

# MICROBIAL BIOTECHNOLOGY

Prof. Utpal Bora  
Department of Biosciences and Bioengineering  
Indian Institute of Technology Guwahati

## Lecture-20

### Lec 20: Microorganisms in pharmaceutical industry

Hello everyone. Welcome back to my course on microbial biotechnology. We are in module 6, where today we will discuss microorganisms in the pharmaceutical industry. We have divided this lecture into five sections. Section 1 will discuss microorganisms overall in the pharmaceutical industry.

Then, number 2: drugs from microorganisms. And number 3: biotransformation of steroids. Number 4: live organisms as therapeutic agents. Section 5 will deal with other therapeutic agents like vaccines, nutrient supplements, and probiotics. So, let us have an overview of pharmaceuticals and microorganisms.

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The profound impact of antibiotics on human health over the past six decades has been substantial, and these stand as one of the most critical groups of compounds synthesized by industrial microorganisms. Other significant healthcare products obtained from microbial fermentations or biotransformations include alkaloids, steroids, toxins, and vaccines. We'll have a detailed discussion of these in module 10. And others, such as vitamins, specific enzymes, and live microbial cell preparations, which are used as probiotics. Advancements in genetic engineering have enabled microorganisms to produce

an extensive array of mammalian proteins and peptides with diverse therapeutic properties, apart from the traditional uses of microorganisms to produce antibiotics and metabolites.

Among the medically significant ones, for example, insulin interference, human growth hormone, and monoclonal antibodies have established markets. Beyond these therapeutic agents that alleviate or treat diseases, many diagnostic products have stemmed from the application of microorganisms. What role do microorganisms play as pharmaceutical or therapeutic agents? They play a crucial role in the pharmaceutical industry across various aspects. For example, in drug development, microorganisms are used in the production of antibiotics, antifungals, and other medications.

## Overview



- The profound impact of antibiotics on human health over the past six decades has been substantial, and these stand as one of the most critical groups of compounds synthesized by industrial microorganisms.
- Other significant health-care products obtained from microbial fermentations or biotransformation include alkaloids, steroids, toxins, vaccines (detailed in [Module 10, lecture 2](#)), vitamins, specific enzymes, and live microbial cell preparations used as probiotics.
- Furthermore, advancements in genetic engineering have enabled microorganisms to produce an extensive array of mammalian proteins and peptides with diverse therapeutic properties.
- Among the medically significant ones, insulin, interferons, human growth hormone, and monoclonal antibodies have established markets.
- Beyond these therapeutic agents that alleviate or treat diseases, many diagnostic products stem from microorganisms.

Examples include penicillin molds for producing penicillin, streptomycetes for streptomycin, and various fungi and bacteria for producing other antibiotics. They are used for vaccine production. Many vaccines, including those against measles, mumps, rubella, and hepatitis, are made using weakened or killed forms of microorganisms or viruses. These stimulate the immune system to produce antibodies without causing disease. Then there are the biological therapeutics, or biotherapeutics.

Microorganisms are employed in the production of biotherapeutics like insulin, growth hormones, and clotting factors, which are used in treating various conditions; genetically engineered vectors in yeast are used to produce these biotherapeutics. Then we have probiotics: beneficial organisms like certain strains of bacteria and yeast are used as probiotics to promote gut health. Then enzymes: microbial enzymes are widely used in pharmaceutical processes, aiding in drug synthesis, purification, and formulation as drugs themselves. Let us discuss the screening for potent bioactive compounds. Several natural products have long been utilized globally in treating various human ailments, gaining recent attention for their therapeutic potential.



Microorganisms play a crucial role in the pharmaceutical industry across various aspects:

- 1. Drug Development:** Microorganisms are used in the development of antibiotics, antifungals, and other medications. For instance, *Penicillium* molds for penicillin, *Streptomyces* for streptomycin, and various fungi and bacteria for producing other antibiotics.
- 2. Vaccine Production:** Many vaccines, including those against measles, mumps, rubella, and hepatitis, are made using weakened or killed forms of microorganisms. These stimulate the immune system to produce antibodies without causing disease.
- 3. Biological Therapeutics:** Microorganisms are employed in the production of bio-therapeutics like insulin, growth hormones, and clotting factors used in treating various conditions. Genetically engineered bacteria and yeast are used to produce these therapeutic proteins.
- 4. Probiotics:** Beneficial microorganisms like certain strains of bacteria and yeast are used as probiotics to promote gut health.
- 5. Enzymes:** Microbial enzymes are widely used in pharmaceutical processes, aiding in drug synthesis, purification, formulation as a drug itself.

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Yet, natural products consisting of numerous chemical elements pose challenges in pinpointing the specific active compounds responsible for their effectiveness. The traditional approach to screening bioactive compounds in natural products involves isolating specific compounds, assessing their pharmacological activity, and studying their pharmacokinetics. However, this method is time-consuming and demanding. Diverse biological screening methods have been used for various targets such as DNA, proteins, and receptors. When biological targets are exposed to natural product extracts,

active compounds have the potential to selectively bind to these targets, while unbound compounds are removed readily. Subsequently, the isolated active compounds can be analyzed using chromatographic techniques paired with mass spectrometry or other detection systems. Some of the methods for screening bioactive compounds involve various techniques aimed at identifying and evaluating compounds with potential therapeutic effects. Some of these methods include HTS, or high-throughput screening. This is a technique used to rapidly test a large number of compounds against specific biological targets, allowing for the identification of potential drug candidates.



- Several natural products have long been utilized globally in treating various human ailments, gaining recent attention for their therapeutic potential. Yet, NPs, consisting of numerous chemical elements, pose challenges in pinpointing the specific active compounds responsible for their effectiveness.
- The traditional approach to screen bioactive compounds in NPs involves isolating specific compounds, assessing their pharmacological activity, and studying their pharmacokinetics. Though effective, this method is time-consuming and demanding.
- Diverse biological screening methods have been developed for various targets such as DNA, proteins, enzymes, and receptors.
- When biological targets are exposed to NP extracts, active compounds have the potential to selectively bind to these targets, while unbound compounds are removed.
- Subsequently, the isolated active compounds can be analyzed using chromatographic techniques paired with mass spectrometry or other detectors.

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Bioassays: these assays involve using living organisms, tissues, cells, or enzymes to test the biological activity of compounds and assess their potential effects on specific biological processes. Chromatography techniques like HPLC, GC, separate and analyze complex mixtures, aiding in isolating and identifying bioactive components. Mass spectrometry, MS identifies and quantifies compounds by measuring mass-to-charge ratios, enabling molecular characterization. Then there are computational methods like molecular docking, which simulates small molecule-target protein interactions, predicting binding affinity and efficacy.

## Methods of screening



Methods for screening bioactive compounds involve various techniques aimed at identifying and evaluating compounds with potential therapeutic effects. These methods include:

- 1. High Throughput Screening (HTS):** A technique used to rapidly test a large number of compounds against specific biological targets, allowing for the identification of potential drug candidates.
- 2. Bioassays:** These assays involve using living organisms, tissues, cells, or enzymes to test the biological activity of compounds and assess their potential effects on specific biological processes.



File: (top) Biological assay in a 96-well plate [Credit: J.N. Eskra, CC-BY-SA-4.0, via Wikimedia Commons]  
(bottom) CyBio-Well and CyBio High-throughput screening device [Credit: Monto, CC-BY-SA-3.0, via Wikimedia Commons]

Then we have cell-based assays. These assays assess compounds' effects on cellular processes like proliferation, apoptosis, and gene expression. Then we have receptor-based screening, disease-valuable compounds for interactions with specific disease-related receptors or proteins. Then we have enzymatic assays. These test compounds' impact on enzyme activity, providing insights into metabolic pathways or disease mechanisms.

Let us now move to section 2, where we will discuss drugs from microorganisms. We get various kinds of therapeutic molecules like antibiotics, antitumor drugs, immunosuppressants, enzyme inhibitors, anti-hypertensives, and antivirals from microorganisms. In this picture, you can see a Crystapen injection, which is basically benzylpenicillin sodium. This is used in the form of an injection. So, numerous drugs are derived from primary and secondary metabolites in microorganisms.

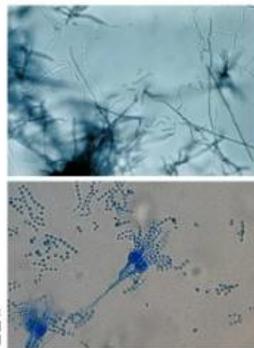


Of over 500,000 bioactive compounds screened, 20 to 25% are microbial in origin, with actinomycetes contributing 45% of these, fungi 38%, and unicellular bacteria around 70%. With rising antibiotic resistance, the need for new drugs is increasing, highlighting the importance of microorganisms in producing antibiotics and other medical substances for treating severe ailments. Let us have a brief discussion on the history of microbial drug discovery. The era of microbial drugs began in 1928 with Alexander Fleming's accidental discovery of penicillin. It was isolated in pure form.

### Use of microorganisms in drug design



- Numerous drugs are derived from primary and secondary metabolites in organisms, including microorganisms.
- Of over 500,000 bioactive compounds screened, 20-25% are microbial, with actinomycetes contributing 45%, fungi 38%, and unicellular bacteria 17%.
- With rising antibiotic resistance, the need for new drugs is increasing, highlighting the importance of microorganisms in producing antibiotics and other medicinal substances for treating severe ailments.



File: Microorganisms in drug design (top): *Streptomyces* sp. (Credit: CDC/Dr. David Berd, Public domain, via Wikimedia Commons)  
 (bottom) *Penicillium* sp. (Credit: Ajay Kumar Chaurasiya, CC BY-SA 4.0, via Wikimedia Commons)

and became a potent active bacterial agent during World War II, with its structure elucidated in 1945 by Fleming, Chain and Florey and you can see here Sir Alexander Fleming was sighted examining a bacterial culture plate in this figure. Fleming's work led to the isolation of other antibiotics like chloramphenicol and streptomycin. By 1997, 42% of top-selling drugs were derived from microbial metabolites. Advances in antimicrobial drugs such as tecoplanin for resistant gram-positive infections and glycylicyclines for tetracycline-resistant bacteria showed promises in combating resistant bacteria. Most

antibiotics are secondary metabolites produced by filamentous fungi and bacteria, particularly actinomycetes.

## Brief history of microbial drug discovery



File: Sir Alexander Fleming examining a bacterial culture plate  
[Credit: Anonymous, CC-BY-4.0, via Wikimedia Commons]

- The era of microbial drugs began in 1928 with Alexander Fleming's discovery of penicillin.
- It was isolated in pure form and became a potent antibacterial agent during World War II, with its structure elucidated in 1945 by Fleming, Chain, and Florey.
- Fleming's work led to the isolation of other antibiotics like chloramphenicol and streptomycin.
- By 1997, 42% of top-selling drugs were derived from microbial metabolites.
- Advances in antimicrobial drugs, such as teicoplanin for resistant Gram-positive infections and glycylicyclines for tetracycline-resistant bacteria, show promise in combating resistant bacteria.

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Although over 4000 antibiotics have been identified, only about 50 are consistently used in antimicrobial therapy with beta-lactams, aminoglycosides and tetracyclines being the most common. Others fail to meet key criteria due to issues like lack of selectivity, toxicity or high production costs. Some antibiotics also serve non-conventional roles. Actinomycin and mitomycin from *Streptomyces pusatus* and *Streptomyces cepitosis* respectively act as anti-tumor agents. Some antibiotics are used to manage microbial diseases in crops.

## Antibiotics



- Most antibiotics are secondary metabolites produced by filamentous fungi and bacteria, particularly actinomycetes.
- Although over 4,000 antibiotics have been identified, only about 50 are consistently used in antimicrobial therapy, with  $\beta$ -lactams, aminoglycosides, and tetracyclines being the most common.
- Others fail to meet key criteria due to issues like lack of selectivity, toxicity, or high production costs.
- Some antibiotics also serve non-conventional roles:
  - actinomycin and mitomycin, from *Streptomyces peucetius* and *S. caepitosus* respectively, act as antitumor agents
  - some antibiotics are used to manage microbial diseases in crops



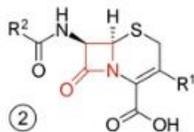
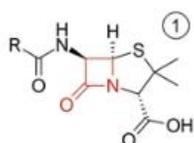
File: (top) Benzylpenicillin, 2,400,000 units, for deep intramuscular injection [Credit: DanZ, CC BY-SA 3.0, via Wikimedia Commons]  
(bottom) Novamoxin, amoxicillin trihydrate capsules [Credit: Bnramon, CC-BY-SA-3.0, via Wikimedia Commons]

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Certain antibiotics are used as growth promoters in animal feed. The beta-lactams. More than 100 beta-lactams, predominantly penicillins and cephalosporins, have obtained approval for human usage, constituting over half of the global antibiotic production. So in this figure 1, you can see the skeletal formula of the basic structure of penicillin. And in figure 2, the basic structure of cephalosporin antibiotics.

And you can see the beta-lactam ring, which is highlighted in red in both cases. Ingrained in both cases. These beta-lactam categories stand out for their broad safety margin. These compounds specifically target the synthesis of peptidoglycan, a crucial component of bacterial cell walls, absent in eukaryotic organisms, ensuring a high level of specificity. Their primary action involves inhibiting the cross-linking transpeptidation process, which leads to the formation of incomplete peptidoglycan, significantly compromising the structural integrity of bacterial cell walls.

## $\beta$ -lactams



- More than 100  $\beta$ -lactams, predominantly penicillins and cephalosporins, have obtained approval for human usage, constituting over half of the global antibiotic production.
- This category stands out for its broad safety margin.
- These compounds specifically target the synthesis of peptidoglycan, a crucial component of bacterial cell walls absent in eukaryotic organisms, ensuring a high level of specificity.
- Their primary action involves inhibiting the cross-linking transpeptidation process, which leads to the formation of incomplete peptidoglycan, significantly compromising the structural integrity of bacterial cell walls (refer to [Module 5 Lecture 4](#)).

File: Skeletal formulae of the basic structures of penicillin (1) and cephalosporin (2) antibiotics, highlighting the beta-lactam ring (red)  
[Credit: Fvasconcelos, Public domain, via Wikimedia Commons]

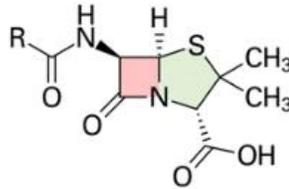
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You can refer to Module 5, Lecture 4, where we have discussed this. Let us now discuss penicillin in a little more detail. The fundamental structure of penicillin is 6-aminopenicillanic acid or 6-APA, which includes a thiazolidine ring combined with a beta-lactam ring. So here you can see the structure of penicillin showing this thiazolidine ring in green and the beta-lactam ring in red. The six-amino position of this structure contains various acyl substituents.

The beta-lactam thiazolidine framework is produced from L-alpha amino adipate, L-cysteine, and L-valine and is shared among penicillins, cephalosporins, and cephamycins. When side-chain precursors are not added to the fermentation medium of *Penicillium notatum* or *Penicillium chrysogenum*, a mixture of natural penicillins, particularly penicillin G and the more acid-resistant penicillin V, is obtained from cultured filtrates. So here in this picture, we can see at the top a penicillin fermentation vessel from the 1940s, and at the bottom, we can see a 1957 bioreactor which was used to grow the *Penicillium* mold.



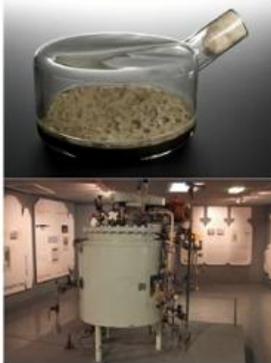
- The fundamental structure of penicillins is **6-aminopenicillanic acid (6-APA)**, which includes a **thiazolidine ring** combined with a  **$\beta$ -lactam ring**.
- The 6-amino position of this structure contains various acyl substituents (refer to Figure).
- This  $\beta$ -lactam-thiazolidine framework is produced from L- $\alpha$ -aminoadipate, L-cystine, and L-valine and is shared among penicillins, cephalosporins, and cephamycins.
- When side-chain precursors are not added to the fermentation medium of *P. notatum* or *P. chrysogenum*, a mixture of natural penicillins, particularly penicillin G (benzyl penicillin) and the more acid-resistant penicillin V (phenoxymethyl penicillin), is obtained from culture filtrates.



File: Structure of penicillin showing thiazolidine ring in green and  $\beta$ -lactam ring in red  
 (Credit: Yikrazuul, Public domain, via Wikimedia Commons)

Previously, penicillin was commercially manufactured using a stationary mat culture, and production was achieved through surface processes, including solid-state fermentation and surface liquid fermentation. Currently, penicillin is produced through suspension culture fermentation, where *Penicillium chrysogenum* is cultivated under controlled agitation. The process is entirely aerobic and utilizes a fed-batch method to maximize efficiency. Here, you can see the diagrammatic representation of the general scheme for penicillin production. First, we prepare the media.

Penicillin fermentation



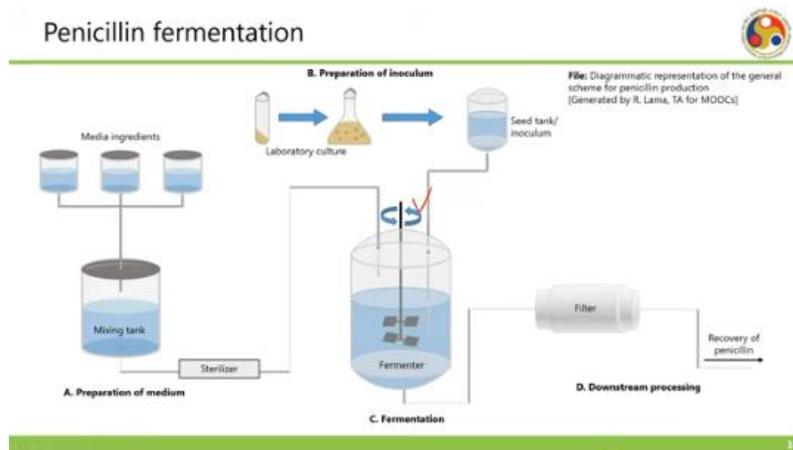
- Previously, penicillin was commercially manufactured using a stationary mat culture and production was achieved through surface processes, including solid-state fermentation and surface liquid fermentation.
- Currently, penicillin is produced through suspension culture fermentation, where *Penicillium chrysogenum* is cultivated under controlled agitation. The process is entirely aerobic and utilizes a fed-batch method to maximize efficiency.

File: (top) Penicillin fermentation vessel from 1940s England  
 (Credit: Medical Photographic Library, CC-BY-4.0, via Wikimedia Commons)  
 (bottom) A 1957 fermentor (bioreactor) used to grow *Penicillium* mold  
 (Credit: Matt Brown, CC BY-2.0, via Wikimedia Commons)

Various ingredients are put into a tank for mixing, sterilized, and then fed into the fermenter or bioreactor. Next, the inoculum is prepared. We start with a small inoculum, then proceed to laboratory culture, which is fed into a seed tank to increase the quantity. This inoculum is then added to the fermenter. Thus, both the media and inoculum are introduced into the fermenter.

The fermentation process takes place, followed by downstream processing and recovery of the penicillin. This, in brief, is the process of penicillin fermentation. Penicillin

fermentation uses glucose, lactose, and corn steep liquor (CSL) in the fermentation media. Fermentation lasts around six days and is conducted at 26 degrees Celsius, with the pH maintained at approximately 6.5. The fermentation occurs in three phases.



During the active growth phase, the nutrients in the medium are rapidly consumed while maintaining a constant pH. Towards the end of the active growth phase, the nutrients are depleted leading to release of ammonia due to deamination reactions causing the pH to rise to 7 to 7.5, an optimal range for penicillin synthesis. The late growth phase is succeeded by the idiophase where penicillin synthesis occurs at growth halts due to nutrient depletion. It is essential to prevent the late idiophase from progressing too far to avoid cell lysis and subsequent penicillin degradation. Penicillin biosynthesis comprises three primary stages, the catalytic step, the oxidative step and the chain exchange.

## Fermentation

- Penicillin fermentation uses glucose, lactose and **cornsteep liquor** (CSL) in the fermentation media. Fermentation lasts for around 6 days, and the fermentation process is usually conducted at 26°C and the pH is maintained at 6.5.
- **Fermentation occurs in three phases:**
  - During the active growth phase, the nutrients in the medium are rapidly consumed while maintaining a constant pH.
  - Towards the end of the active growth phase, the nutrients are depleted, leading to release of ammonia due to deamination reactions, causing the pH to rise to 7-7.5, an optimal range for penicillin synthesis.
  - The late growth phase is succeeded by the **idiophase**, where penicillin synthesis occurs **as growth halts due to nutrient depletion**. It's essential to prevent the late idiophase from progressing too far to avoid cell lysis and subsequent penicillin degradation.

During the catalytic phase, ACV synthetase enzyme facilitates the condensation of cysteine, valine and alpha-amino adipate into ACV tripeptide. This particular molecule is produced by the condensation of these three molecules. The tripeptide ACV then undergoes

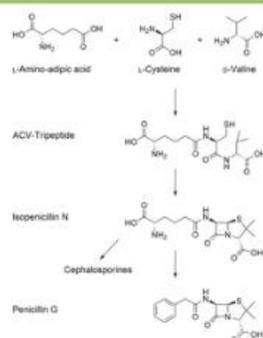
oxidative ring closure forming a bicyclic ring facilitated by the isopenicillin N synthase to give isopenicillin N. So, due to this ring closure the isopenicillin N is produced.

The final step involves the substitution of L-aminoadipate. The conversion of isopenicillin N into penicillin G is achieved by utilizing a two enzyme system comprising acyl coenzyme synthetase and acyl coenzyme racemase. So this is the final product, the penicillin G coming out as a result of these two enzyme system interactions. Now apart from these natural penicillin, we have many derivatives of penicillin. Semisynthetic penicillins are created through a combination of microbial, enzymatic or chemical processes.

## Synthesis of penicillin



- Penicillin biosynthesis comprises three primary stages: the catalytic step, oxidative process, and chain exchange.
- During the catalytic phase, the **ACV synthetase enzyme** facilitates the condensation of **cysteine, valine, and alpha-aminoadipate** into the tripeptide ACV.
- The tripeptide ACV then undergoes oxidative ring closure, forming a bicyclic ring facilitated by the **isopenicillin N synthase**, to give **isopenicillin N**.
- The final step involves the substitution of **L-aminoadipate**. The conversion of isopenicillin N into **Penicillin G** is achieved by utilizing a two-enzyme system comprising **Acyl-CoA synthetase** and **Acyl-CoA racemase**.



File: Pathway for penicillin biosynthesis [Credit: Cacycle, CC-BY-SA-3.0, via Wikimedia Commons]

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The key intermediary in this production is 6-APA, essential in the synthesis of semisynthetic penicillin. For commercial production of 6-APA, hydrolysis of penicillin G and V is carried out using an enzyme like penicillin acylase or amides. Microbial hydrolysis of natural penicillins occurs optimally at higher temperatures and at alkaline pH. Once obtained, 6-APA is chemically bonded with the desired side chain. Let us now discuss the anti-tumor drugs which are produced by microbial fermentation.

## Derivatives of penicillin



- Semi-synthetic penicillins are created through a combination of microbial, enzymatic, or chemical processes.
- The key intermediary in this production is **6-aminopenicillanic acid (6-APA)**, essential in the synthesis of semi-synthetic penicillin.
- For commercial production of 6-APA, hydrolysis of penicillin G and V is carried out using an enzyme like **penicillin acylase** or **amidase**.
- Microbial hydrolysis of natural penicillins occurs optimally at higher temperatures and an alkaline pH.
- Once obtained, 6-APA is chemically bonded with the desired side chain.

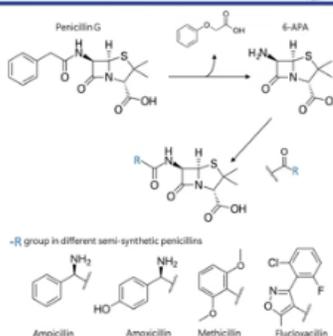


Figure: Examples of semi-synthetic penicillin  
[Generated by R. Lama, TA for MOOCs]

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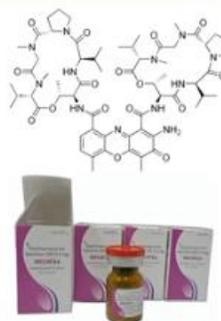
Microbial metabolites play a crucial role in cancer therapy. Over 60% of approved small-molecule drugs derived from initial products or their modified forms often originate from streptomycetes, initially screened for tumor cell activity. Here we can see actinomycin D, a widely used chemotherapeutic drug which is marketed as dactinomycin. The use of microbial metabolites as anti-tumor agents dates back to the discovery of actinomycin in the 1940s. Since then, numerous compounds with anti-cancer properties have been isolated from natural sources.

Notable among these approved drugs are actinomycin D, anthracyclines, bleomycin, mitomycin C, anthracenones, enediynes, taxol, and epothilones. Here we can see a list of anti-tumor drugs like mitomycin, anthracyclines, streptozotocin, bleomycins, epothilones, taxol, etc. We can also see the various organisms which produce these drugs. For example, mitomycin is produced by *Streptomyces caespitosus*. The mechanism of action involves forming covalent bonds across the DNA strands, affecting hypoxic cells in certain neoplasms like gastric, colorectal, and lung cancer.

## Antitumor drugs



- Microbial metabolites play a crucial role in cancer chemotherapy, with over 60% of approved small molecule drugs derived from natural products or their modified forms, often sourced from *Streptomyces*, initially screened for tumor cell activity.
- The use of microbial metabolites as anti-tumor agents dates back to the discovery of actinomycin in the 1940s.
- Since then, numerous compounds with anticancer properties have been isolated from natural sources.
- Notable approved drugs include **actinomycin D**, **anthracyclines** (daunorubicin, doxorubicin, epirubicin, pirarubicin, valrubicin), **bleomycin**, **mitomycin C**, **anthracenones** (mithramycin, streptozotocin, pentostatin), **enediynes** (calicheamicin), **taxol**, and **epothilones**.



File: Actinomycin D, a widely used chemotherapy drug, marketed as Dactinomycin  
[Credit: Edgar, Public Domain, via Wikimedia Commons]

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Similarly, we have the anthracyclines produced by *S. peucetius*, and they mostly act through intercalation, inserting between adjacent base pairs of DNA, making them effective against lung cancer. Then you have streptozotocin. Produced by *Streptomyces achromogenes*, it causes DNA alkylation, which is toxic to cells by damaging DNA and is effective against pancreatic islet cancer cells. Then we have the bleomycins, produced by *Streptomyces verticillus*. They induce DNA strand breaks and are effective against carcinomas and lymphomas.

Then we have Epothilones, produced by *Sorangium cellulosum*. Their mechanism of action includes stabilizing microtubules and inhibiting P-glycoprotein efflux pumps, making them effective against breast cancer. Then we have Calicheamicins, which are produced by *Micromonospora*. They act by cleaving double-stranded DNA and are effective against acute myelogenous leukemia.

Then we have Taxol or paclitaxel, produced by various organisms like *Taxomyces* and *Nodulosporium*. It acts by promoting tubulin polymerization and interfering with microtubule breakdown during cell division, making it effective against refractory ovarian cancer, breast cancer, and advanced forms of Kaposi's sarcoma. Let us discuss another class of compounds obtained from microbes. These are the immunosuppressants. Immunosuppression refers to reducing the immune response to prevent graft or transplant rejection or to treat autoimmune diseases.

### Some anti-tumor drugs



Drug	Organism	Mechanism of action	Effective against
Mitomycins	<i>Streptomyces caespitosus</i>	forming covalent bonds across DNA strands	against hypoxic cells in certain neoplasms like gastric, colorectal, and lung cancer
Anthracyclines	<i>Streptomyces peucetius</i>	intercalating function and inserts in between the adjacent base pair of DNA	lung cancer
Streptozotocin	<i>Streptomyces achromogenes</i>	alkylating agent, toxic to cells by causing damage to DNA	pancreatic islet cell cancer
Bleomycins	<i>Streptomyces verticillus</i>	induce DNA strand nicks	carcinomas and lymphomas
Epothilones	<i>Sorangium cellulosum</i>	stabilizing microtubulins and inhibiting P-glycoprotein efflux pumps	Breast cancer
Calicheamicins	<i>Micromonospora echinospora</i>	cleavage of double-stranded DNA	cute myelogenous leukemia
Taxol (Paclitaxel)	<i>Taxomyces andrieanae</i> , <i>Nodulosporium sylviforme</i>	promoting tubulin polymerization and interfering with normal microtubule breakdown during cell division	refractory ovarian cancer, breast and advanced forms of Kaposi's sarcoma

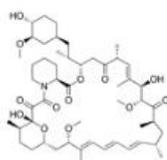
Table 15.2: Bioactive compounds from microorganisms with potent anti-tumor activities

Several microbial compounds have been identified with the capability to suppress the immune response. Here, you can see the structure of sirolimus, which is marketed as a drug and is a known immunosuppressant. Cyclosporine, derived from the fungus *Tolypocladium inflatum*, is a calcineurin inhibitor and has been used since the 1980s. Then, there is

rapamycin from *Streptomyces*. It complexes with FK protein 12 to deactivate the mammalian target of rapamycin.

Then, we have tacrolimus from *Streptomyces tsukubensis*. This is also a calcineurin inhibitor but shows 100 times more potency than cyclosporine. All these actions ultimately affect the secretion of interleukin-2, blocking the activation of B cells and T cells. Another important class of compounds is the enzyme inhibitors, which are obtained from microbes. Enzyme inhibition is a mechanism of disease therapy by blocking certain disease pathways.

## Immunosuppressants



File: Structure of sirolimus with a marketed drug [Credit: Vaccinationist, Public Domain, via Wikimedia Commons]

- **Immunosuppression** refers to reducing the immune response to prevent graft or transplant rejection or to treat autoimmune diseases.
- Several microbial compounds have been identified with the capability to suppress the immune response:
  - **Cyclosporine**, derived from the fungus *Tolypocladium inflatum*, is a calcineurin inhibitor and has been used since the 1980s.
  - **Rapamycin** (Sirolimus), from *Streptomyces hygroscopicus*, complexes with FKprotein12 to deactivate the mammalian target of rapamycin (mTOR).
  - **Tacrolimus**, from *S. tsukubensis*, is also a calcineurin inhibitor but shows 100 times more potency than cyclosporine.
- All these actions ultimately affect the secretion of IL-2, blocking the activation of B-cells and T-cells.

Enzyme inhibitors are valuable tools for studying enzyme structure, reaction mechanisms, as well as for applications in medicine and agriculture. Microbes produce various enzyme inhibitors with industrial uses, including clavulanic acid, a beta-lactamase inhibitor, and statins, which are cholesterol-lowering drugs.

Other inhibitors target enzymes such as glucosidases, amylases, lipases, proteases, and xanthine oxidases. Here is a table of various enzyme inhibitors obtained from microbial sources, which also shows the particular enzyme inhibited by each inhibitor used as a drug. In this column, you can find the pharmacological importance of these enzyme inhibitors, which are used as drugs. For example, the inhibitor clavulanic acid, produced by *Streptomyces clavuligerus*, inhibits beta-lactamases. Its pharmacological importance lies in its ability to inhibit beta-lactamases, helping overcome antibiotic resistance against beta-lactam antibiotics.

## Enzyme inhibitors



- Enzyme inhibitors are valuable tools for studying enzyme structures and reaction mechanisms, as well as for applications in medicine and agriculture.
- Microbes produce various enzyme inhibitors with industrial uses, including **clavulanic acid** (a  $\beta$ -lactamase inhibitor) and **statins** (cholesterol-lowering drugs).
- Other inhibitors target enzymes such as **glucosidases**, **amylases**, **lipases**, **proteases**, and **xanthine oxidase**.



**File:** (top) Amoxiclav clavulanic acid solution: produced by two generic brands Sandoz and Mylan (**Credit:** Barbirosia, CC-BY-SA-3.0, via Wikimedia Commons) (bottom) A package and pill of atorvastatin 40mg (Lipitor) (**Credit:** Panthro, CC-BY-SA-3.0, via Wikimedia Commons)

Other examples include Acarbose, produced by Actinoplanes, which inhibits alpha-glucosidases and sucrase. This reduces starch breakdown in the intestine, making it useful in combating diabetes. Amylase inhibitors, produced by Streptomyces, inhibit amylase, preventing dietary starches from being absorbed—useful for diabetes, obesity, and hyperlipidemia. These are currently very important lifestyle diseases. Lipstatin, produced by Streptomyces toxytricini, inhibits pancreatic lipase, interfering with gastrointestinal fat absorption.

Protease inhibitors like antipain, leupeptin, and chymostatin are produced by various Streptomyces species. They target the enzyme protease. Their pharmacological importance includes uses against emphysema, arthritis, pancreatitis, cancer, and AIDS. Hydroxyakalone, produced by Agrobacterium aurantiacum, targets xanthine oxidase, preventing excessive uric acid accumulation. As shown here, different enzyme inhibitors help address various common human diseases.

### Sources and pharmacological importance of enzyme inhibitors



Drug	Organism	Enzyme inhibited	Pharmacological importance
Clavulanic acids	<i>Streptomyces clavuligerus</i>	$\beta$ -lactamases	Inhibitor of $\beta$ -lactamases, helps in overcoming antibiotic resistance against $\beta$ -lactam group of antibiotics
Acarbose	<i>Actinoplanes</i> sp. SE50	$\alpha$ glucosidase and sucrase	Decreased starch breakdown in the intestine, useful in combating diabetes
Amylase inhibitors	Paim	<i>Streptomyces corchorushii</i>	prevent dietary starches from being absorbed by the body, useful diabetes, obesity and hyperlipemia
	TAI-A, TAI-B	<i>Streptomyces calvus</i> TM-521	
Lipstatin	<i>Streptomyces toxytricini</i>	pancreatic lipase	interferes with the gastrointestinal absorption of fat
protease inhibitors	antipain	<i>Streptomyces yolosukaeensis</i>	emphysema, arthritis, pancreatitis, cancer and AIDS
	leupeptin	<i>Streptomyces roseochromogenes</i>	
	chymostatin	<i>Streptomyces hygroscopicus</i>	
hydroxyakalone	<i>Agrobacterium aurantiacum</i>	xanthine oxidase	Prevents excessive accumulation of uric acid

**Table 15.3:** Examples of enzyme inhibiting drugs

The next important type of compounds produced with the help of microbes or obtained from microbes are the anti-hypertensives. Particularly, statins are potent anti-hypertensive drugs which work by reducing cholesterol levels through the inhibition of HMG coenzyme A reductase. So, blocking these enzymes in the liver activates low-density lipoprotein receptors, leading to increased LDL clearance from the bloodstream and decreased blood cholesterol levels. Under the class of drugs, the zaragozic acids act as competitive inhibitors of squalene synthase, blocking the binding of farnesyl pyrophosphate. This inhibits the conversion of farnesyl pyrophosphate to squalene, which ultimately leads to a decrease in cholesterol synthesis.

This cholesterol-lowering action mitigates the risk of cardiovascular disease and prevents stroke. So here you can see the statin which is inhibiting the HMG coenzyme A reductase. And then here it is the zaragozic acid, which is a competitive inhibitor of the squalene synthase, thereby blocking the binding of farnesyl pyrophosphate and inhibiting the conversion of farnesyl pyrophosphate to squalene, which ultimately leads to a decrease in cholesterol synthesis. What are the sources of anti-hypertensives? The first statin, compactin, was initially derived from *Penicillium brevicompactum* and later from *Penicillium citrinum* as an antibiotic product.

## Antihypertensives

- **Statins** are potent antihypertensive drug which work by reducing cholesterol levels through the **inhibition of HMG-CoA reductase**.
- Blocking of this enzyme in the liver activates low-density lipoprotein (LDL) receptors, leading to increased LDL clearance from the bloodstream and decreased blood cholesterol levels.
- Another class of drugs, the **zaragozic acids**, act as **competitive inhibitor of squalene synthase**, blocking the binding of farnesyl pyrophosphate. This inhibits the conversion of farnesyl pyrophosphate to squalene, which ultimately leads to a decrease in cholesterol synthesis.
- This cholesterol-lowering action mitigates the risk of cardiovascular disease and prevents stroke.

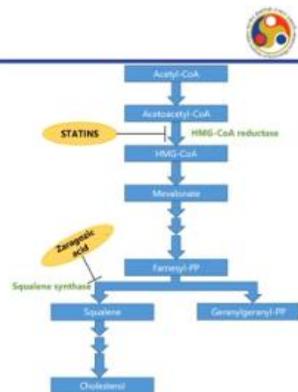


Figure: Synthesis of cholesterol and action of statins and zaragozic acids  
[Figure generated for usage in MOOCs in Bioengineering Research Laboratory (BERL), IIT

Derivatives of compactin, such as lovastatin, an ethylated form, were isolated in the 1970s from *Monascus ruber* and *Aspergillus terreus* broths, becoming the first commercially marketed statin approved by the FDA in 1987. Another statin, pravastatin, is produced through distinct biotransformation methods from compactin by *Streptomyces carbophilus* and *Actinomadura* species. Zaragozic acids A, B, and C were initially identified from distinct sources and are the identifiable sterile fungal cultures, *Sporormiella intermedia* and *Leptodontidium elatius*, near the European city of Zaragoza, Spain, hence the name

zaragozic acid. And *Leptodontidium elatius* near the European city of Zaragoza, Spain, hence the name zaragozic acid. Now, let us discuss some of the metabolites which act as antivirals.

### Sources of antihypersensitives



- The first statin, **compactin (mevastatin)**, was initially derived from *Penicillium brevicompactum* and later from *Penicillium citrinum* as an antibiotic product.
- Derivatives of compactin, such as **lovastatin (monacolin K, mevinolin)**, an ethylated form was isolated in the 1970s from *Monascus ruber* and *Aspergillus terreus* broths, becoming the first commercially marketed statin, approved by the FDA in 1987.
- Another statin, **pravastatin**, is produced through distinct **biotransformation methods** from compactin by *Streptomyces carbophilus* and *Actinomadura sp.*
- **Zaragozic acids A, B, and C** were initially identified from distinct sources: an unidentifiable sterile fungal culture, *Sporormiella intermedia*, and *Leptodontidium elatius*, near the European city of Zaragoza, Spain (hence the name).

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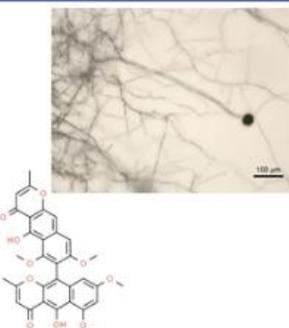
Since the 1980s, HIV/AIDS has posed a huge risk to human health, especially to vulnerable communities in society. And recently, with the COVID-19 pandemic, It has become much more apparent that viruses pose a huge risk to human health, and as such, there is a need for potent antiviral compounds. This has led to an increase in the study of antiviral drugs from various sources, and more than a hundred different drugs from microbial sources are now known. Recent studies indicate that natural compounds synthesized by microorganisms, such as aurasperone A,

neochinin A and B, aspulvinone D, M, and R, exhibit significant in vitro potency against the SARS-CoV-2 virus. Here, you can see that aurasperone A is an anti-SARS-CoV-2 drug obtained from *Aspergillus niger*. These are some of the bioactive compounds which have antiviral activities. You have the metabolite name in this column here. Then you have the organisms from which these metabolites are obtained.

## Antivirals



- Since the 1980s HIV-AIDS has posed a huge risk to human health, especially the vulnerable communities of the society
- With the recent Covid-19 pandemic, it has become much apparent that viruses pose a huge risk to human health and as such, there is need for potent antivirals.
- This has led to an increase in study of antiviral drugs from various sources and more than a hundred different drugs from microbial sources are now known.
- Recent studies indicate that natural compounds synthesized by microorganisms, such as aurasperone A, neochininulin A and B, and aspulvinone D, M, and R, exhibit significant in vitro potency against SARS-CoV-2.



**File:** Aurasperone A is an anti-SARS-CoV-2 drug obtained from *Aspergillus niger* (pictured)  
**[Credit:** Anonymous, CC-BY-SA-3.0, via Wikimedia Commons]

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And you can see the name of the virus against which these are used. Here, you can see the mechanism of action. Let us now move on to section 3, where we will discuss the biotransformation of steroids. We will first have an overview of the structure of steroids and the general scheme for steroid biotransformation, and then finally, the industrial-scale biotransformation. Steroids are known as cyclopentanoperhydrophenanthrene.

### Some bioactive compounds with anti-viral activities



Metabolite	Group	Source organism	Effective against	Mechanism of action
Rubrumlines A-O	Peptide	<i>Eurotium rubrum</i>	H1N1	Hemagglutinin
Sorbicatechol A and B	Polyketone	<i>Penicillium chrysogenum</i>	H1N1	
Diketopiperazines	Peptide	<i>Aspergillus versicolor</i>	HCV	HCV protease
Oleanolic acid	Terpenoids	<i>Saccharomyces cerevisiae</i>	HCV	Inhibition of genome replication and transcription
Abtarnariol	Polyketone	<i>Pleospora tarda</i>	HSV	Viral replication
Balticolid	Polyketone	Ascomyceteous strain	HSV1	Viral replication
Betulinic acid	Terpenoids	<i>S. cerevisiae</i> , <i>Yarrowia lipolytica</i>	HIV	Viral release inhibition
Sclerotiorin	Polyketone	<i>Penicillium sclerotiorum</i>	HIV	HIV-1 integrase and protease
Semicochlidinol A and B	Alkaloid	<i>Chrysosporium merdarium</i>	HIV	HIV protease
Aspulvinone D, M and R	Polyphenol	<i>Cladosporium</i> sp.	SARS-CoV-2	Viral replication
Aurasperone A	Polyphenol	<i>Aspergillus niger</i>	SARS-CoV-2	Viral replication
Neochininulin A	Alkaloid	<i>Aspergillus fumigatus</i>	SARS-CoV-2	Viral replication

**Table 15.4:** Bioactive compounds from microorganisms with potent anti-viral activities

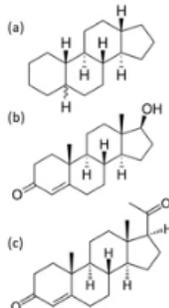
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These are natural compounds with various therapeutic properties. In this figure, we can see certain structures of common steroids with a steroid nucleus, including testosterone and progesterone. More than 250 steroids and their derivatives are found in various organisms, such as insects, plants, vertebrates, and fungi. It has been observed that even minor alterations in the molecular structure of steroids can significantly impact their biological activity. Consequently, extensive research has been undertaken to enhance the activity of existing steroid compounds and synthesize novel steroidal compounds with pharmacological efficacy.

## Overview



- Steroids, also known as cyclo-pentano-per-hydro-phenanthrene, are natural compounds with various therapeutic properties.
- More than 250 steroids and their derivatives are found in various organisms:
  - Insects (e.g., Ecdysteroids)
  - Plants (e.g., Phytosterols, diosgenin)
  - Vertebrates (e.g., Cholesterol, corticosteroids, sex hormones, bile acids)
  - Fungi (e.g., Ergosterol, ergosteroids)
- It has been observed that even minor alterations in the molecular structure of steroids can significantly impact their biological activity.
- Consequently, extensive research has been undertaken to enhance the activity of existing steroid compounds and synthesize novel steroidal compounds with pharmacological efficacy.



File: Structures of common steroids (a) steroid nucleus; (b) testosterone; (c) progesterone  
[Generated by R. Lama, TA for MOOCs]

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**Steroid Biotransformation** Steroid transformation converts initial steroids into drug intermediates, which are then processed into active compounds through simple chemical or microbial methods. Chemical synthesis of steroids often requires numerous steps and hazardous reactions, and creates environmental challenges. For example, producing cortisone from deoxycholic acid involves 37 steps under extreme conditions, resulting in costs exceeding \$200 per gram.

## Steroid biotransformation



- Steroid transformation converts initial steroids into drug intermediates, which are then processed into active compounds through simple chemical or microbial methods.
- Chemical synthesis of steroids often requires numerous steps, hazardous reagents, and creates environmental challenges.
- For example, producing cortisone from deoxycholic acid involves 37 steps under extreme conditions, resulting in costs exceeding \$200 per gram.



File: Conversion of deoxycholate to cortisone: the chemical process for this is energy extensive and involves multiple steps  
[Generated by R. Lama, TA in MOOCs]

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What are the processes involved in biotransformation? Numerous processes are involved, starting with hydroxylation, followed by dehydrogenation, epoxidation, ring aromatization, side chain degradation, reduction of aldehydes and ketones to alcohol, hydrolysis, esterification, and degradation of the steroid nucleus. In hydroxylation, there is a substitution of a hydroxyl group for hydrogen. In dehydrogenation, there is an introduction of a C=C double bond on the rings by the removal of water. In epoxidation, there is the addition of a single oxygen atom across a C=C double bond.

takes place in ring aromatization introduction of aromatic ring through oxidation happens in the side chain degradation selective removal of aliphatic side chain without breakdown of the steroid nucleus happens in the hydrolysis addition of water by acetylase to degrade steroidal acetates takes place in the esterification it generally involves acetylation And then here you have various examples like for example cortisone to hydrocortisone by *Curvularia lunata* and so on. Let us look for the general scheme steroid production slowly through biotransformation is not generally feasible because of the multiple steps involved and thus a combination of microbial transformation And chemical reaction is employed. For instance, conversion of stigmasterol from soy to cortisol involves the following steps.

Processes involved in biotransformation 

Process	Mechanism	Example
Hydroxylation	Substitution of hydroxyl group for hydrogen	Cortisone to hydrocortisone by <i>Curvularia lunata</i>
Dehydrogenation	Introduction of C=C double bond on the rings by removal of water	Cortisol to prednisone by <i>Corynebacterium simplex</i>
Epoxidation	Addition of a single oxygen atom across a C=C double bond	14, 15-epoxidation of 14(15)-dehydrocompounds using <i>C. lunata</i>
Ring A Aromatization	Introduction of aromatic ring in ring A through oxidation	19-nortestosterone to estrone by <i>Pseudomonas testosterone</i>
Side chain degradation	Selective removal of aliphatic side chain without breakdown of steroidal nucleus	Yield of C-17 keto steroids by <i>Nocardia</i> sp
Reduction of aldehydes and ketones to alcohol		Estrone to estradiol by <i>Streptomyces</i> sp.
Hydrolysis	Addition of water by acetolases to degrade steroidal acetates	Estradiol acetate to estradiol by <i>Flavobacterium dehydrogenans</i>
Esterification	Generally involves acetylation	Androstenedione to testosterone acetate by <i>Saccharomyces fragilis</i>
Degradation of steroid nucleus		Degradation of cholesterol by mycobacteria

The first step is the chemical conversion of stigmasterol to progesterone. And biotransformation of progesterone to yield 11 alpha-hydroxyprogesterone by *Rhizopus nigricans*. Then chemical conversion of 11 alpha-hydroxyprogesterone to cortisol. Similarly, diosgenin from Mexican barbasco plant can yield cortisol in the following steps. chemical conversion of diosgenin to Reichstein's substance, biotransformation of Reichstein's substance to cortisol by *Curvularia lunata*.

And then cortisol can be biotransformed to prednisone by the organism *Corynebacterium simplex*, while cortisol derived chemically from cortisol can be biotransformed by *Corynebacterium simplex* to produce prednisone. So, here starting with stigmasterol to progesterone and using this *Rhizopus nigricans*, we get the 11-hydroxyprogesterone which is converted to hydrocortisone. And then this with the help of *Corynebacterium simplex* can be converted to prednisone. And also, if we start from diosgenin, it is converted to Reichstein's substances and with the help of *Curvularia lunata*, it is converted to hydrocortisone cortisol from which cortisone is obtained. And on these, the *Corynebacterium simplex* can act and produce prednisone.

## General scheme



- Steroid production solely through bio-transformation is not generally feasible and thus a combination of microbial transformation and chemical reactions is employed.
- For instance, conversion of **stigmasterol** from soy to cortisol involves the following steps:
  1. Chemical conversion of stigmasterol to progesterone
  2. Bio-transformation of progesterone to yield 11  $\alpha$ -hydroxyprogesterone by *Rhizopus nigricans*
  3. Chemical conversion of 11  $\alpha$ -hydroxy-progesterone to cortisol (hydrocortisone)
- Similarly, **diosgenin** from Mexican barbasco plant can yield cortisol in the following steps:
  4. Chemical conversion of diosgenin to Reichstein's substance
  5. Bio-transformation of Reichstein's substance to cortisol by *Curvularia lunata*
- Furthermore, cortisol can be biotransformed to prednisolone by the organism *Corynebacterium simplex*, while cortisone derived chemically from cortisol can be biotransformed by *Corynebacterium simplex* to produce prednisone.

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So, these are the steps involved in the combined chemical microbial transformation of steroids. Then cholesterol derivatives may be obtained from the biodegradation of cholesterol by enzymes produced by microbacterium species. Microbacterium utilizes cholesterol as a carbon and energy source, breaking it down into simpler metabolites through a series of enzymatic reactions. These reactions typically start with the oxidation of the steroid ring structure or side chains by enzymes such as cholesterol oxidases. Microbacterium species often possess multiple enzymes and pathways that act sequentially to break down the cholesterol molecule into metabolites like cholest-4-en-3-one, pregnenolone and other intermediary products.

## Biotransformation of cholesterol by mycobacterium

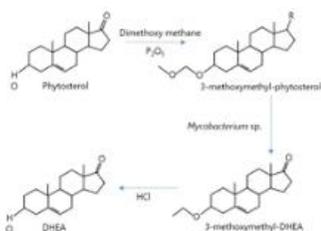
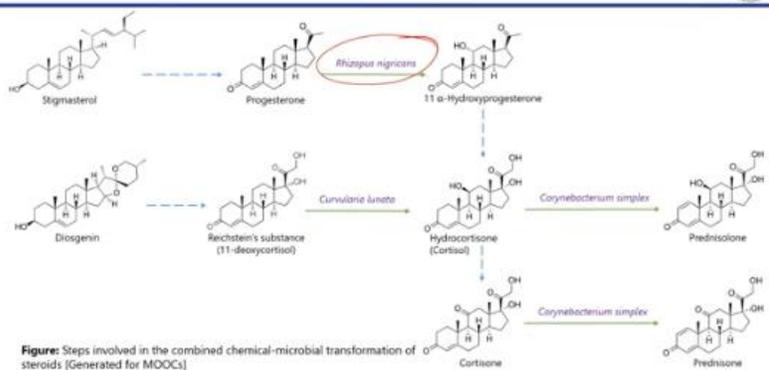


Figure: Microbial transformation of dehydroepiandrosterone from phytosterols by *Mycobacterium* sp. [Generated for MOOCs]

- Cholesterol derivatives may be obtained from the biodegradation of cholesterol by enzymes produced by *Mycobacterium* species.
- *Mycobacterium* utilizes cholesterol as a carbon and energy source, breaking it down into simpler metabolites through a series of enzymatic reactions.
- These reactions typically start with the oxidation of the steroid ring structure or side chains by enzymes such as cholesterol oxidase.
- *Mycobacterium* species often possess multiple enzymes and pathways that act sequentially to break down the cholesterol molecule into metabolites like cholest-4-en-3-one, pregnenolone, and other intermediary products.

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In an industrial scale, large-scale biotransformations use low-cost sterols like diosgenin from Mexican yam or stigmasterol from soybean oil. Microbial transformations employ cells, spores, enzymes or their immobilized forms. Active cultures grow 6 to 24 hours before adding substrate or a large inoculum allows immediate substrate addition. Solubilizers like tween or solvent aid poorly soluble compounds. Sterilization, aeration and progress monitoring via chromatography or spectroscopy ensure the process ends at a maximum titer.



#### Section I: Microorganisms in pharmaceuticals

- Overview
- Screening for potent bioactive compounds
- Genetic engineering

#### Section II: Drugs from microorganisms

- Antibiotics
  - Case study: Industrial production of penicillin
- Antitumor drugs
- Immunosuppressants
- Enzyme inhibitors
- Antihypersensitives
- Antivirals

#### Section III: Biotransformation of steroids

- Overview: Structure of steroid
- General scheme for steroid biotransformation
- Industrial scale biotransformation

#### Section IV: Live organisms as therapeutic agents

- Phage therapy: Isolation, screening, mode of administration, clinical trials
- Oncolytic viruses: Mode of action, clinical trials, approved therapeutic agents

#### Section V: Other therapeutic agents

- Vaccines, nutrient supplements and probiotics

Let us now discuss about the application of live organisms as therapeutic agents. One of the important approach is the phage therapy. phage therapy or phagotherapy involves using bacteriophages to treat bacterial infections. It emerged in the early 20th century but was largely replaced by antibiotics after World War II. Some of the key advantages are reduced side effects, lower risk of bacterial resistance compared to antibiotics, high specificity sparing the host and beneficial bacteria like gut microbiota.

Phage therapy also has a high therapeutic index meaning it causes minimal side effects even at higher doses. How does these phase therapy works? Phage therapy works by using bacteriophages viruses that target harmful bacteria. The process involves number one bacteriophages attached to bacterial cells. and inject their genetic material in step one.

## Phage therapy



Phage therapy, or phagotherapy, involves using bacteriophages to treat bacterial infections. It emerged in the early 20th century but was largely replaced by antibiotics after World War II.

Key advantages include:

- Reduced side effects.
- Lower risk of bacterial resistance compared to antibiotics.
- High specificity, sparing the host and beneficial bacteria like gut microbiota.
- Phage therapy also has a high therapeutic index, meaning it causes minimal side effects even at higher doses.

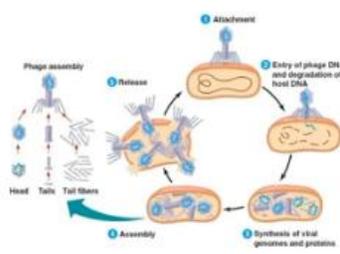


File: Bacteriophages in action  
[Credit: Prof. Graham Beards, CC-BY-SA-3.0, via Wikimedia Commons]

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We can see that the genetic material produce new phages inside the bacteria. So there is entry of the phage and degradation of the host DNA. Then degradation, the bacterial cell being destroyed as the new phages are released. The process holds the bacterial infection by preventing the bacteria from multiplying. We need to isolate a virus for this application.

## Mechanism of action



File: Schematic representation of phage injection and replication  
[Author: xxoverflowed, CC-BY-2.0, via Wikimedia Commons]

Phage therapy works by using bacteriophages, viruses that target harmful bacteria.

The process involves:

1. Bacteriophages attaching to bacterial cells and injecting their genetic material.
2. The genetic material producing new phages inside the bacteria.
3. The bacterial cell being destroyed as the new phages are released.

This process halts the bacterial infection by preventing the bacteria from multiplying.

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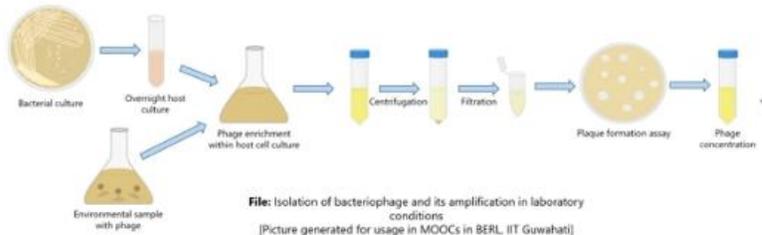
In a typical process of phage isolation, a bacterial sample is combined with an environmental sample from effluent outlets, sewage or even soil and allowed to incubate usually overnight. So here we have the environmental sample with the Phage and then we have the bacterial culture. We are culturing it by phage enrichment with the host culture. Then finally we go for centrifugation, filtration and then we go for plaque formation assay and again the phage concentration to obtain the phage After incubation, the remaining

bacteria are separated from the culture using techniques like centrifugation or filtration and the resulting filtrate is assessed for the presence of phages.

## Isolation of viruses



- In a typical process of phage isolation, a bacterial sample is combined with an environmental sample (from effluent outlets, sewage, or even soil) and allowed to incubate, usually overnight.
- After incubation, the remaining bacteria are separated from the culture using techniques like centrifugation or filtration, and the resulting filtrate is then assessed for the presence of phages.



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Then, testing the isolated virus is very important, so we go for plaque testing. These mixtures of phage filtrate dilutions with bacteria are applied to a plate via spreading or a soft agar overlay. Incubation reveals plaques indicating phage activity. Then, we proceed with culture lysis. Here, phage filtrate is introduced into a broth culture containing bacteria and incubated.

The culture is observed for cell lysis, which is indicated by the loss of culture turbidity. Alternatively, metabolic dyes can be employed to evaluate bacterial metabolic activity instead of turbidity. Let us now discuss the screening of viruses. While a detailed biological characterization of a phage may seem useful for phage therapy, practical screening typically focuses on a few key characteristics, like the ability to eliminate target bacteria. Phages must be virulent and productive enough

## Testing the isolated viruses

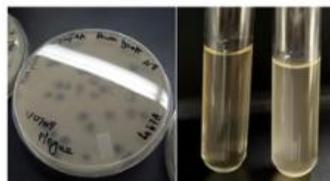


### Plaque testing:

- Plaque testing mixes dilutions of phage filtrate with bacteria, applied to a plate via spreading or a soft agar overlay.
- Incubation reveals plaques, indicating phage activity.

### Culture lysis:

- In culture lysis, phage filtrate is introduced into a broth culture containing bacteria and incubated.
- The culture is observed for cell lysis, which is indicated by the loss of culture turbidity.
- Alternatively, metabolic dyes can be employed to evaluate bacterial metabolic activity instead of turbidity.



**File:** Methods of testing isolated bacteriophages  
(left) Plaque testing on a *Mycobacterium smegmatis* plate;  
(right) Testing by culture lysis  
[Credit: Anonymous, Public Domain, via Wikimedia Commons]

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to outpace bacterial growth and prevent bacterial overgrowth. They should have strictly lytic behavior. Lytic phages are preferred in therapy because temperate phages may carry toxin genes, which could increase the virulence of bacteria through genetic transfer. Host range: phages that can infect a wider range of hosts within a specific pathogen species are more likely to be useful for treating infections caused by that pathogen. Phages with these ideal traits are stored in phage banks in bacterial medium.

## Screening of viruses



While a detailed biological characterization of a phage may seem useful for phage therapy, practical screening typically focuses on a few key characteristics:

**Ability to eliminate target bacteria:** Phages must be virulent and productive enough to outpace bacterial growth and prevent bacterial overgrowth.

**Strictly lytic behavior:** Lytic phages are preferred in therapy because temperate phages may carry toxin genes, which could increase the virulence of bacteria through genetic transfer.

**Host range:** Phages that can infect a wider range of hosts within a specific pathogen species are more likely to be useful for treating infections caused by that pathogen.

Phages with these ideal traits are stored in phage banks in bacterial medium.

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The mode of treatment and administration. Phages are highly specific to certain bacteria, often necessitating patient swabs and cultures before treatment. However, clinics maintain Phage cocktails for common bacterial strains, periodically updating them to address emerging strains. In Eastern countries, these cocktails are available in pharmacies. Phages are administered orally, topically on wounds or on surfaces.

They are often freeze dried into pills with some types stable up to 55 degree centigrade and a shelf life of about 14 months. Oral administration is more effective with an antacid to improve survival through the stomach while topical application involves applying Phages on gauze placed over the treatment site. Funding for Phage therapy research and trials is challenging due to complexities in patenting bacteria-first products and the limited public awareness. Several trials have been conducted to evaluate Phage therapy's efficacy against specific infections like *Pseudomonas aeruginosa* and *Escherichia coli*. Locust Biosciences has developed a CRISPR-modified Phages cocktail, showing promise in early trials against *E. coli* urinary tract infections.

## Mode of treatment and administration



- Phages are highly specific to certain bacteria, often necessitating patient swabs and cultures before treatment. However, clinics maintain phage cocktails for common bacterial strains, periodically updating them to address emerging strains.
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- Phages are administered orally, topically on wounds, or on surfaces.
- They are often freeze-dried into pills, with some types stable up to 55°C and a 14-month shelf life.
- Oral administration is more effective with an antacid to improve survival through the stomach, while topical application involves applying phages on gauze placed over the treatment site.



File: PYO Phage therapy cocktail from Eliava Institute  
(Credit: Ashvienis, CC-BY-SA-4.0, via Wikimedia Commons)

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The FDA has recently approved Various clinical trials including intravenous and nebulized Phages therapy for pseudomonas aeruginosa infections in cystic fibrosis patients and trials for urinary tract infections and chronic prosthetic joint infections conducted by adaptive fast therapeutics in collaboration with the Mayo Clinic. Phages in biofilm treatment. Phagetherapy is highly effective against biofilm infections, particularly those caused by Pseudomonas aeruginosa and Staphylococcus aureus. In a report of 78 biofilm infection cases, 96% of patients showed clinical improvement while 52% achieving complete relief or bacterial eradication.

## Trials



- Funding for phage therapy research and trials is challenging due to complexities in patenting bacteriophage products and the limited public awareness.
- Several trials have been conducted to evaluate phage therapy's efficacy against specific infections like *Pseudomonas aeruginosa* and *Escherichia coli*.
- Locus Biosciences developed a CRISPR-modified phage cocktail, showing promise in early trials against *E. coli* urinary tract infections.
- The FDA has recently approved various clinical trials, including intravenous and nebulized phage therapy for *P. aeruginosa* infections in cystic fibrosis patients [Clinical trial number NCT04684641], and trials for urinary tract infections [Clinical trial number NCT04287478] and chronic prosthetic joint infections conducted by Adaptive Phage Therapeutics in collaboration with the Mayo Clinic [Clinical trial number NCT04787250].

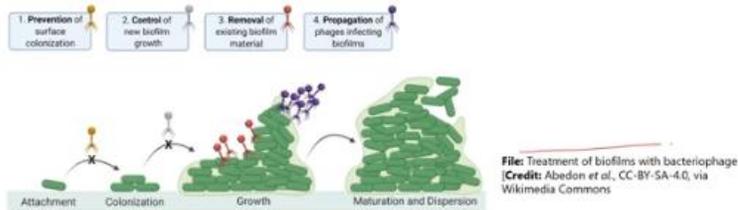
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Challenges such as low antibiotic penetration due to structural barriers and enzymatic deactivation of antibiotics make phage therapy an attractive alternative for managing SARS infections. Here, you can see in the first case there is an attachment, and then after that, there is colonization. There is growth, and there is maturation and dispersion taking place. So, this is basically showing the treatment of biofilms with bacterial phages. Another interesting application is in cancer.

## Phages in biofilm treatment



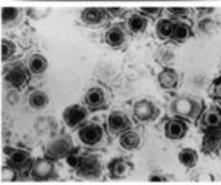
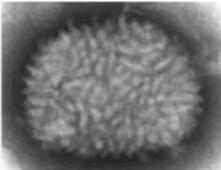
- Phage therapy is highly effective against biofilm infections, particularly those caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
- In a report of 78 biofilm infection cases, 96% of patients showed clinical improvement, with 52% achieving complete relief or bacterial eradication.
- Challenges such as low antibiotics penetration due to structural barriers, enzymatic deactivation of antibiotics make phage therapy an attractive alternative for managing such infections.



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We have certain oncolytic viruses. An oncolytic virus refers to a virus that selectively targets and eradicates cancer cells. As it infects cancer cells, it induces oncolysis, leading to the destruction of these cells while producing new virus particles that contribute to further tumor elimination. Several viruses, including adenovirus, reovirus, measles, herpes simplex, Newcastle disease virus, and vaccinia, have undergone clinical testing as oncolytic agents. While most oncolytic viruses are engineered for tumor selectivity, naturally occurring viruses like reovirus and Senecavirus have also been tested in clinical trials.

## Oncolytic viruses



- An **oncolytic virus** refers to a **virus that selectively targets and eradicates cancer cells**.
- As it infects cancer cells, it induces **oncolysis**, leading to the destruction of these cells while producing new virus particles that contribute to further tumor elimination.
- Several viruses, including adenovirus, reovirus, measles, herpes simplex, Newcastle disease virus, and vaccinia, have undergone clinical testing as oncolytic agents.
- While most oncolytic viruses are engineered for tumor selectivity, naturally occurring viruses like reovirus and senecavirus have also been tested in clinical trials.

File: (top) A TEM micrograph of Vaccinia virions [Credit: CDC/Cynthia Goldsmith, Public domain, via Wikimedia Commons] (bottom) Transmission electron micrograph of herpes simplex virus [Credit: CDC/Dr. Erskine Palmer, Public domain, via Wikimedia Commons]

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The mechanism of action is immunity as an obstacle. Oncolytic viruses selectively target tumor cells due to the latter's defective antiviral response. The interaction between oncolytic viruses and the immune system revolves around two key aspects. The first one is immunity as an obstacle. The patients

The immune system poses a significant challenge to oncolytic viruses as it naturally works to neutralize any virus present. To address pre-existing immunity, less common human

pathogenic viruses can be used, yet subsequent antibody generation remains unavoidable. Strategies like coating viral vectors with polymers such as polyethylene glycol have been attempted to shield them from antibodies, although this also interferes with their interaction with host cells. Another approach involves hiding viruses within macrophages, exploiting their natural migration to areas of tissue destruction, particularly low-oxygen regions associated with cancer growth. Here, another mechanism of action is immunity as an ally.

### Mechanism of action: Immunity as an Obstacle



Oncolytic viruses selectively target tumor cells due to the latter's defective antiviral response. The interaction between oncolytic viruses and the immune system revolves around two key aspects.

#### **Immunity as an Obstacle:**

- The patient's immune system poses a significant challenge to oncolytic viruses as it naturally works to neutralize any virus present.
- To address pre-existing immunity, less common human pathogenic viruses can be used, yet subsequent antibody generation remains unavoidable.
- Strategies like coating viral vectors with polymers such as polyethylene glycol have been attempted to shield them from antibodies, although this also interferes with their interaction with host cells.
- Another approach involves hiding viruses within macrophages, exploiting their natural migration to areas of tissue destruction, particularly low-oxygen regions associated with cancer growths.

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Despite impeding viral activity, the patient's immune system can also aid in fighting tumors. Infection caused by oncolytic viruses attracts the immune system's attention to the tumor, potentially generating beneficial and enduring anti-tumor immunity. Tumor lysis releases substances like tumor-associated antigens and danger-associated molecular patterns, triggering an anti-tumor immune response akin to a personalized cancer vaccine. So here you can see the virus infecting a tumor cell and replicating within the tumor cell. This results in lysis of the tumor cell and release of viruses, leaving behind certain debris, and then the virus goes on to attack other tumor cells.

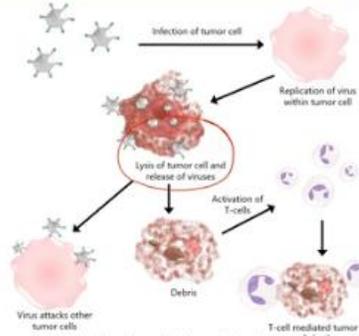
This debris will activate T cells and then lead to T cell-mediated tumor cell death. Naturally occurring oncolysis. Vaccinia virus has been studied for oncolysis since the 1920s when Levaditi and Nicolau demonstrated its tumor-inhibiting effect in mice and rats. Vesicular stomatitis virus shows promise for brain tumors, inducing regression and durable cures in animal models. Reoviruses efficiently replicate in cancer cells, leading to lysis and forming the basis of Reolysin.

## Mechanism of action: Immunity as an Ally



### Immunity as an Ally:

- Despite impeding viral activity, the patient's immune system can also aid in fighting tumors.
- Infection caused by oncolytic viruses attracts the immune system's attention to the tumor, potentially generating beneficial and enduring antitumor immunity.
- Tumor lysis releases substances like tumor-associated antigens and danger-associated molecular patterns (DAMPs), triggering an antitumor immune response akin to a personalized cancer vaccine.



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Then there is Seneca virus, an oncolytic picornavirus being explored for cancers like small cell lung cancer and pediatric solid tumors. There is Rigvir. And Echo7 Ecovirus is approved for cancer treatment in Latvia, Georgia, and Armenia. Other viruses include Maraba virus, Coxsackievirus, modified influenza viruses, and Sendai virus, which are under investigation for their oncolytic properties. Then we have the genetically engineered oncolytic viruses.

## Naturally occurring oncolysis



- **Vaccinia virus (VACV)** has been studied for oncolysis since the 1920s, when Levaditi and Nicolau demonstrated its tumor-inhibiting effects in mice and rats.
- **Vesicular stomatitis virus (VSV)** shows promise for brain tumors, inducing regression and durable cures in models.
- **Reoviruses** efficiently replicate in cancer cells, leading to lysis and forming the basis of **Reolysin**.
- **Senecavirus**, an oncolytic picornavirus, is being explored for cancers like small cell lung cancer and pediatric solid tumors.
- **RIGVIR**, an **ECHO-7 echovirus**, is approved for cancer treatment in Latvia, Georgia, and Armenia.
- Other viruses, including **maraba virus**, **Coxsackievirus A21 (Cavatak)**, modified **influenza A viruses**, and **Sendai virus**, are also under investigation for their oncolytic properties.

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These are specialized viruses that have been modified through genetic manipulation to target and attack cancer cells selectively while sparing healthy cells. These viruses are designed to exploit the defective pathways present in cancer cells, allowing them to replicate and lyse the malignant cells specifically. The genetic modification in these viruses often involves altering their viral genomes to enhance their tumor-specific replication and destruction capabilities. These modifications can include tumor-selective replication, enhanced tumor cell killing, immunomodulation, and reduced virulence in healthy cells. And there are preclinical and clinical trials going on.



- Genetically engineered oncolytic viruses are specialized viruses that have been modified through genetic manipulation to target and attack cancer cells selectively while sparing healthy cells.
- These viruses are designed to exploit the defective pathways present in cancer cells, allowing them to replicate and lyse the malignant cells specifically.
- The genetic modifications in these viruses often involve altering their viral genomes to enhance their tumor-specific replication and destruction capabilities. These modifications can include:

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Chen showed that CB706, a prostate-specific adenovirus combined with radiotherapy, enhanced cell death and viral burst size in prostate cancer mice. Then there is HSV1716. Seprehvir demonstrated synergy with cancer chemotherapies, while bevacizumab, an anti-VEGF antibody, reduced inflammation and boosted oncolytic HSV virotherapy in mice. Then there are certain things in clinical trials; for example, Onyx-015 combined with chemotherapy showed improved responses but was discontinued due to inconclusive results. Then vaccinia virus GL-ONC1 was tested with chemo- and radiotherapy in head and neck cancer patients as part of the standard of care.

### Pre-clinical trials and clinical trials



#### **Pre-Clinical Investigations:**

- Chen et al. (2001) showed that CV706, a prostate-specific adenovirus, combined with radiotherapy, enhanced cell death and viral burst size in prostate cancer mice.
- SEPREHVIR (HSV-1716) demonstrated synergy with cancer chemotherapies, while bevacizumab, an anti-VEGF antibody, reduced inflammation and boosted oncolytic HSV virotherapy in mice.

#### **Clinical Trials:**

- Onyx-015 combined with chemotherapy showed improved responses but was discontinued due to inconclusive results.
- Vaccinia virus GL-ONC1 was tested with chemo- and radiotherapy in head and neck cancer patients as part of the Standard of Care.

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Then we have certain approved therapeutic agents like talimogene, laherparepvec, known as T-Vec and developed by Amgen, successfully concluded phase 3 trials for advanced melanoma in March 2013. known as T-Vac and developed by Amgen, successfully concluded phase 3 trials for advanced melanoma in March 2013. In October 2015, the US FDA granted approval to T-Vec, marketed as Imlygic, specifically for treating melanoma in patients with inoperable tumors. This marked its status as the first approved oncolytic agent in the Western world, leveraging the herpes simplex virus as its foundation. Now

certain things are in phase 1 trials for pancreatic cancer and phase 3 trial for head and neck cancer combined with cisplatin, chemotherapy and radiotherapy.

Then there is TAsERpATUREV known as DELYTACT and developed by Daiichi Sankyo stands as the inaugural approval oncolytic virus therapy sanctioned by the Japan Ministry of Health, Labor and Welfare. DELYTACT is genetically modified oncolytic herpes simplex virus type 1 has gained approval for treating malignant glioma in Japan. Let us now discuss about Other therapeutic agents, which include vaccines, nutrient supplements and probiotics. Microorganisms play a vital role in vaccine production and are employed to produce antigens or components that trigger the desired immune response.

### Approved therapeutic agents



- Talimogene laherparepvec (OncoVEX GM-CSF), known as T-vec and developed by Amgen, successfully concluded phase III trials for advanced melanoma in March 2013.
- In October 2015, the US FDA granted approval to **T-VEC**, marketed as Imlygic, specifically for treating melanoma in patients with inoperable tumors. This marked its status as the first approved oncolytic agent in the western world, leveraging the **herpes simplex virus** (HSV-1) as its foundation.
- It has also undergone Phase I trials for pancreatic cancer and a Phase III trial for head and neck cancer, combined with cisplatin chemotherapy and radiotherapy.
- **Teserpaturev** (G47Δ), known as **Delytact** and developed by Daiichi Sankyo, stands as the inaugural approved oncolytic virus therapy sanctioned by the Japan Ministry of Health, Labour, and Welfare (MHLW).
- **Delytact**, a genetically modified oncolytic herpes simplex virus type 1 (HSV-1), has gained approval for treating malignant glioma in Japan.

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Bacteria yeast. viruses are manipulated to express specific proteins or genetic material that mimic the antigens present in pathogens, aiding in the development of vaccines. With the aid of recombinant DNA technology, these microorganisms are harnessed to create safer and more effective vaccines, offering a controlled and reliable means of antigen production without the need to handle the actual disease causing pathogens. Then we have the food supplements like vitamins, certain bacteria and fungi are capable of synthesizing vitamins like B vitamins like riboflavin, cobalamin and biotin are produced by microorganisms through fermentation processes and also amino acids are produced by bacteria and yeast and some of these include glutamic acid, lysine and others through fermentation. These are essential components in various food products, animal feed and in pharmaceuticals.



- Microorganisms play a pivotal role in vaccine production and are employed to produce antigens or components that trigger the desired immune response.
- Bacteria, yeast, and viruses are manipulated to express specific proteins or genetic material that mimic the antigens present in pathogens, aiding in the development of vaccines.
- With the aid of recombinant DNA technology, these microorganisms are harnessed to create safer and more effective vaccines, offering a controlled and reliable means of antigen production without the need to handle the actual disease-causing pathogens. (dealt in detail in **Module 10, Lecture 2**)

File: (top) Production and packaging of COVIran, Covid-19 vaccine from Iran [Credit: Tasnim News Agency, CC BY 4.0, via Wikimedia Commons] (bottom) Oxford-AstraZeneca Covid-19 vaccine, a DNA vaccine [Credit: Julieth Méndez, CC BY-SA 4.0, via Wikimedia Commons]

## Food supplements

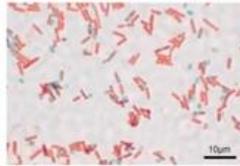
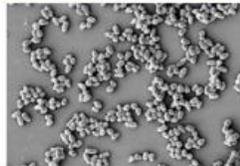


### Vitamins:

- Certain bacteria and fungi are capable of synthesizing vitamins.
- For instance, various B vitamins like riboflavin (B2), cobalamin (B12), and biotin are produced by microorganisms through fermentation processes.

### Amino Acids:

- Microorganisms like bacteria and yeast are used to produce amino acids such as glutamic acid, lysine, and others through fermentation.
- These amino acids serve as essential components in various food products, animal feed, and pharmaceuticals.



File: Some bacteria used in production of food supplements: (top) *Corynebacterium glutamicum*, used in mass scale production of glutamic acid [Credit: Veder1941, CC-BY-SA-4.0, via Wikimedia Commons] (bottom) *Bacillus subtilis*, often used in production of riboflavin precursors [Credit: Y tambe, CC-BY-SA-3.0, via Wikimedia Commons]

Then another important application is the probiotics. These are live microorganisms that, when administered in adequate amounts, offer health benefits to the host. At the top, we can see yogurt and then kimchi. These are traditional foods rich in probiotics, while at the bottom is Yakult. It is arguably the most popular probiotic brand today.

The most common probiotics include strains of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, found in various fermented foods like yogurt, kefir, kimchi, sauerkraut, and some types of seeds. These probiotics are known to promote a healthy balance of gut bacteria, aiding in digestion, immune system regulation, and potentially reducing the risk of certain conditions such as diarrhea, irritable bowel syndrome, and certain allergies. So, with this, we come to the end of this lecture.

## Probiotics



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- Most commonly, probiotics include strains of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, found in various fermented foods like yogurt, kefir, kimchi, sauerkraut, and some types of cheese.
- Probiotics are known to promote a healthy balance of gut bacteria, aiding in digestion, immune system regulation, and potentially reducing the risk of certain conditions such as diarrhea, irritable bowel syndrome, and certain allergies.

**File:** (top) yogurt (L) and kimchi (R) are traditional food rich in probiotics, while (bottom) Yakult is arguably the most popular probiotics brand  
**Credit:** (top-L) Anonymous, CC-BY-SA-3.0, via Wikimedia Commons (top-R) chomjong, CC-BY-2.0, via Wikimedia Commons (bottom) Tbatb, CC-BY-SA-3.0, via Wikimedia Commons