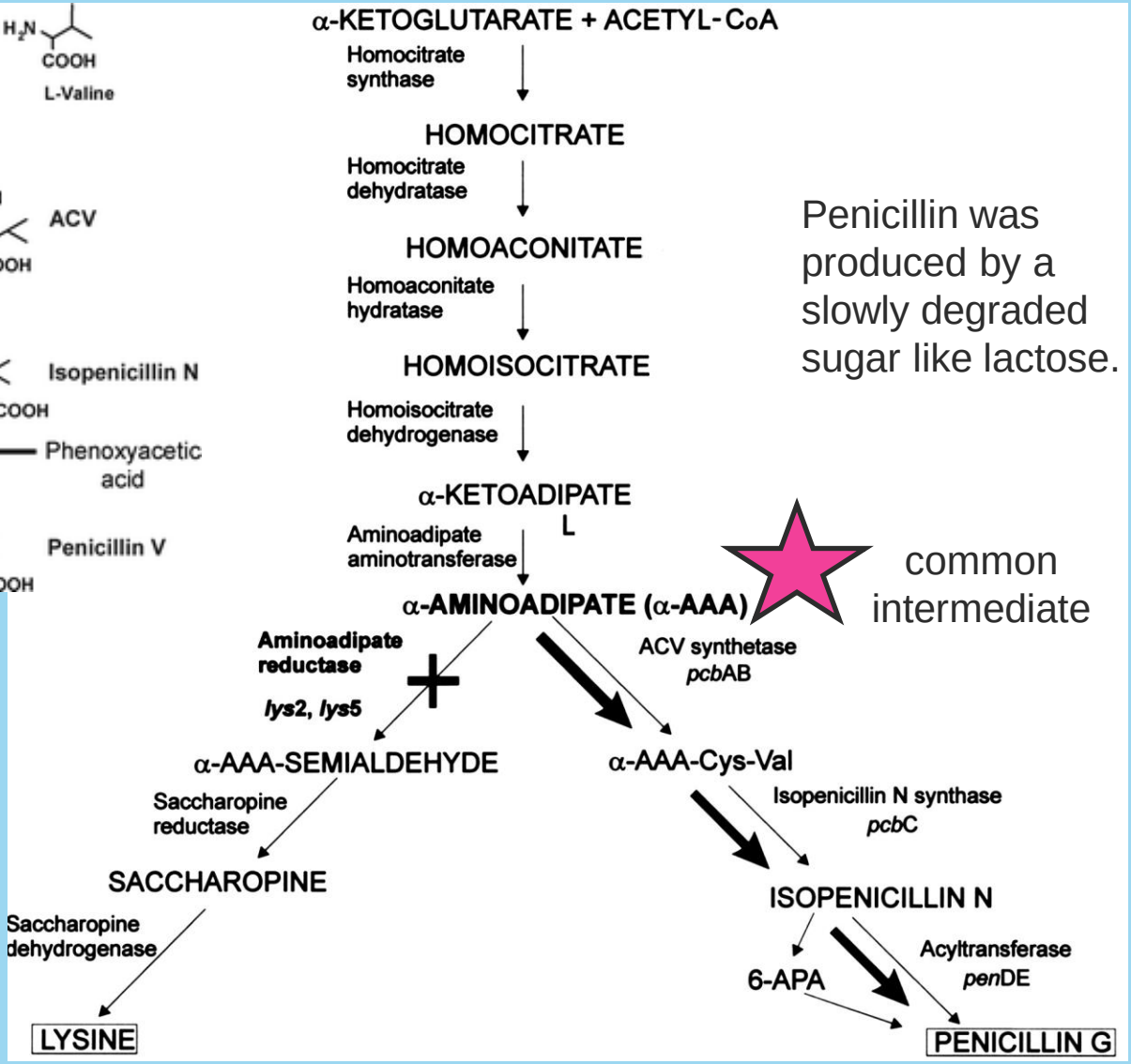
L-α-Aminoadipic acid combines with L-cysteine, and then with L-valine to form a α-L-aminoadipyl cysteinyl valine.
This compound undergoes cyclization to form isopenicillin which reacts with phenyl acetyl CoA (catalysed by the enzyme acyltransferase) to produce penicillin G (benzyl penicillin).
In this reaction, aminoadipic acid gets exchanged with phenyl acetic acid.



REGULATION OF BIOSYNTHESIS



- Lysine: feedback inhibition
- Phosphate concentration
- Catabolite repression by glucose and ammonia

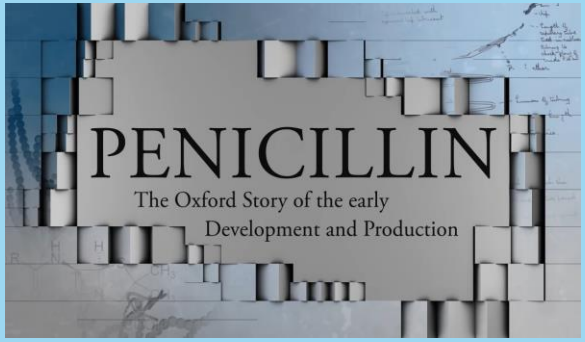


Penicillin was produced by a slowly degraded sugar like lactose.

BIOPROCESS: PENICILLIN

- Production of penicillin G : either natural by adding side chain precursors i.e. phenyl acetate or semisynthetic derivatives by genetic engineering
- Methods of cultivation
  - Surface or submerged cultivation
  - Commercial scale: fed batch with 2 phases @ 25°C, pH6.5-7.7, O_2 60mmol
- Downstream: extracellular → remove mycelium, extract & precipitate, freeze dry

1. A medium of corn steep liquor (a by product of starch manufacture), yeast extract and others substrates added to the fermenter.
2. After 40 hours, Penicillin begins to be secreted by the fungus
3. The mould mycellium (cell matter) is filtered from the harvested product.
4. Penicillin is extracted in the organic solvent: butylacetate, in which it dissolves.
5. Potassium salts are added and a penicillin precipitate is formed, this is washed and dried.



PRODUCTION PROCESS OF PENICILLIN

- Penicillin production is an aerobic process and a continuous supply of O_2 to the growing culture is very essential.
- The required aeration rate is 0.5-1.0 vvm. The pH is maintained around 6.5, and the optimal temperature is in the range of 25-27°C.
- Penicillin production is usually carried out by **submerged processes**. The medium used for fermentation consists of corn steep liquor (4-5% dry weight) and carbon source (usually lactose). An addition of yeast extract, soy meal or whey is done for a good supply of nitrogen.
- Sometimes, ammonium sulfate is added for the supply of nitrogen.
- **Phenyl acetic acid** (or phenoxyacetic acid) which serves as a precursor for penicillin biosynthesis is continuously fed.
- Further, continuous feeding of **sugar** is advantageous for a good yield of penicillin.

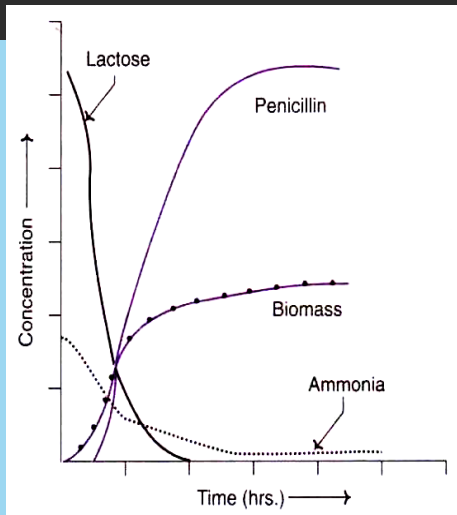


Fig. 25.4 : Penicillin production in relation to substrates utilization and biomass formation.

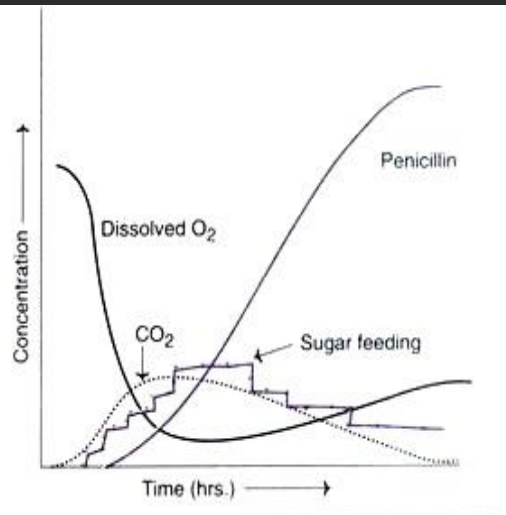
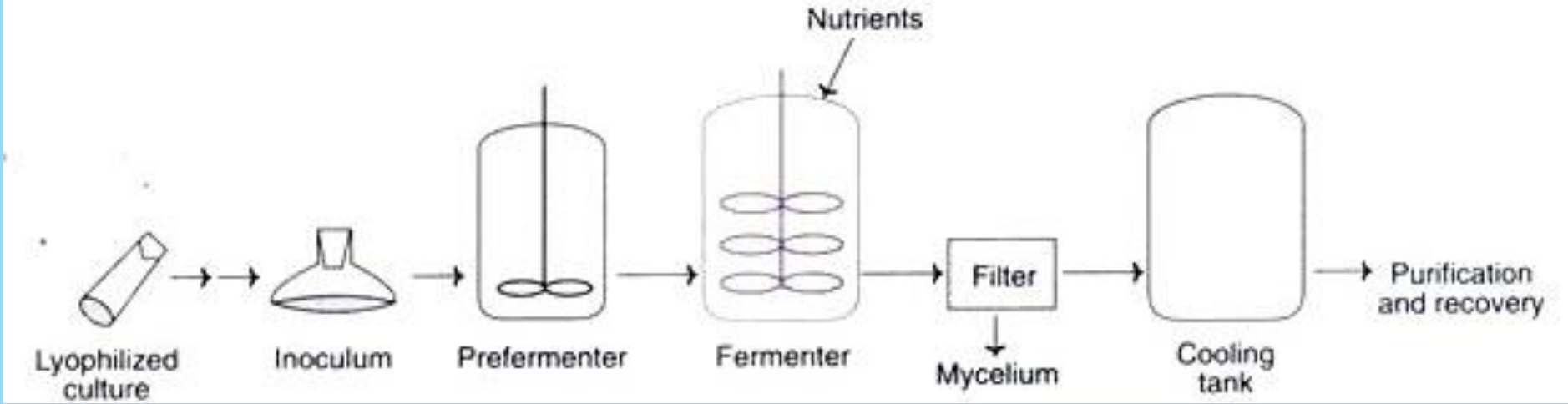


Fig. 25.5 : Penicillin production in relation to continuous feeding of sugar, O_2 utilization, and CO_2 formation.

- 10% of the metabolized carbon contributes to penicillin production, while 65% is utilized towards energy supply and 25% for growth of the organisms.
- For efficient synthesis of penicillin, the growth of the organism from spores must be in a loose form and not as pellets.
- The growth phase is around 40 hours with a doubling time of 6-8 hours. The penicillin production phase then can be extended to 150-180 hours.

RECOVERY OF PENICILLIN

- As the fermentation is complete, the **broth containing about 1% penicillin** is processed for extraction. The mycelium is removed by filtration. Penicillin is recovered by solvent (n-butyl acetate or methyl ketone) extraction at low temperature (<10°C) and acidic pH (<3.0). By this way, the chemical and enzymatic (bacterial penicillinase) degradations of penicillin can be minimized.
- The penicillin containing solvent is treated with activated carbon to remove impurities and pigments. Penicillin can be recovered by adding potassium or sodium acetate. The potassium or sodium salts of penicillin can be further processed (in dry solvents such as n-butanol or isopropanol) to remove impurities. The yield of penicillin is around 90%.
- As the water is totally removed, **penicillin salts** can be crystallized and dried under required pressure. This can be then processed to finally produce the pharmaceutical dosage forms. Penicillins G and H are fermented from the fungus *Penicillium chrysogenum*.



6-AMINO PENICILLANIC ACID

- The penicillins G and H are mostly used as the starting materials for the production of several synthetic penicillins containing the basic nucleus namely 6-amino penicillanic acid (6-APA).
- About 10 years ago, only chemical methods were available for hydrolysis of penicillins to produce 6-APA. Now a days, enzymatic methods are preferred.
- **Immobilized penicillin amidases enzymes** have been developed for specific hydrolysis of penicillin G and penicillin V. Penicillin salt of either G or V can be used for hydrolysis by immobilized enzyme system. The pH during hydrolysis is kept around 7-8, and the product 6-APA can be recovered by bringing down the pH to 4.
- At pH 4, 6-amino penicillanic acid gets precipitated almost completely in the presence of a water immiscible solvent. In general, the enzymatic hydrolysis is more efficient for penicillin V than for penicillin G. However, penicillin G is a more versatile compound, as it is required for ring expansions.

CEPHALOSPORIN

*Not effective vs. Enterococcus or Listeria

1st Generation	Gram (+)
2nd Generation	Decreasing Gram (+) and Increasing Gram (-)
3rd Generation	Gram (-), but also some GPC
4th Generation	Gram (+) and Gram (-)

- They have improved stability against β-lactamases, and are more active against Gram-negative bacteria. Cephalosporin's are broad spectrum antibiotics with low toxicity. Basically cephalosporin's have a β-lactam ring fused with a dihydrothiazine ring.
- **Cephalosporin C** was first discovered in the cultures of fungus *Cephalosporium acremonium* (later renamed as *Acremonium chreysogenum*). The other organisms employed are *Emericeliopsis* sp, *Paecilomyces* sp and *Streptomyces* sp.
- Several mutants of *C. acremonium* have been developed for improved production of cephalosporin. Mutants with defective sulfur metabolism or those with resistance to sulfur analogs have high yielding capacity. Certain regulatory genes of cephalosporin biosynthesis (e.g., isopenicillin N synthetase) have been cloned and genetic manipulations carried out for increased production of cephalosporin's.

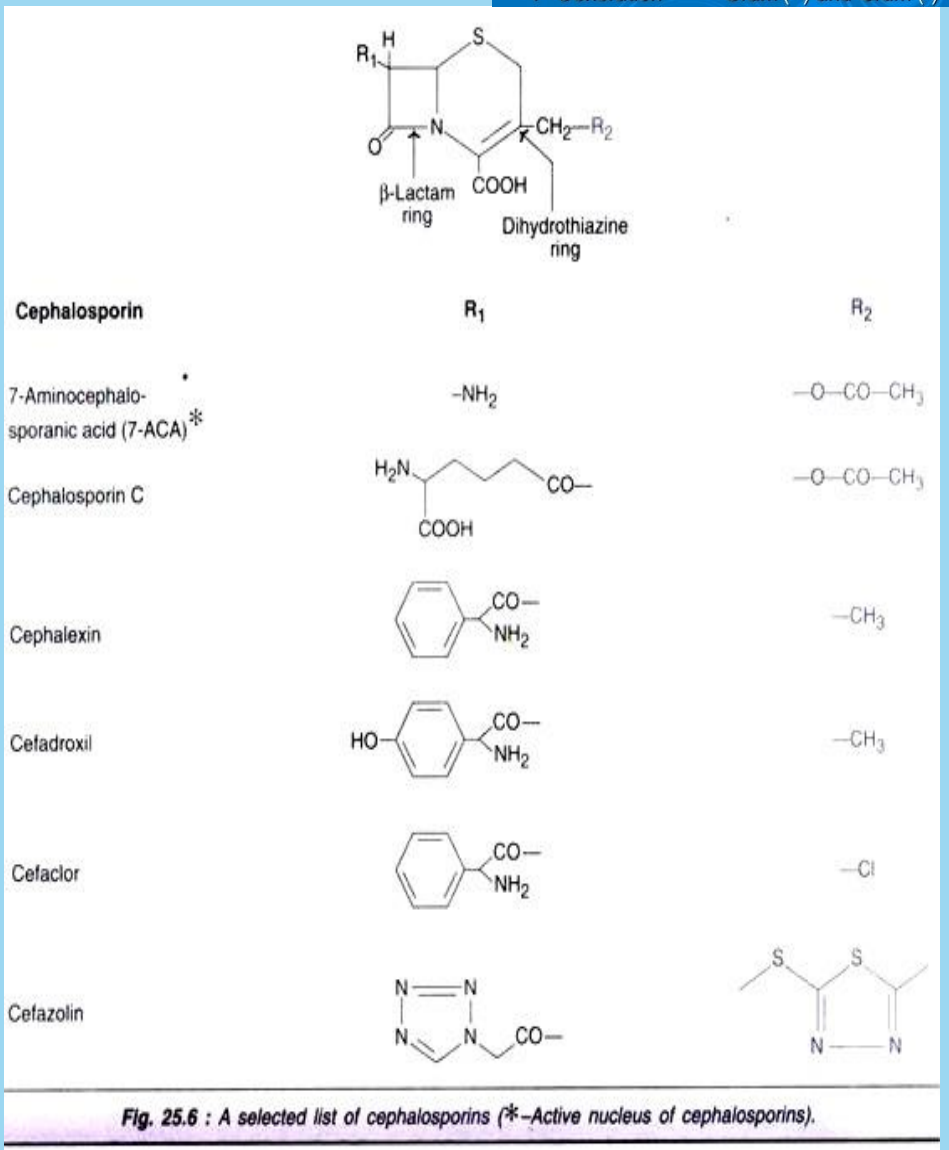


Fig. 25.6 : A selected list of cephalosporins (–Active nucleus of cephalosporins).*

CEPHALOSPORIN PRODUCTION

- These have been synthesized by chemical splitting to form 7 aminocephalospioranic acid (7-ACA) with subsequent chemical acylation as well as by modification on the C-3 site.

- By the action of epimerase, penicillin N is formed from isopenicillin N. Then, penicillin N gets converted to cephalosporin C by a three stage reaction catalysed by three distinct enzymes namely expandase, hydroxylase and acetyl transferase.

Regulation

- A low concentration of lysine promotes cephalosporin synthesis. The inhibitory effect of lysine at a higher concentration can be overcome by adding L-aminoadipic acid.
- The carbon sources that get rapidly degraded (e.g. glucose, glycerol) reduce cephalosporin production.
- Methionine promotes cephalosporin synthesis in *C. acremonium*, but has no influence on *Streptomyces*'s.

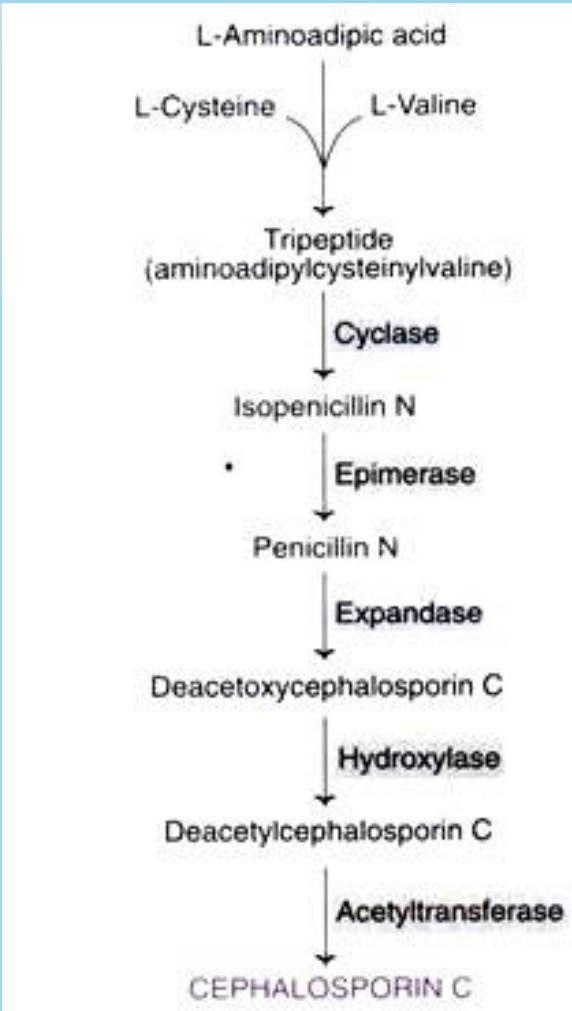
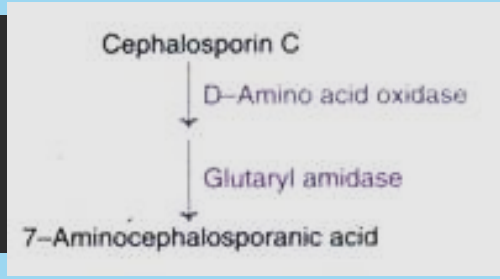


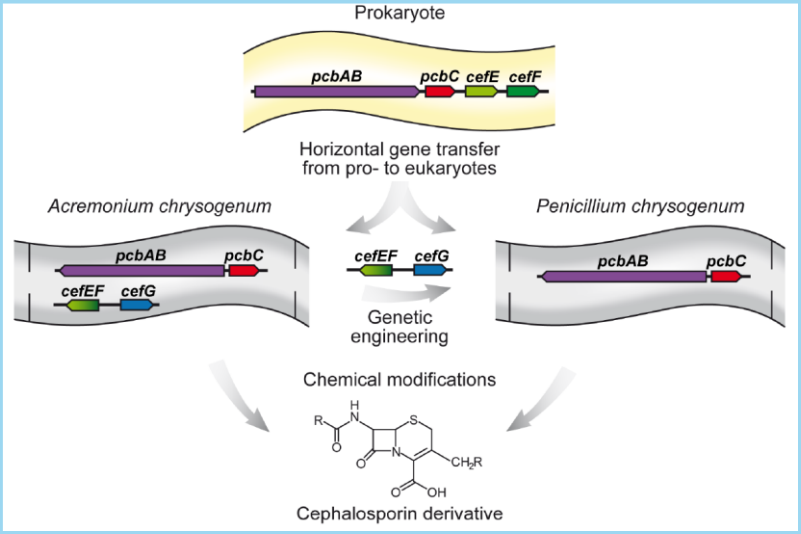
***Fig. 25.7** : Biosynthesis of cephalosporin C by A. chrysogenum.*

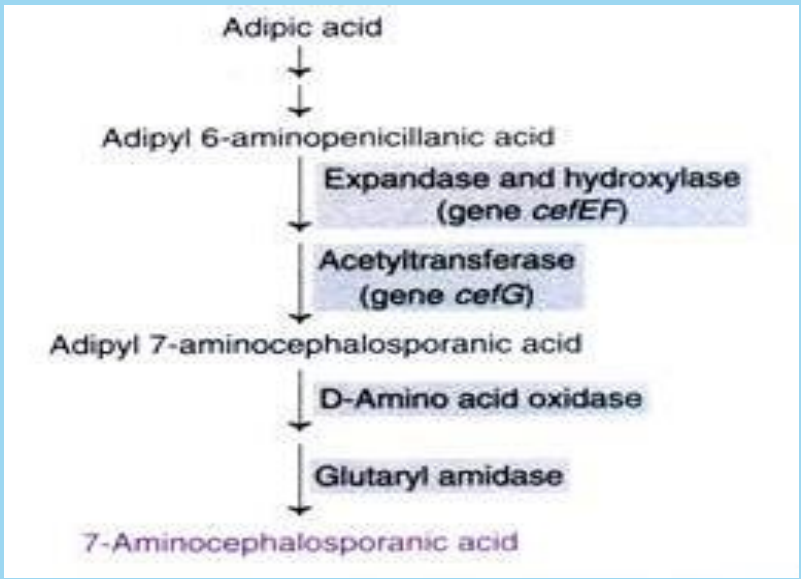
PRODUCTION OF CEPHALOSPORIN

- The fermentation process concerned with the production of cephalosporin is similar to that of penicillin. The culture media consists of corn steep liquor and soy flour-based media in a continuous feeding system. The other ingredients of the medium include sucrose, glucose and ammonium salts. Methionine is added as a source of sulfur.
- The fermentation is carried out at temperature 25-28°C and pH 6-7. The growth of microorganisms substantially increases with good O_2 supply, although during production phase, O_2 consumption declines.
- **Cephalosporin C** from the culture broth can be recovered by ion-exchange resins, and by using column chromatography. Cephalosporin C can be precipitated as zinc, sodium or potassium salt, and isolated.

7-AMINOCEPHALOSPORANIC ACID







- 7-ACA is the nucleus structure present in all the cephalosporin's. Cephalosporin C, produced by fermentation, can be subjected to chemical hydrolysis to form 7-ACA, tedious and drawbacks.
- Recently, immobilized enzymatic hydrolysis of cephalosporin C to 7-ACA has been developed. This is mainly carried out by **two enzymes-D-amino acid oxidase (isolated from *Trigonopsis variabilis*) and glutaryl amidase (source-*Pseudomonas* sp)**.
- By inserting expandase and hydroxylase gene (*cefEF*), and acetyl transferase gene (*cefG*) from *S. clavuligerus* into *P. chrysogenum*. Further, the genes responsible for the enzymes D-amino acid oxidase (from *Pseudomonas diminuta*) have also been inserted into *P. chrysogenum*. Both these enzymes act on adipyl-7-ACA to produce 7-amino- cephalosporanic acid.

AMINOGLYCOSIDES

- Aminoglycosides are oligosaccharide (carbohydrate) antibiotics. They contain an aminocyclo-hexanol moiety which is bound to other amino sugars by glycosidic linkages (>100 aminoglycosides).
- **Streptomycin** was the first aminoglycoside that was successfully used to treat tuberculosis against *Mycobacterium tuberculosis*. They are reserve antibiotics, resistance is easily.
- Aminoglycoside antibiotics are produced by ***Actinomyces* sp.**
- Recombinant DNA techniques have been used to produce hybrid aminoglycosides, and for increasing the fermentation yield.
- All the ring structures in the molecules of aminoglycosides are ultimately derived from glucose.

Biosynthesis

- More than 30 enzymatic steps have been identified. **Glucose 6-phosphate** obtained from glucose takes three independent routes to respectively produce streptidine 6-phosphate, L-dehydrostreptose and N- methyl glucosamine.
- The former two compounds condense to form an intermediate later combines with methyl glucosamine to produce di-hydro-streptomycin-6-phosphate. This compound then gets converted to streptomycin.

TABLE 25.2 Selected examples of aminoglycosides with the organisms responsible for their production

Aminoglycoside	*Organism*
Streptomycin	*Streptomyces griseus*
Neomycin B and C	*S. fradiae*
Kanamycin A, B and C	*S. kanamyceticus*
Hygromycin B	*S. hygroscopicus*
Gentamicin	*Micromonospora purpurea*
Sisomicin	*M. inyoensis*

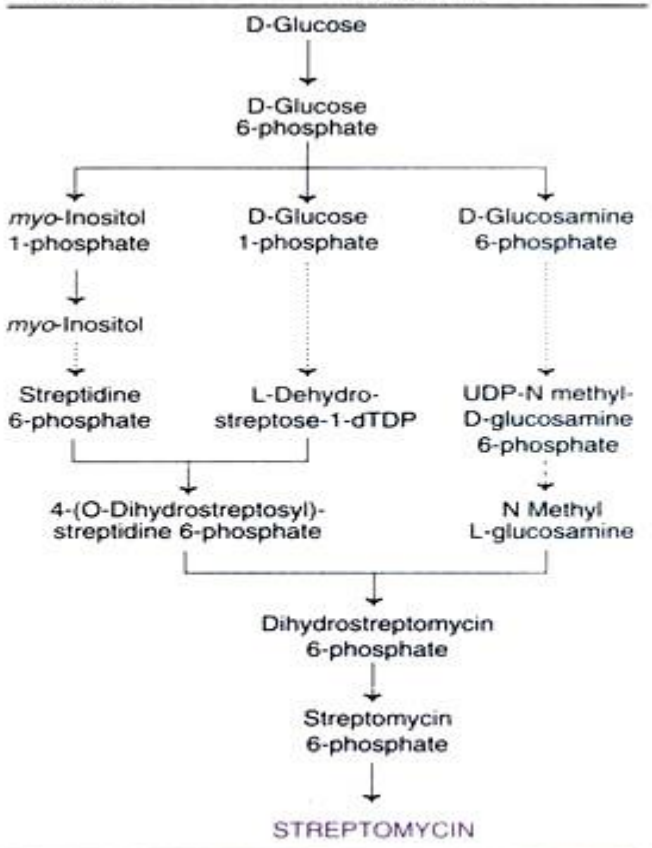


Fig. 25.9 : An outline of streptomycin biosynthesis.

AMINOGLYCOSIDES

Regulation of biosynthesis:

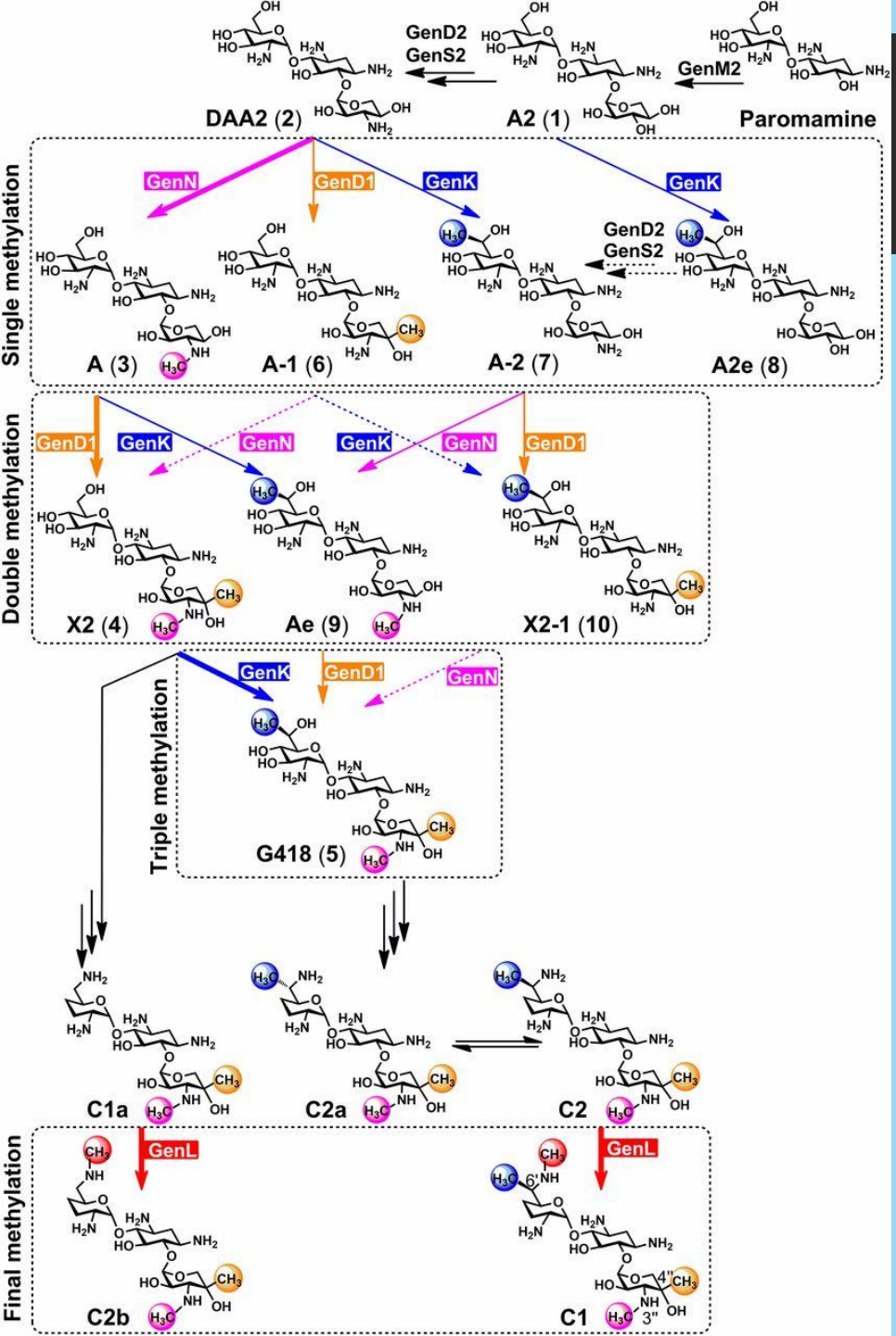
- **Factor A**, isolated from streptomycin-producing strains of ***S. griseus***, promotes streptomycin production. The nutrient sources-carbohydrates (**glucose**), **ammonia** and **phosphate** also regulate (by feedback mechanism) streptomycin production.

Production Process of Streptomycin:

- The medium used for streptomycin usually consists of soy meal or soy flour or corn syrup that can supply glucose at a slow rate (amylase activity is poor in *Streptomyces* sp). The initial supply of nitrogen (NH_3) and phosphate is also obtained from soy meal. This is required since glucose, ammonia and phosphate in high quantities inhibit streptomycin synthesis.
- The fermentation conditions for optimal production of streptomycin are — temperature 27-30°C, pH 6.5-7.5, aeration rate 0.5-1.0 vvm. The duration of fermentation process depends on the strain used, and is between 6 to 8 days.

Recovery of Streptomycin:

- Streptomycin or other aminoglycosides are **basic** in nature. They can be recovered by *weak cationic exchange resins* in an ion-exchange column. Treatment with activated carbon is often necessary to remove impurities. Streptomycin can be precipitated in the form of sulfate salt.



METHYLTRANSFERASES OF GENTAMICIN BIOSYNTHESIS

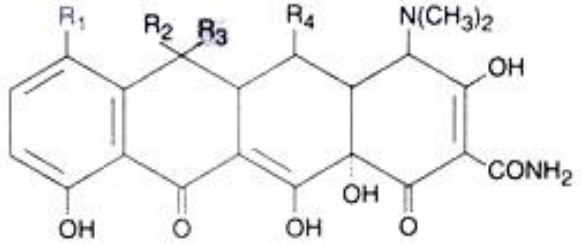
https://www.pnas.org/content/115/6/1340

- Gentamicin C complex from *Micromonospora echinospora*
- The complex consists of five components differing in their methylation pattern at one or more sites in the molecule. We show here, using specific gene deletion and chemical complementation, that the gentamicin pathway up to the branch point is defined by the selectivity of the methyltransferases GenN, GenD1, and GenK. Unexpectedly, they comprise a methylation network in which early intermediates are ectopically modified.
- Using whole-genome sequence, we have also discovered the terminal 6′-*N*-methyltransfer required to produce gentamicin C2b from C1a or gentamicin C1 from C2, an example of an essential biosynthetic enzyme being located not in the biosynthetic gene cluster but far removed on the chromosome.

TETRACYCLINE

- Tetracyclines are broad spectrum antibiotics with widespread medical use. They are effective against Gram-positive and Gram-negative bacteria (also mycoplasmas, chlamydias rickettsias). They are used to combat stomach ulcers (against *Helicobacter pylori*). Tetracyclines inhibit protein biosynthesis by blocking the binding of aminoacyl tRNA to ribosomes (A site).

The basic structure of tetracyclines is composed of a naphthalene ring (a four ring structure). **Chlortetracycline and oxy-tetracycline** are most commonly used.



Tetracycline	R_1	R_2	R_3	R_4	Examples of producing organisms
Tetracycline	H	CH_3	OH	H	*Streptomyces aureus, S. flavus, S. antibioticus*
Chlortetracycline	Cl	CH_3	OH	H	*S. aureofaciciens, S. viridifaciens, S. flavus*
Oxytetracycline	H	CH_3	OH	OH	*S. antibioticus, S. cellulosae S. parvus, S. rimosus*
Minocycline	$N(CH_3)_2$	H	H	H	Semisynthetic
Doxycycline	H	CH_3	H	OH	Semisynthetic

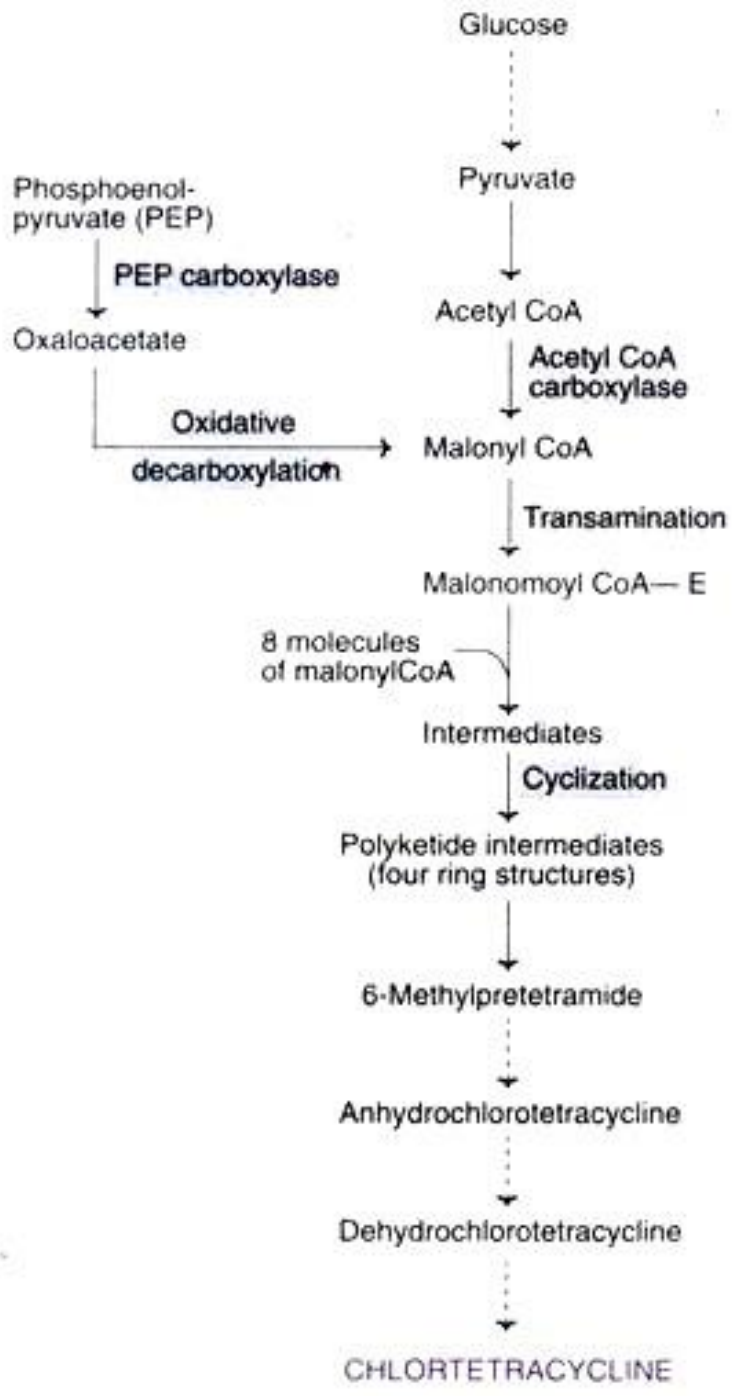
***Fig. 25.10** : Structures of some important tetracyclines along with the examples of organisms for their production.*

TETRACYCLINE

- Chlortetracycline was firstly isolated from the cultures of ***Streptomyces aureofaciens*** (in 1945). There are at least 20 streptomycetes identified now that usually produces a mixture of tetracyclines.

- High-yielding strains of *S. aureofaciens* and *S. rimosus* have been developed by using ultraviolet radiation and/or other mutagens (nitrosoguanidine). Such strains are very efficient for the production of chlortetracycline. Genetically engineered *S. rimosus* developed for increased synthesis of oxytetracycline.

- The pathway for the biosynthesis of tetracyclines is very complex. There are at least *72 intermediates* formed during the course of chlortetracycline biosynthesis, some of them have not been fully characterized.



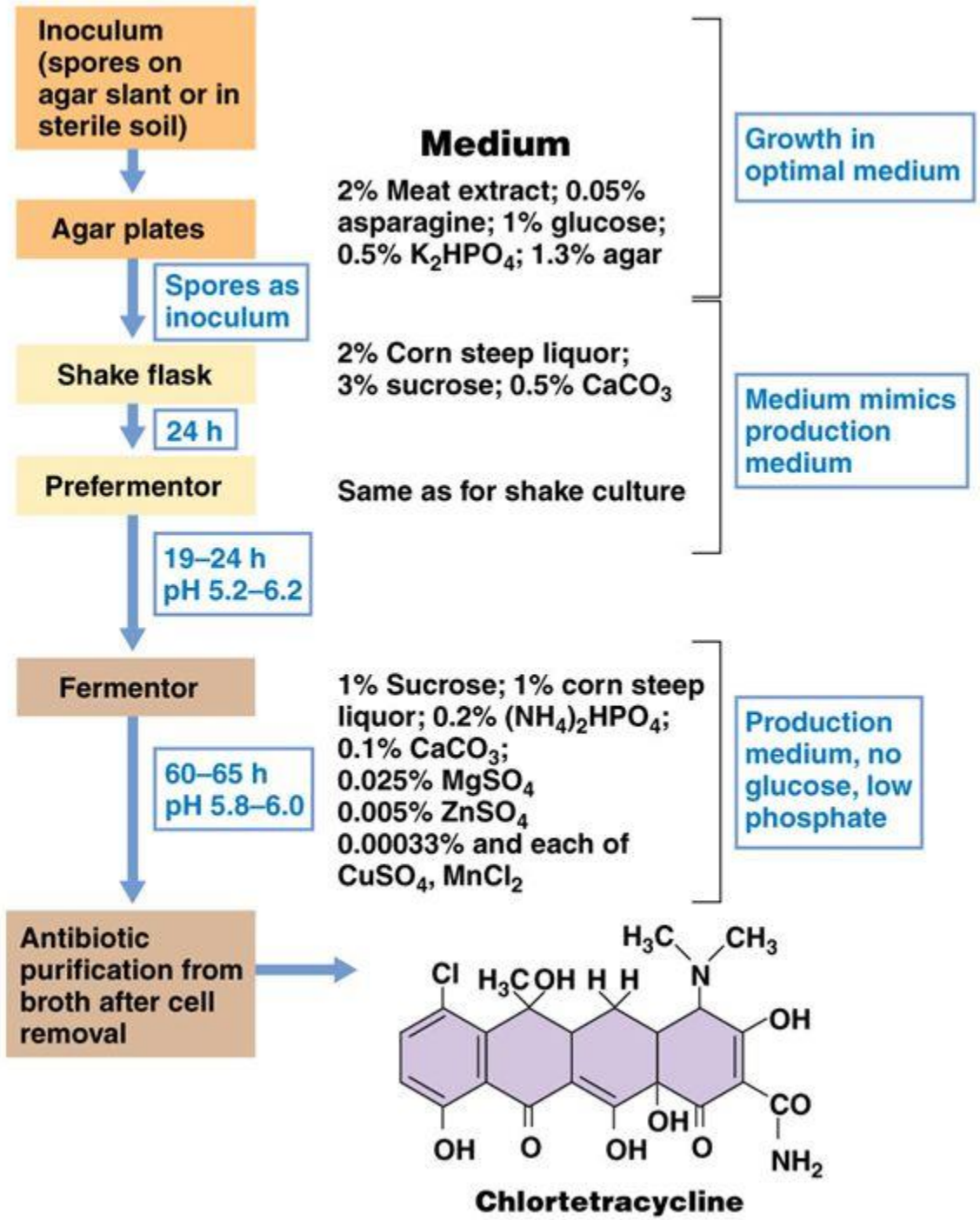
POLYKETIDE ANTIBIOTIC SYNTHESIS

- It refers to a group of antibiotics including tetracyclines that are synthesized by successive **condensation of small carboxylic acids** such as acetate, butyrate, propionate and malonate. The synthesis of polyketide antibiotics is comparable to that of long chain.
- As glucose gets oxidized, it forms **acetyl CoA** and then malonyl CoA. On transamination, the later gives malonomoyl CoA. The enzyme anthracene synthase complex binds to malonomoyl CoA and brings out the condensation of 8 molecules of malonyl CoA to form a polyketide intermediates (four ring structures). These intermediates undergo a series of reactions to finally produce chlortetracycline.

Regulation of biosynthesis

- Carbohydrate metabolism (particularly glycolysis) controls chlortetracycline synthesis.
- For more efficient synthesis of the antibiotic, glycolysis has to be substantially low.
- The addition of phosphate reduces chlortetracycline production.

Tetracycline Production



CHLORTETRACYCLINE PRODUCTION

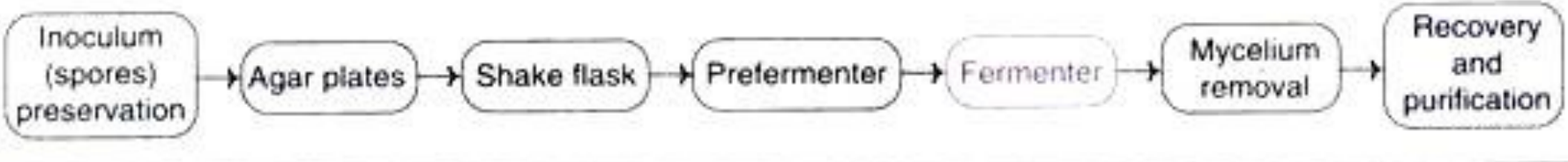


Fig. 25.12 : An outline of production chart for chlortetracycline.

- The fermentation medium consists of corn steep liquor, soy flour or peanut meal for the supply of nitrogen and carbon sources.
- Continuous feeding of carbohydrate is desirable for growth and antibiotic production. This can be done by supplying glucose or starch. For more efficiency, ammonium and phosphate has to be maintained at a low concentration.
- The ideal fermentation conditions are — temperature 27-30°C, pH-6.5-7.5, and aeration 0.8-1.0 vvm. The duration of fermentation is around 4 days.

Recovery of chlortetracycline:

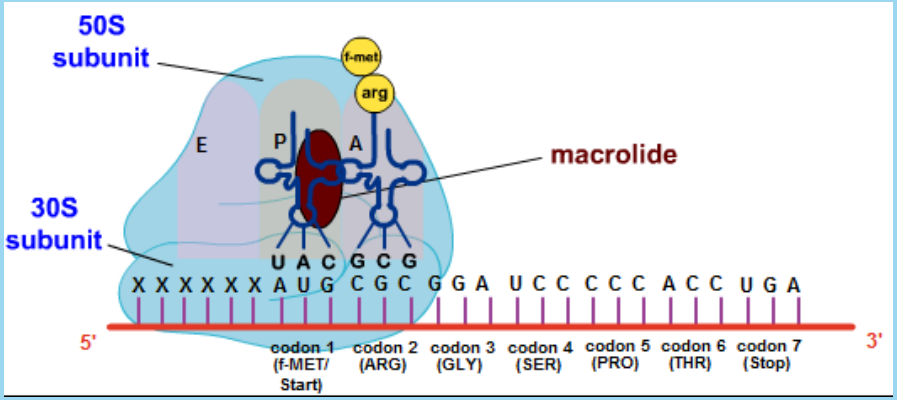
- At the end of the fermentation, the culture broth is filtered to remove the mycelium. The filtrate is treated with n-butanol or methylisobutylketone in acidic or alkaline condition for extracting the antibiotic. It is then absorbed to activated charcoal to remove other impurities. Chlortetracycline is eluted and crystallized.

MACROLIDES

- Macrolides are a group of antibiotics with **large lactone rings** (i.e. macrocylic lactone rings). They consist of 12-, 14-, or 16-membered lactone rings with 1-3 sugars linked by glycosidic bonds. The sugars may be 6-deoxyhexoses or amino sugars.

- **Erythromycin** and its derivative clarithromycin are the most commonly prescribed microlides. They are effective against Gram-positive bacteria. Clarithromycin is currently used to combat stomach ulcers caused by *H. pylori*. The macrolides inhibit the protein biosynthesis by binding to 50S ribosome. **Polyene macrolides** is a very large ring macrolides that many contain lactone rings in the range of 26-28. e.g. nystatin, amphotericin. These polyene macrolides are antifungal.

- Produced by actinomycetes as shown



Macrolid antibiotic	*Producing organism*
Erythromycin	*Streptomyces erythreus*
Oleandomycin	*S. antibiotics*
Pikromycin	*S. felleus*
Megalomicin	*Micromonospora inositola*
Tylosin	*S. fradiae*
Carbomycin A	*S. halstedii*
Leucomycins	*Streptoverticillium kitasatoensis*

BIOSYNTHESIS OF ERYTHROMYCIN

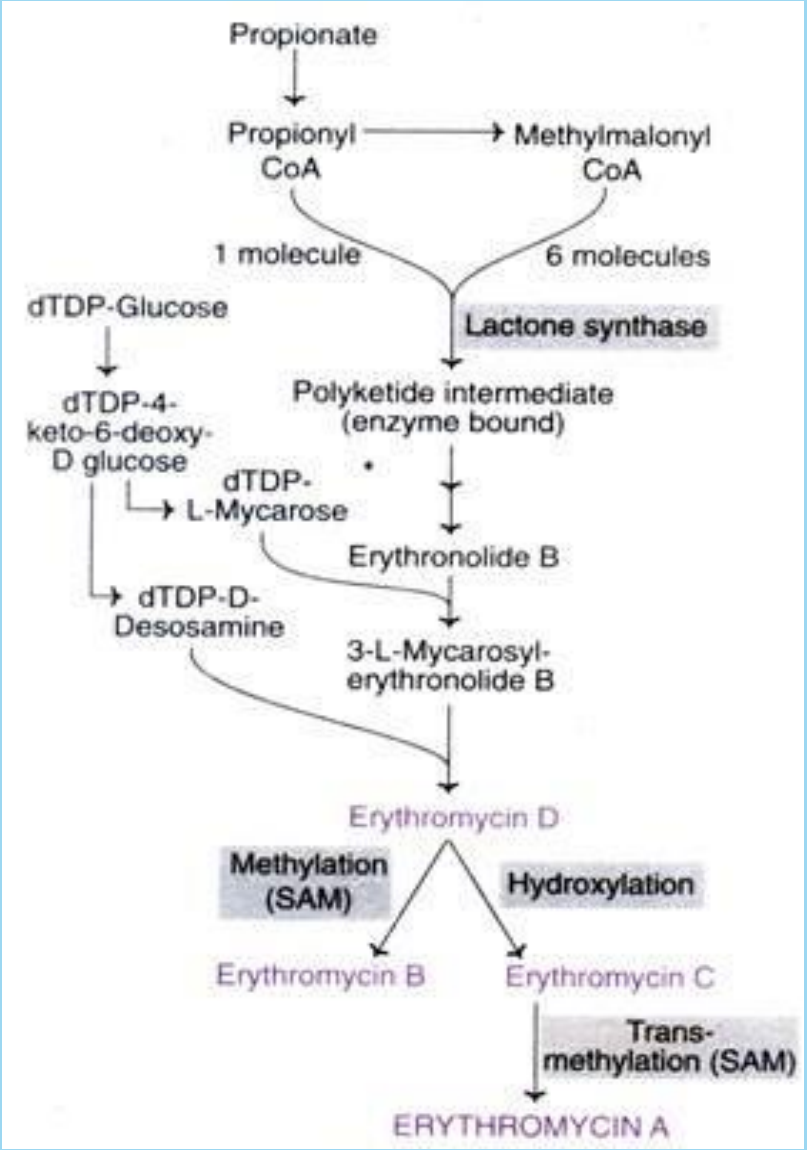
- In the biosynthesis of erythromycin, the lactone rings are contributed by acetate, propionate or butyrate while the sugar units are derived from glucose. Macrolide biosynthesis is a complex process which is analogous to fatty acid biosynthesis. The enzyme **lactone synthase** is a multi-enzyme complex which is comparable in its structure and function to fatty acid synthase complex.

Regulation of biosynthesis:

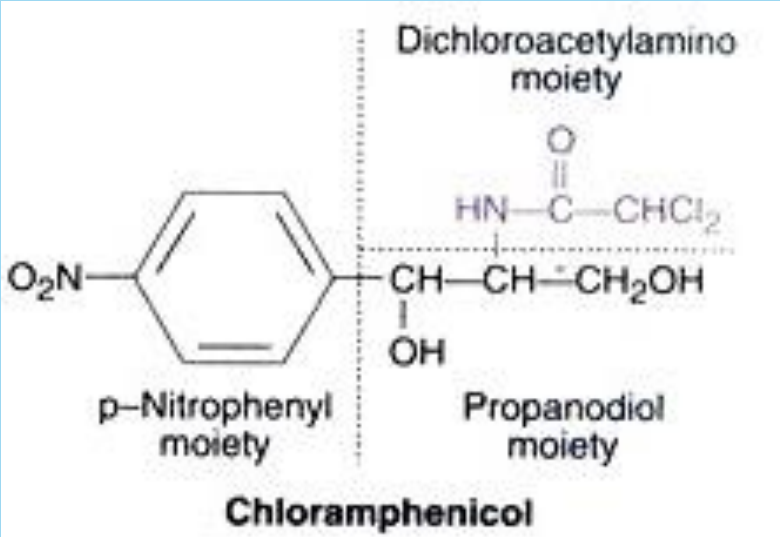
- End product inhibition is well documented. Erythronolide B inhibits the enzyme lactone synthase. The final product erythromycin has also been shown to inhibit transmethylase.
- Addition of propanol to the culture medium induces the synthesis of acetyl CoA carboxylase, and almost doubles the production of erythromycin.

Production Process:

- Industrial production of erythromycin is carried out by aerobic submerged fermentation. The culture medium mainly consists of soy meal or corn steep liquor, glucose (or starch), yeast extract and ammonium sulfate. Fermentation is carried out at 30-34°C for about 3-7 days. Conventional methods are used for the recovery and purification of erythromycin.



AROMATIC ANTIBIOTICS



Chloramphenicol

Griseofulvin

Chloramphenicol

- Chloramphenicol is a broad spectrum antibiotic that can act against several bacteria. But it is associated with side effects, damage to bone-marrow. It is a reserve antibiotic and selectively used. Chloramphenicol binds to 50S ribosomal subunit and blocks (peptidyltransferase reaction) protein biosynthesis.
- Chloramphenicol can be produced by ***Streptomyces venezuelae*** and ***S. omiyanesis***. However, chemical synthesis is mostly preferred for the commercial production of chloramphenicol.

Griseofulvin

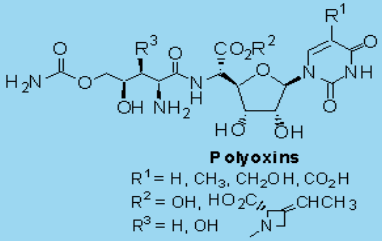
- Griseofulvin is an antibiotic that acts specifically on fungi with chitinous cell walls. It also treats plant diseases caused by *Biotrytis* and *Alternaria solani.*
- Commercial production of griseofulvin is carried out by employing ***Penicillium patulum***. Chemical synthesis is less used due to high cost. The fermentation is carried out by an **aerobic submerged process with a glucose rich** medium. Nitrogen is supplied by sodium nitrate. The optimal conditions for fermentation are—temperature 23-26°C, pH 6.8-7.3, aeration 0.8-1 vvm, and the period is 7-10 days.

NUCLEOSIDE ANTIBIOTICS

- There are several antibiotics (>200) which have nucleoside like structures. Nucleoside antibiotics have diverse structures and biological activities.
  - Puromycin is used to understand the ribosomal function in protein biosynthesis.
  - Neplanosin possesses antiviral activity.
  - Blasticidin S is a fungicide antibiotic used in plant pathology.

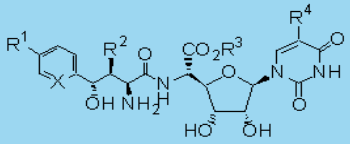
Producers:

- Puromycin — *Streptomyces alboniger*
- Neplamosin A — *Ampullariella regularis*
- Blasticidin S — *S. griseochromogenes*
- Polyoxins — *S. cacaoi*



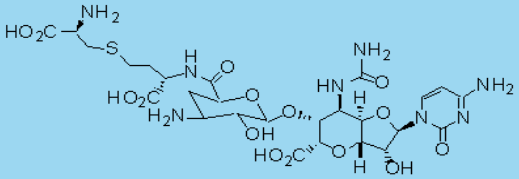
Polyoxins

R^1 = H, CH_3, CH_2OH, CO_2H
R^2 = OH, HO_2C, $CHCH_3$
R^3 = H, OH



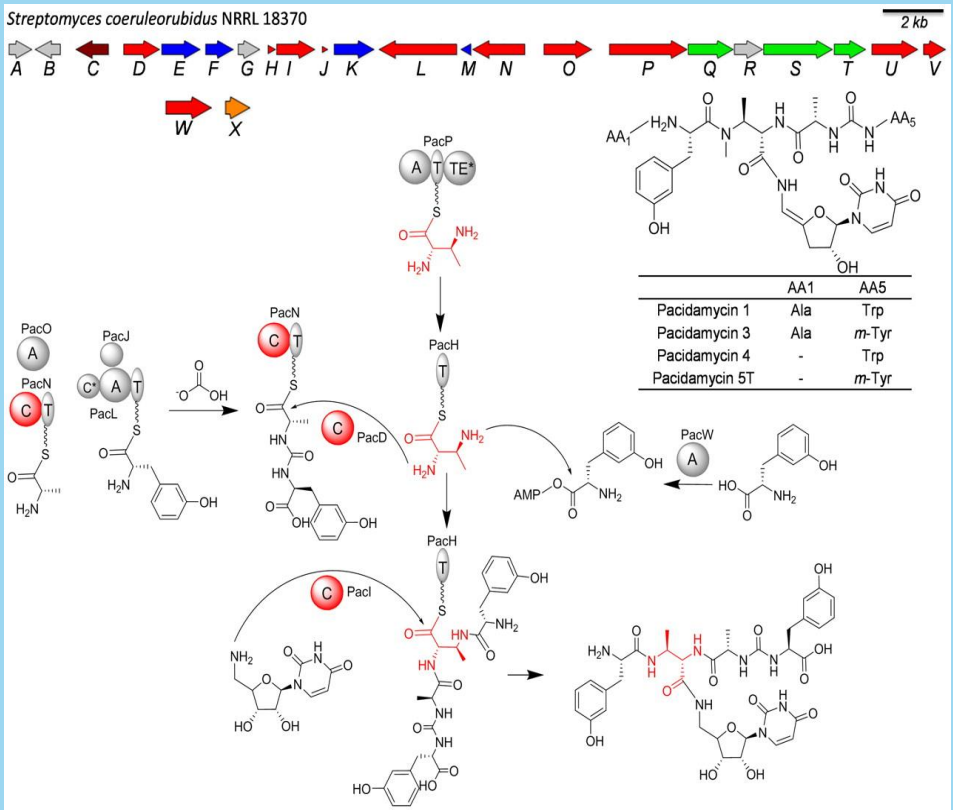
Nikkomycins

X = N, CH; R^1 = H, OH; R^2 = H, Me;
R^3 = OH, Glu, homo-Ser; R^4 = H, Me, CH_2OH



Ezomycins

A_1: Shown
A_2: No cystathionine
B_1: Uracil nucleobase
B_2: Uracil; no cystathionine

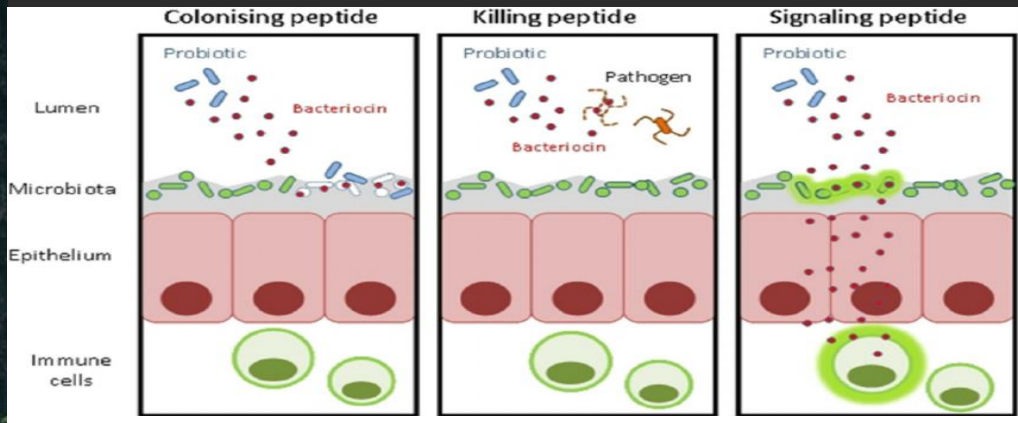


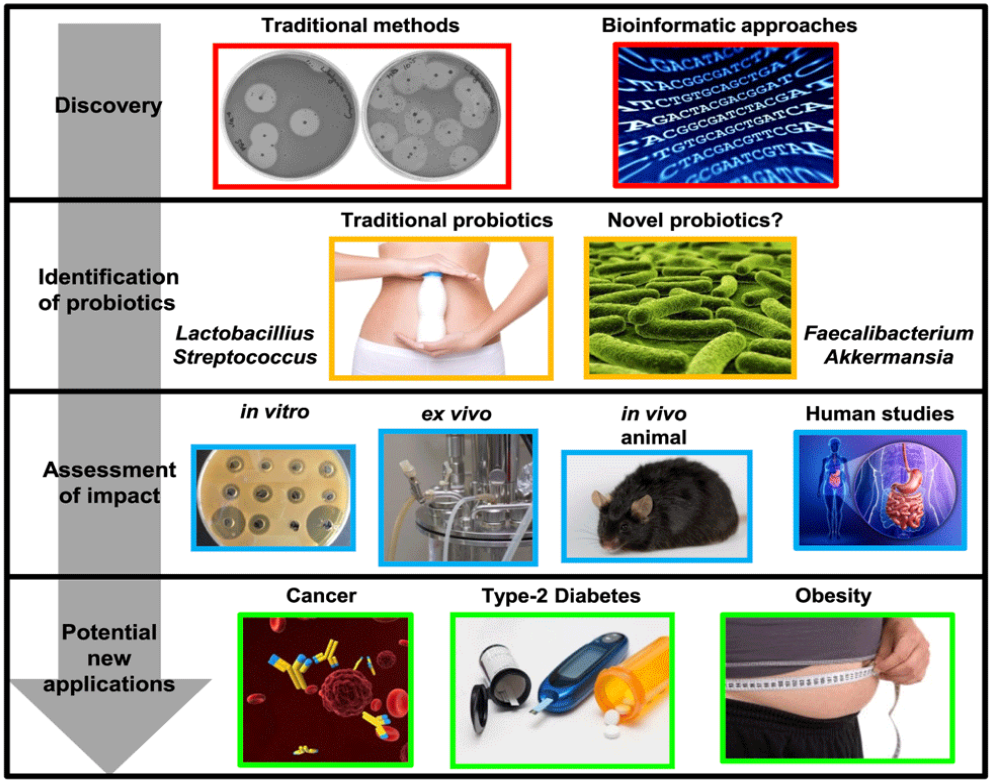
Bacteriocin vs Antibiotic

More Information Online WWW.DIFFERENCEBETWEEN.COM

	Bacteriocin	Antibiotic
DEFINITION	Bacteriocin is a proteinaceous toxin produced by bacteria against closely related bacterial strains	Antibiotic is an antimicrobial substance that kills or inhibits the growth of bacteria
ANTIBACTERIAL ACTIVITY	Narrow-spectrum	Broad-spectrum
PRODUCTION	Bacteriocins synthesis occurs in ribosomes by translation process as they are polymers of amino acids	Antibiotics are secondary metabolites resulting from their metabolic pathways
MOLECULAR WEIGHT	Usually have a high molecular weight	Usually have a low molecular weight
RIBOSOMAL ORIGIN	Ribosomal origin	Non-ribosomal origin
SUSCEPTIBILITY OF BACTERICIDAL AGENTS	Bacteriocin producers are insusceptible to bactericidal agents	Antibiotic producers are susceptible to bactericidal agents
MECHANISM OF ACTION	Bind to cell walls of sensitive microbes, motive ionic imbalances, and produce spores	Destroy cell walls, destroy cell membranes, inhibit protein synthesis and inhibit DNA and RNA synthesis

BACTERIOCIN

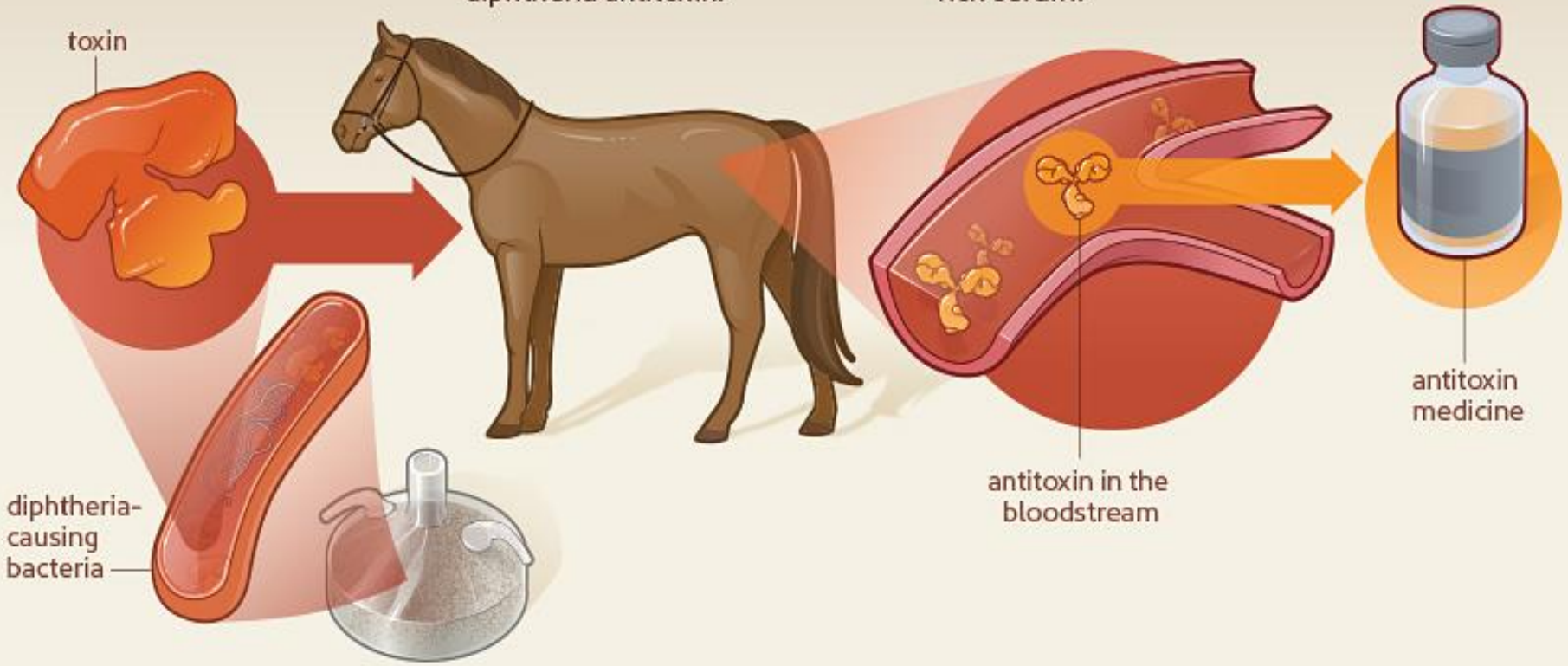




HOW DID THEY MAKE DIPHTHERIA ANTITOXIN?

SCIENTISTS LEARNED TO HARNESS THE IMMUNE SYSTEMS of some animals to produce antitoxin serums to use as medicines. Diphtheria antitoxin was one of these medicines. Doctors used diphtheria antitoxin to treat and prevent diphtheria, an often deadly childhood disease.

1. Scientists grow diphtheria-causing bacteria in the laboratory and harvest its toxin.
2. Next, researchers inject horses with the diphtheria toxin. As an immune response, the animals' blood produces diphtheria antitoxin.
3. Scientists collect blood from the horses and separate out the antitoxin rich serum.
4. Then, researchers purify the antitoxin serum for use as a medicine for people.



Mahidol University
Faculty of Science

SCBT431

ANTIBIOTICS PRODUCTION IN INDUSTRIES

Questions and Discussion

Asst.Prof. Dr.Adisak Romsang

Dept. Biotechnology, Fac. Science, Mahidol University
Room K610 Tel. 02201-5962 Email: adisak.rom@mahidol.ac.th

REFERENCES

- http://www.orthobullets.com/basic-science/9059/antibiotic-classification-and-mechanism
- http://www.biologydiscussion.com/antibiotics/antibiotics-types-top-7-types-of-antibiotics-with-diagram/10330
- http://www.emedexpert.com/classes/antibiotics.shtml
- http://www.biotopics.co.uk/microbes/penici.html
- http://www.madehow.com/Volume-4/Antibiotic.html
- http://www.yourarticlelibrary.com/essay/antibiotics-commercial-production-of-antibiotics/23351/
- https://www.scribd.com/doc/151871319/Fermentation-How-Antibiotics-are-Produced-By-Fermentation-Technology

FURTHER READING

Main article:

http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0290(19970705)55:1%3C216::AID-BIT22%3E3.0.CO;2-I/epdf

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5039525/
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4962027/
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4538407/
4. http://link.springer.com/article/10.1007%2Fs00253-015-6947-9
5. http://www.sciencedirect.com/science/article/pii/S1740674915000050
6. http://onlinelibrary.wiley.com/doi/10.1002/ardp.201500073/full
7. http://www.sciencedirect.com/science/article/pii/S0167799914002169
8. http://www.sciencedirect.com/science/article/pii/S0966842X14002170
9. http://link.springer.com/article/10.1007%2Fs00253-014-6228-z
10. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4166954/