Lecture II

Ecology of microorganisms. Normal microbiota of human organism. Microbiota of oral cavity and saliva. Influence of external environmental factors (physical, chemical and biological) to microorganisms. Sterilization and disinfection. Bacteriophages. Genetics of microorganisms, types of genetic variability. The base of antibiotic therapy, chemical therapeutic drugs. Antibiotics.

Ecology of microorganisms

- Microorganisms are widely spread in environment – in soil, water, air, human, animal and plants..
- Ecology (greek, *eikos* –home)of microorganisms investigates their distribution pattern in environment. (yunanca, *eikos* – yaşayış yeri)

Ecosystem and its components

- The main research object of ecology – **ecosystem** consists of biotic and abiotic components.
- **Biotic components** consist of biocenoses – microbial populations with various number and species.
- Physical and chemical factors of ecosystem form **abiotic components**

Microorganisms of ecosystem

- Ecosystem microorganisms are divided on two categories – autochtonous and allochtonous.
- ***Autochtonous microorganisms –*** *permanent residents of ecosystem (exp. gut, soil). These ecosystmes provide all needed conditions for mciroorganims surviving.*
- ***Allochtonous (zymogenic)*** *are not permanent representatives of ewcosystem. They present in ecosystem when necessary conditions exist for their survival.*
- For example, bifidobacteria always present in intestinal tract because they are permanent (autochthonous) intestinal microorganisms. However, Candida species are considered as allochthonous inhabitants of the intestines

Mutual relationship types of microorganisms

- Microorganisms live in environment and host organisms in form of niocenoses.Coexistence of two and more organisms is called symbiosis. Organisms living in symbiosis are called symbionts.
- Depending on form of mutual relationship three forms of symbiosis exist:
- ***mutualism***
- ***antagonism***
- ***neutralism***

Mutual relationship types of microorganisms

- **Mutualism is beneficial relationship for symbionts. Organisms provide each other with essential nutritional components.** An example of a mutualism is the symbiosis of blue-green algae (cyanobacteria) with fungi.

  There variants of mutualistic symbiosis:

  - *Metabiosis*- one of the microorganisms uses metabolic products of other organism

  - *Commensalism*- one of the symbionts benefits while the other is unaffected - *Satellitism* – the growth of one microorgasnism stimulates the growth other During **antagonism** one microorganism suppress the growth of the other, even sometimes causing its destruction

Microorganisms and environment. Fundamentals of sanitary microbiology

- **Sanitary microbiology** is a branch of medical microbiology, studying microorganisms in the environment (soil, water, air, food, etc.) and the processes they cause.
- The main purpose of the **sanitary microbiology** is detection of infectious agents in the environment and development of measures to prevent contamination of the environment with microorganisms, and prevention of spread of infectious diseases.
-

The normal microbiota of human organism

- The representatives of the normal microflora are saprophytes – commensal microorganisms which do not have harmful effect on human organism.
- Normal flora colonizes skin and mucous membranes – upper respiratory tract, gastrointestinal tract, genitourinary tract, etc.
- Microflora of mucous membranes have specific colonization pattern. Distal zones of mucous membranes are risch with microorganisms as they are in close contact with environment.
- Tissue and organs which are normally have no contact with environment are sterile (blood, lympha, inner organs, cerebrospinal fluid, brain, etc.).

The normal microbiota of human organism

- Normal flora is divided to 2 groups: obligate and facultative microflora. **Obligate microflora** is also called permanent, residual, indigenous or autochtonous flora. It consists of saprophyte and opportunistic pathogens adapted to live and permanently isolated from host organism.
- ***Facultative, allochtonous flora*** is isolated from organism during certain period of time (temporarily). These microorganisms enter host organism and leave it after certain period of time.

Skin microflora

Microorganism	Morphological features
Staphylococcus epidermidis	Gram positive cocci(grape clusters)
Staphylococcus aureus	Gram positive cocci(grape clusters)
Propionobacterium acne	Gram negative pleomorphic rods
Corynebacterium spp. (diphteroids)	Gram positive pleomorphic rods
Lactobacillus spp.	Gram positive rods
Streptococcus pyogenes	Gram positive cocci (in chains)
Candida spp.	Yeastlike fungi
Malassezia furfur	Yeastlike fungi

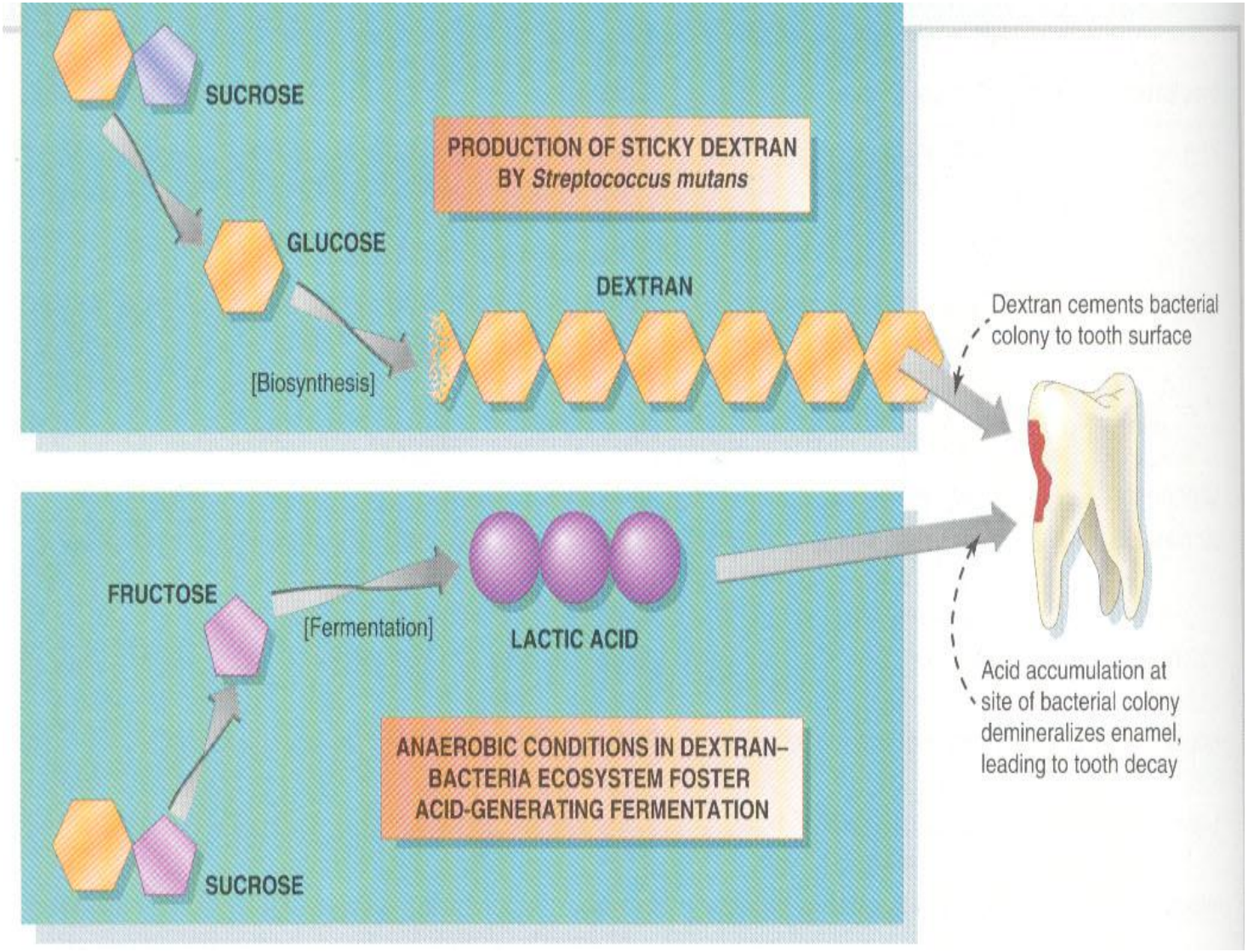
Microflora of respiratory airways

Anatomical area	Microorganism	Morphological features
Upper respiratory tractnasal cavirtyand naso-pharynx)	Staphylococcus epidermidis Staphylococcus aureus Yaşıllaşdıran streptokoklar Streptococcus pneumoniae Branhamella catarrhalis Corynebacterium cinsi (difteroidlər) Haemophilus cinsi Bacteroides cinsi Actinomyces cinsi	Gram positive cocci(grape clusters) Gram positive cocci(grape clusters) Gram positive cocci (in chains) Gram positive diplococci Gram negative coccobacteria Gram positive pleomorphic rods Gram negative pleomorphic rods Gram negative pleomorphic rods Gram positive rods, branching micelia
Lower repiratory tract(trachea, bronchi, bronchioles, lungs)	No microorganisms	

Digestive tract microflora

Anatomical area	Microorganism	Morphological features
Oral cavity		
Saliva and teeth	Streptococcuss pp. Lactobacillus spp. Veilonella spp. Bacteroides spp. Fusobacteria Actinomyces spp.	Gram positive cocci (in chains) Gram positive cocci Gram negative diplococci Gram negative pleomorphic rods Gram negative rods Gram positive rods, branching micelia
Pharynx(tonsils)	Streptococcus spp. Branhamella catarrhalis Corynebacterium spp.(diphteroids) Staphylococcus spp.	Gram positive cocci (in chains) Gram negative coccobacteria Gram positive pleomorphic rods Gram positive cocci(grape clusters)
Esophagus	Saliva and food microrogansims	
Stomach	Lactobacillus spp Corynebacterium spp.(diphteroids) Candida spp.	Gram positive rods Gram positive pleomorphic rods Yeastlike fungi

The etiological role of microorganisms in the formation of caries



Digestive tract microbiota

Anatomical site	Microorganism	Morphological features
Small intestine	Lactobacillus spp. Enterococcus spp. Bacteroides spp. Candida spp.	Gram positive cocci Gram positive diplococci Gram negative pleomorphic rods Yeastlike fungi
Large intestine	Bacteroides spp. Bifidobacterium spp. Enterobacteriaceae Enterococcus spp. Clostridium spp. Fusobacteria Lactobacillus spp. Staphylococcus spp. Peptostreptococcus spp. Candida spp. Entamoeba coli Trichomonas	Gram negative pleomorphic rods Gram positive rods Gram negative rods Gram positive diplococci Gram positive sporeforming rods Gram negative rods Gram positive rods Gram positive cocci(grape clusters) Gram positive cocci(in chains) Yeastlike fungi Protozoa Protozoa

Large intestine microbiota

- Large intestine is extremely rich with microorganisms. Its upper parts - cecum and transverse colon have 10^8-10^{10} microbial cells per 1gr of intestinal content.
- Distal zone of large intestine has the highest number of microorgansims 10^{10} /gr which (20-30% of all stool microbiota).
- In general, large intestine microbiota includes up to 500 microorganism species. Thus, it is also called as microbial reservoir of organism.

Large intestine microbiota

- **Obligate microbiota**of large intestine generally consists of anaerobic bacteria (96-99%).
- Anaerobic microorganisms number is 1000-foulds higher than other microorganisms (*Bacteroides, Bifidobacterium,* anaerobic lactobacteria).
- 1-4% of microflora respresented by other obligate microbiota(*E.coli*, *Enterococcus, Lactobacillus*) and
- ***Facultative microbiota***(*Enterobacteriaceae, Clostridium*, *Fusobacterium, Staphylococcus*, *Peptostreptococcus* spp., *Candida* spp., etc.)

Mucous microbiota
Lumen microbiota

- Mucous membrane of intestinal tract and mucus surrounding it has special microflora called **mucous microbiota**. Microbiotasurrounding mucous membrane prevents microorganism invasion of intestinal wall cells. Mucous microbiota is stabile
- In contrast, the lumen microbiota, which represents the microbiota of the intestinal contents, is relatively more volatile. Under the influence of various factors, the number and composition of microorganisms in the intestinal microbiota may change. As a result, there are cases called dysbiosis and dysbacteriosis

Age-related changes in gut microbiota

- ***The intestinal tract of newborns is sterile.*** The normal flora is formed from the first hours of life through nutrition of newborn.
- ***In breastfed infants,*** it is represented by large amounts of lactic acid streptococci and lactobacilli.
- In contrast*,* ***non-breastfed infants*** have a more complex intestinal microflora, with fewer lactobacilli.
- At the end of the first year of life in healthy children, the normal microflora is the same as in adults.

Normal microbiota of the urogenital tract

Anatomical site	Microorganism	Morphological features
Urinary tract (lower part)	Micrococcus spp. Staphylococcus epidermidis Streptococcus spp. Mycobacterium smegmatis Corynebacterium spp.(diphteroids) Bacteroides spp. Neisseria cinsi Enterobacteriaceae	Gram positive cocci Gram positive cocci(grape clusters) Gram positive cocci(in chains) Gram positive acid resistant rods Gram positive pleomorphic rods Gram negative pleomorphic rods Gram negative diplococci Gram negative rods
Renal pelvis, ureter, bladdr, urethra	No microorganisms	
Cervix	Lactobacillus spp. Corynebacterium spp. (diphteroids) Streptococcus spp. Staphylococcus spp. Enterobacteriaceae Candida spp. Trichomonas vaginalis	Gram positive rods Gram positive pleomorphic rods Gram positive cocci(in chains) Gram positive cocci(grape clusters) Gram negative rods Yeastlike fungi Protozoa
Ovaries, fallopian tubes, uterus	No microorganisms	

Significance of normal microbiota

- Normal microbiota, especially obligate microflora representative are **antagonists** of obligate and opportunistic pathogenic bacteria.
- This feature is possible due to release of organic acids, antibiotics and bacteriocins.
- Thus, normal flora prevents **colonization** of mucous membranes by pathogenic microorganisms.
- Normal microbiota– is one of the nonspecific factors of organism.

Significance of normal microbiota

- Normal microbiota being antigen for immune system cells plays significant role in formation of **natural immunity**.
- Normally the pool of serum antibodies is induced by normal microbiota.
- The normal intestinal microflora plays role in the **digestive process**, **metabolism**, as well as in the **synthesis of some biologically active substance**s, **vitamins** (vitamin K, B vitamins).

Significance of normal microbiota

- The significance of normal microbiota is well studied on animals without microbes(gnotobionts).
- These animal do not have microorganisms and are kept under special(without microorganisms) condition.
- Gnotobionts have poorly developed lymphoid tissue, thus they are susceptible to infections and can not survive under normal conditions.

Disbiozsis and disbacteriosis

- There is a balance betweem obligate and facultative normal microbiota representatives.
- This balance is primarily due to the antagonistic effect of obligate microflora on the facultative microflora.
- Impact of various factors may lead to violation of this balance –**disbacteriosis** and **disbiosis**.

Factors causing disbacteriosis and disbiosis

- Wide and irrational use of antibiotics
- Other factors – underlying diseases, esp. intestinal infections, helmynth and parasite invasions, hormonal and chemical therapy, stress, etc.
- Worsening of ecological conditions in modern era –another cause of spread of disbacteriosis.

Mechanism of disbiosis and disbacteriosis

- The development of disbacteriosis is due to decrease of number of **obligate microbiota**.
- As a result, the number of opportunistic pathogens – staphylococci, *Proteus*, *Pseudomonas*, *Candida* increases which leads to development of diseases.
- Depending on etiology fungal, staphylococcal, proteus etc. disbiosis exist.
- Sometimes dysbiosis is also classified according to its location (oral, intestinal, uterine, etc.).

Disbiosis and related diseases

- Longterm alteration of normal microbiota composition and function leads to various symptoms.
- Among them diarrhea, constipation, colitis, cancer, allergy, hypo- and hypercholesterinemy, hypo- hypertensy, caries, arthritis, liver pathologies, etc. can be given as examples.

Treatment of dysbiosis and dysbacteriosis

- The main principle is determination and elimination of factors causing dysbiosis.
- One of the important approach is removal of opportunistic pathogens(selective decontamination).
- Probiotics (eubiotics) are used to restore the microflora.
- Eubiotics - obligate representatives of the normal intestinal microflora - bifidobacteria, lactobacilli, E.coli, enterococci, etc. bacteria are prescribed.
- Bacterial preparations are used in the form of lyophilized dry powder, tablets,extracts.

Environmental factors

- Physical, Chemical and biological environmental factors have bactericide, bacteriostatic and mutagen influence on microbes.

Physical factors

- Temperature
- Atmosphere pressure
- Drying
- Light (UV-rays)
- Ionizing radiation
- Ultrasound

High pressure. Bacteria's and mostly their spores

are resistant to mechanical pressure. Bacteria's are found in nature that are living in deep ocean at the depth 1000- 10 000 m under the pressure of 100-900 atmospheres. Some types of bacteria withstand 3000-5000 atm, but bacterial spores are resistant even 20 000 atm

Drying.

- Water is needed for normal life activity of microorganisms. Drying can cause dehydration of cytoplasma, violates integrity of cytoplasmic membrane, which brings to death of the cell.
- Some microorganisms under the influence of drying die after a couple of minutes: those are mostly cocci’s.

Liofilization drying.

- The essence of this method is that microorganisms are freeze first from -25 to -73 Celsius, and then dry with the + temperature in the vacuum. Wherein the cytoplasma of bacteria is frozen and becomes ice and then this ice evaporates under positive temperature and the cell stays alive (transfer of water from frozen condition into gaseous, bypassing the liquid phase- sublimation).

Ray energy

- In nature, microorganisms are constantly exposed to solar radiation. Direct sunlight causes the death of many microorganisms within a few hours, with the exception of photosynthetic bacteria (green and purple sulfur bacteria). The destructive effect of sunlight is due to the activity of ultraviolet rays (UV rays). They inactivate cell enzymes and damage DNA. Pathogenic bacteria are more sensitive to UV rays than saprophytes. Therefore, it is better to store microbial cultures in the laboratory in the dark. In this regard, Buchner's experience is demonstrative.

Buchner's experiment.

In a Petri cup with a thin layer of agar, an abundant inoculation of any bacterial culture is performed. Then the letters cut out of black paper and is glued onto the outer surface of the seeded cup, forming, for example, the word "typhus".
The cup, turned upside down, is exposed to direct sunlight for 1 hour. Then the pieces of paper are removed, and the cup is put in a thermostat for a day at 37 ° C. Bacterial growth is observed only in those places of the agar that were protected from the action of UV rays by glued letters. The rest of the agar remains transparent, i.e. there is no growth of microorganisms.

- X-ray radiation, Alfa, beta and ultraviolet rays have a devastating effect on microorganisms only in big quantities. Those rays destroy cells. In recent years with the use radiation method Petri's cups, syringes, suture materials are sterilized for one time usage.
- UVH-energy. Causes heating of environment, haves devastating effect on microorganisms and damages cells.

-Influences on genetic features of microorganisms

-Influences on intensity of cell division

-activity of some ferments

-hemolytic features

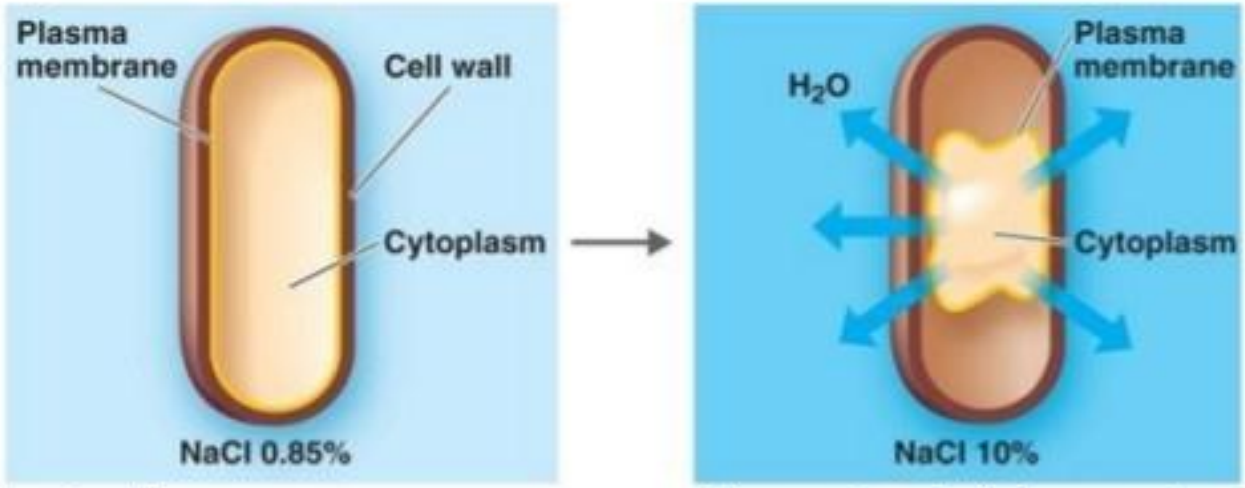
- Ionizing radiation. The main feature of this radiation is causing the ionization process.

Ultrasound.

- Haves a big amount of energy and with the help of this energy they can cause physical, chemical and biological phenomenon. With the help of US waves we can cause inactivation of ferments, vitamins, toxins, destroy different materials and substances, multicellular and unicellular organisms.

Effect of Osmotic Pressure

- Osmotic pressure is the pressure exerted on bacterial cells by their environment



- **Isotonic**
- **Hypertonic (plasmolysis)**

- **Hypotonic:** the bacterial cell gains water and swells to the limit of its cell wall
- Some opportunistic pathogens are **facultative halophiles**
  - ***Staphylococcus aureus* - colonizes the surface of the skin (salt)**

Sterilization

- **Sterilization- it is a process of total destruction of microorganisms.**
- **Methods of sterilization**
  - **Heating**
  - **Chemical**
  - **Ray or beam affection**
  - **Mechanical (filtration)**

Dezincification, aseptic and antiseptics.

- **Dezincification – process of destruction of pathogen microorganisms**
- **Aseptic – set of measures directed to prevent microbes to get into the wounds or organs during surgery and other medical procedures.**
- **Antiseptics – меры, направленные на уничтожение микробов в ране или патологическом очаге. Set of measures needed to destroy microbes in the wounds and pathological focus.**

Influence of chemical factors on microorganisms.

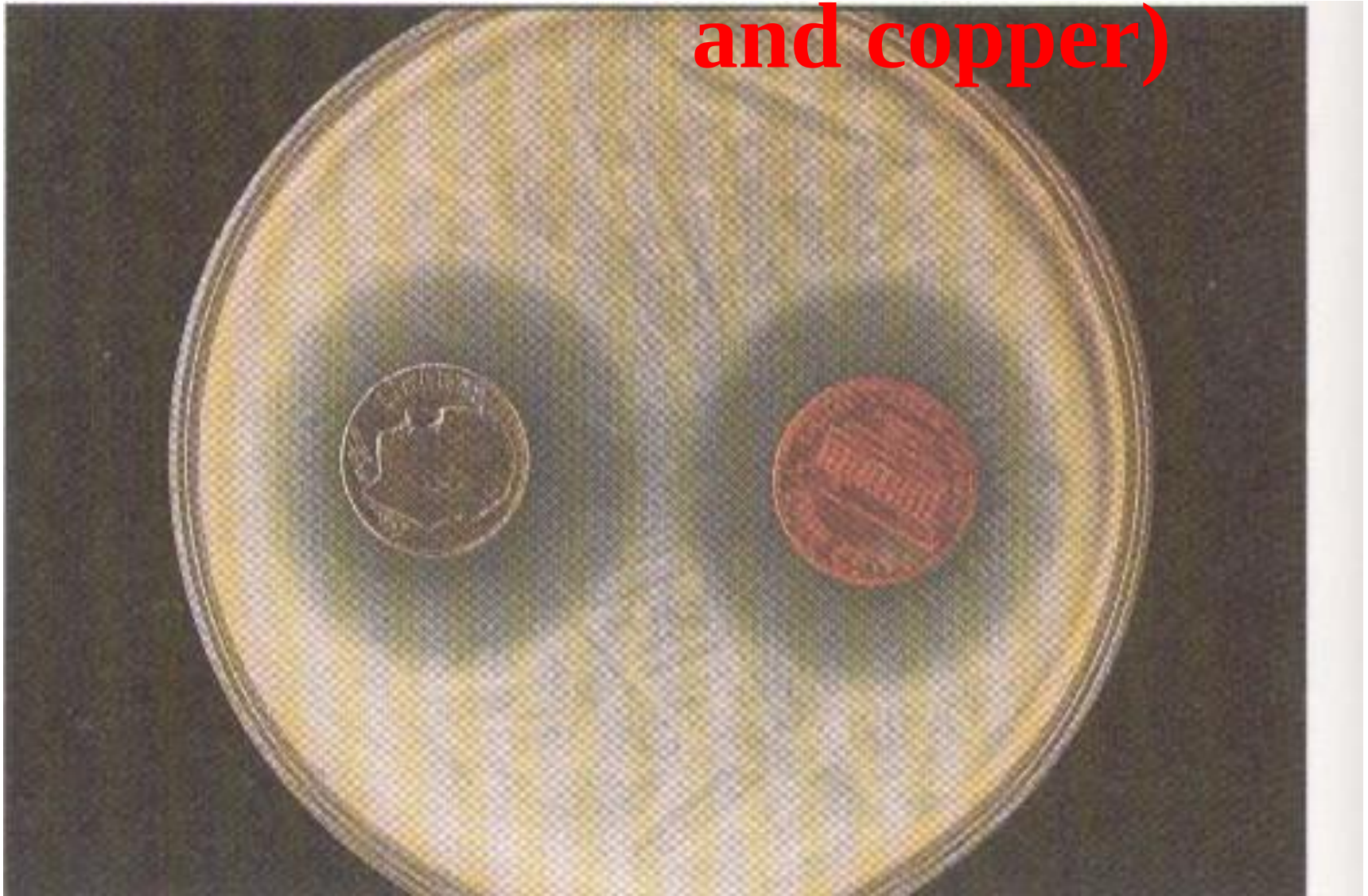
- **Main groups of antiseptics:**
- surfactants (detergents)
- - acids and their salts (boric, salicylic) and alkalis (ammonia)
- - alcohols (70-80 about ethanol)
- - phenol and its derivatives
- - aldehydes (formaldehyde)
- - oxidizing agents (hydrogen peroxide, potassium permanganate)
- - halogens (iodine, chlorine preparations)
- - salts of heavy metals
- - derivatives of 8-hydroxyquinolone
- - derivatives of nitrofuran (furatsilin)
- - dyes (diamond green)

Influence of chemical factors on microorganisms

The main groups of disinfectants:

- Chlorine-containing compounds (bleach, sodium and calcium hypochlorite, chloramine B, dichloro-1, sulfochlantin, etc.)
- Phenolic compounds (lysol, chloro-beta-naphthol, hexachlorophen, etc.)
- Quaternary ammonium compounds (nirtan, ampholan, etc.)
- Peroxide compounds (perhydrol, deoxone-1)

Antimicrobial salt activity of heavy metals (silver and copper)



Influence of biological factors on microorganisms.

Symbiosis is the coexistence of various organisms.

Forms of symbiosis:

- - metabiosis
- -mutualism
- - commensalism
- - satelliteism
- - synergism
- - antagonism

Metabiosis.

- One of the microbes uses product of other life activity, creates conditions for progress.

Ammonifiers —(ammonia)→ nitrification

Cellulose-breaking bacteria —(carbohydrates)→ nitrogen fixing

Mutualism

Cohabitation of microorganisms is –Mutualism.

Commensalism is

One microbe lives off the other, without harming him.

Normal micro flora of animals

Epiphytic microbes

Satelliteism

- Stimulation of growth and multiplication of one microbe with the products of other living activity.

Factors of growth, vitamins gr.B

Hay stick → Hemophilic bacteria

Synergism.

- Amplification of physiological features and functions during co-cultivation.

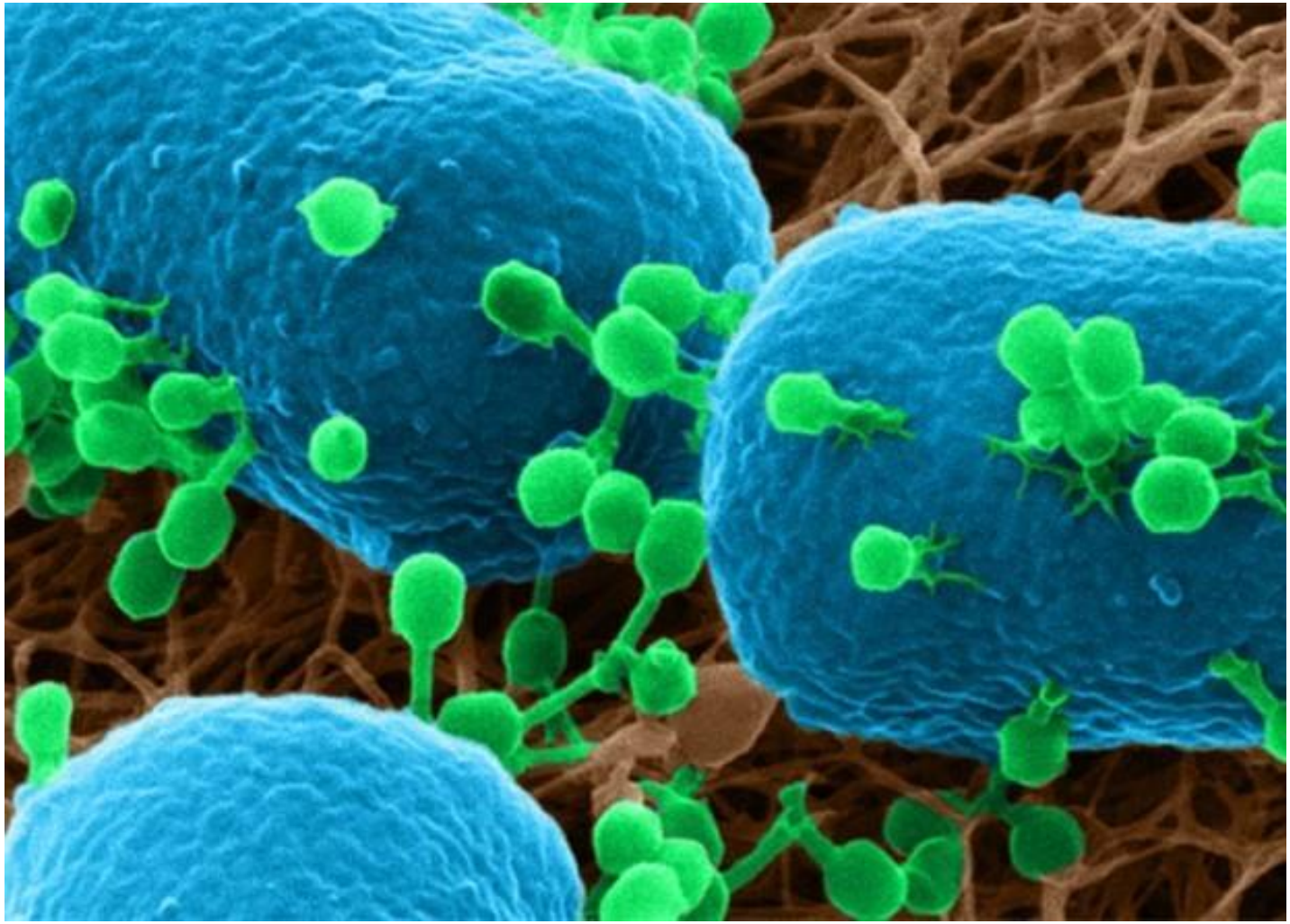
revitalization of lactic acid bacteria

Hay stick ⟷ Lactic acid bacteria

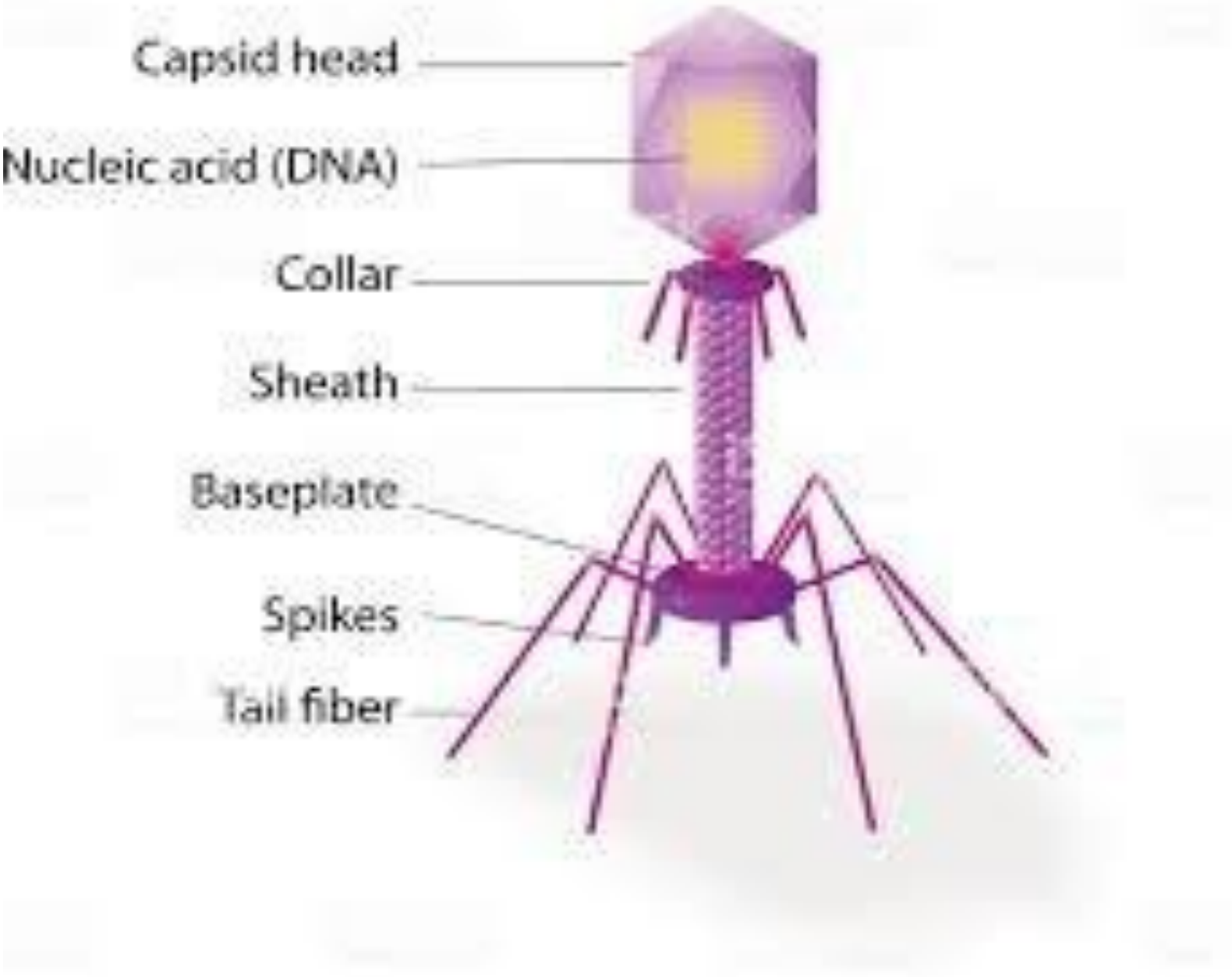
Bacteriophages.

- Bacteriophages (Greek "faqos" -fatting) are viruses that specifically penetrate bacteria that multiply inside them, as a result of which a bacterial cell dies or becomes a prophage carrier.
- Opened in 1917 scientist D ‘Errell.

Attack of bacteriophages on bacteria.



Structure of bacteriophage



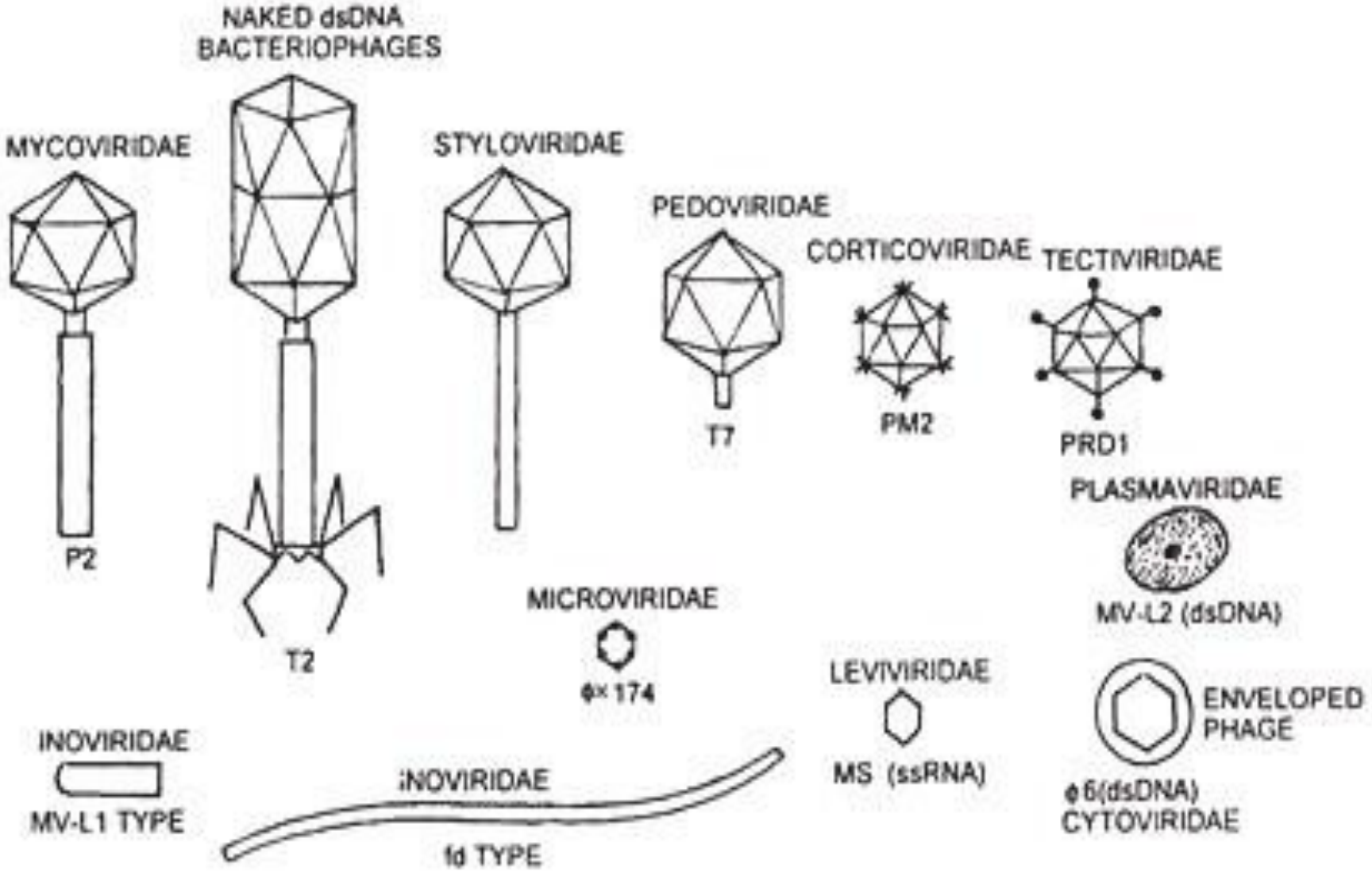


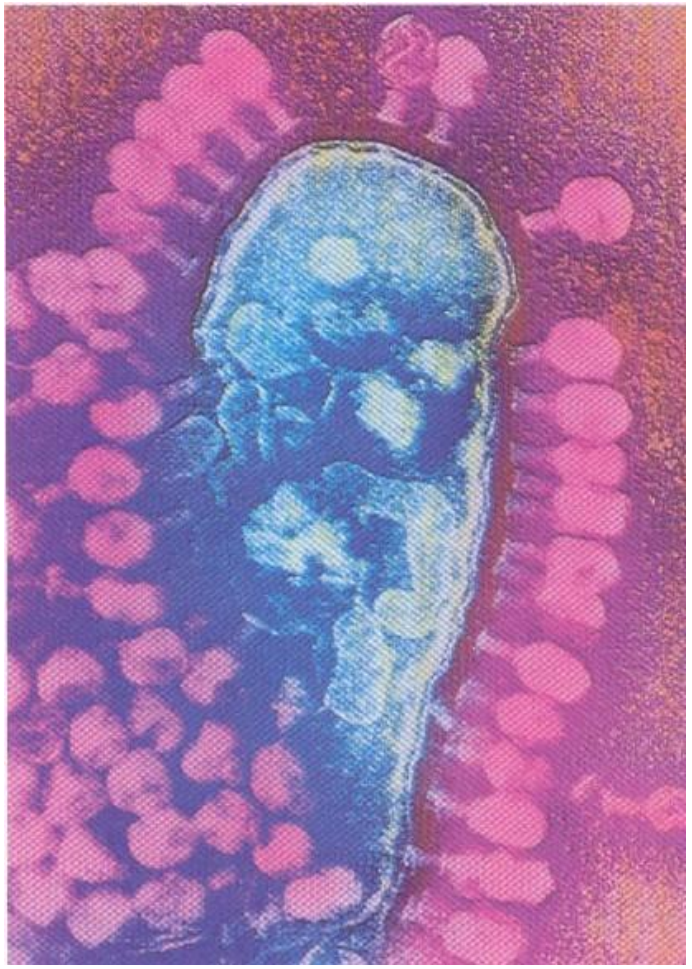
FIG. 13.1. Families of bactenophages (diagrammatic).

Types of bacteriophages

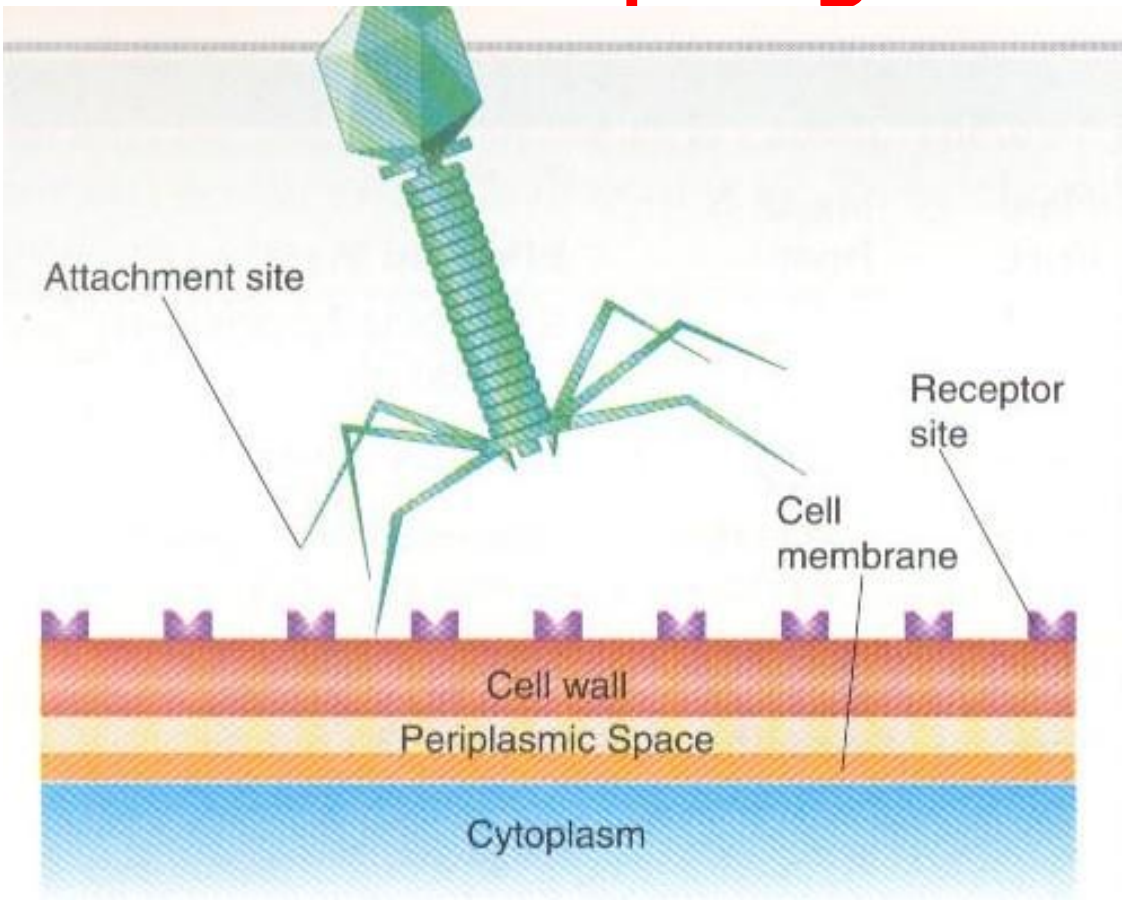
- Virulent bacteriophages (a productive type of interaction with a bacterial cell)
- Moderate bacteriophages (integrative type of interaction)

Phases of interaction of a virulent phage with a sensitive cell.

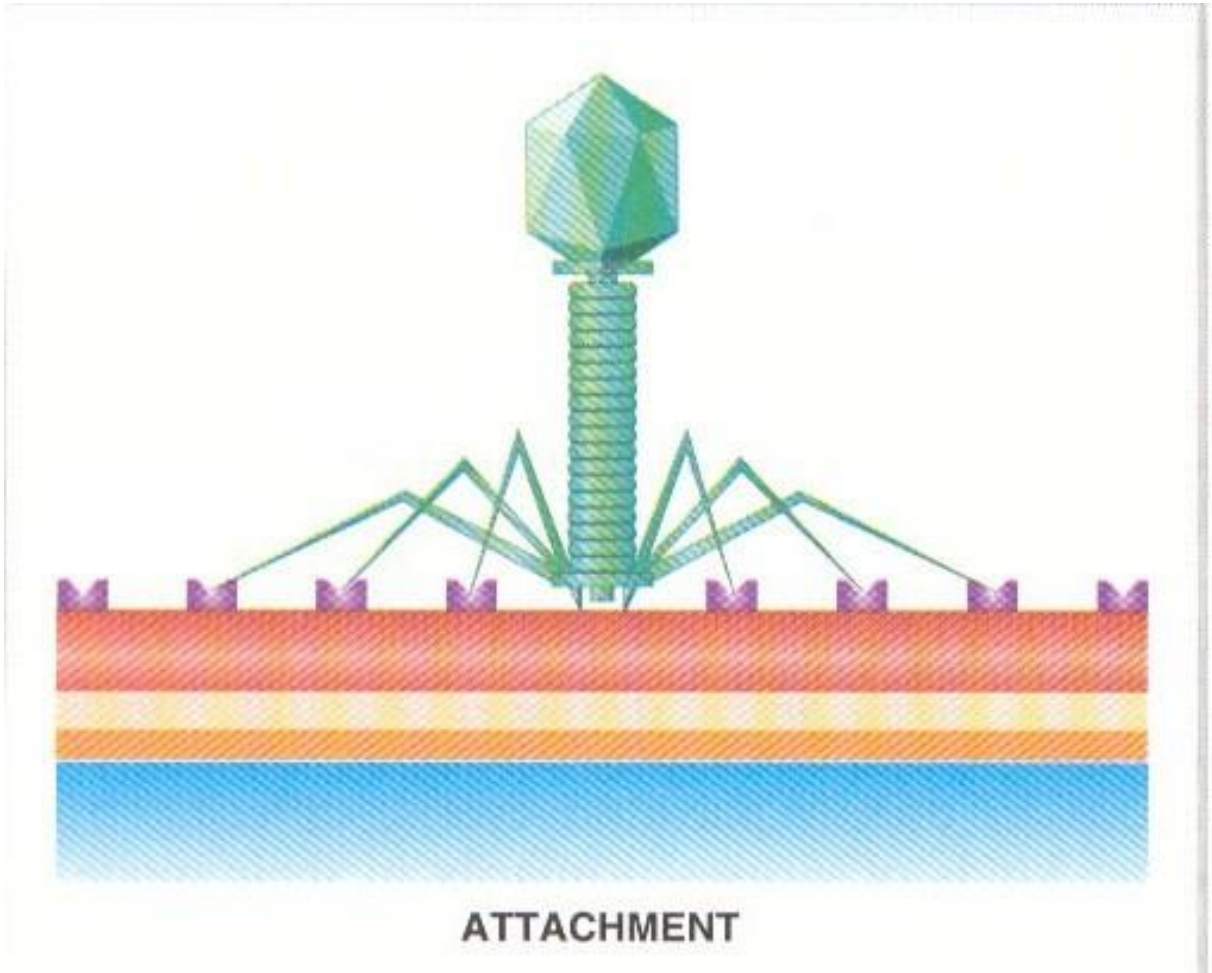
- Adsorption of phage on the surface of a bacterial cell
- Penetration of the phage into the bacterial cell
- Reproduction of phages in a bacterial cell
- Lysis of the bacterial cell and the release of phage offspring



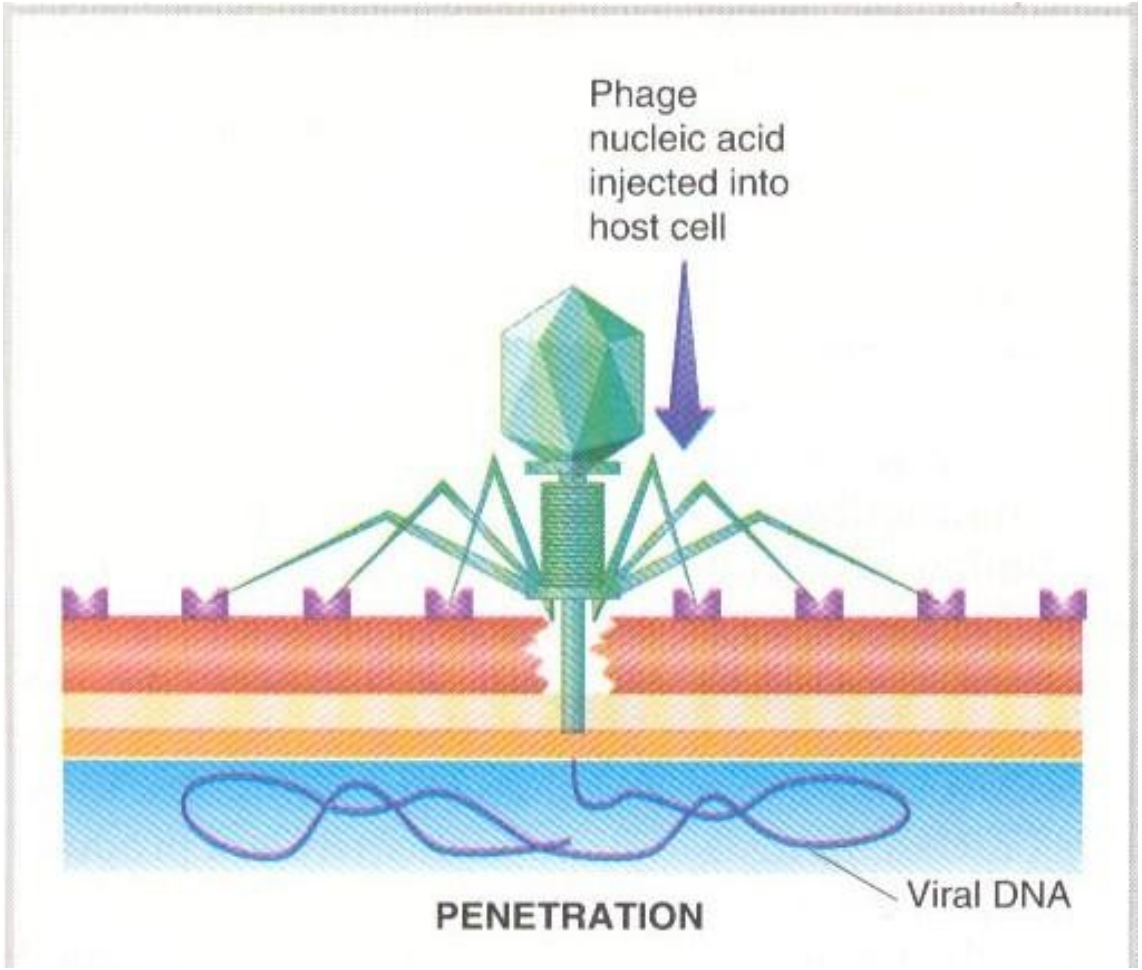
The mechanism of specific adsorption of a bacteriophage on a



Irreversible adsorption of bacteriophage on the cell wall.



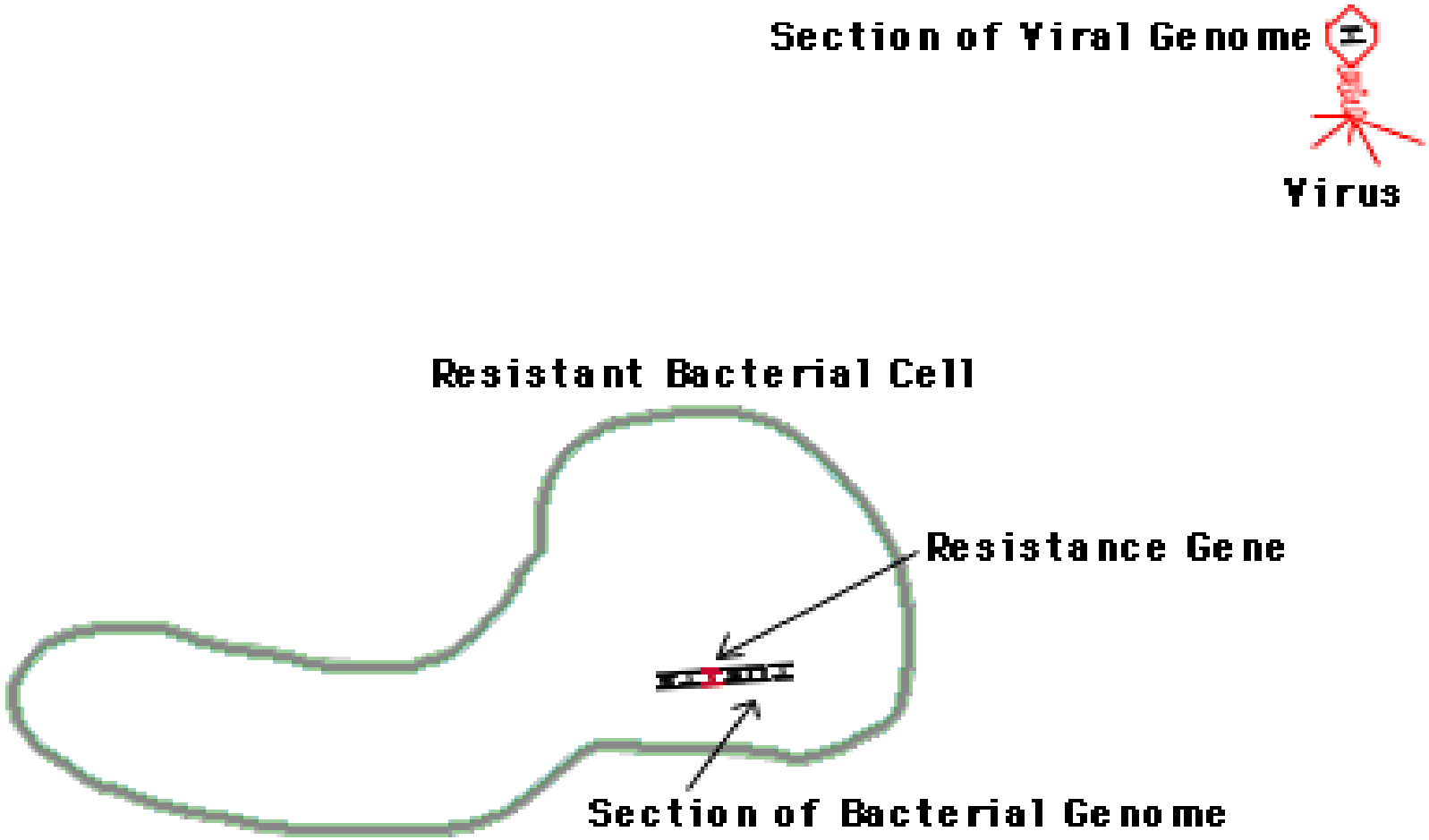
Penetration of nucleotide acid phages into bacterial cell.



Phases of interaction of a moderate phage with a sensitive cell.

- Adsorption of phage on the surface of a bacterial cell
- Penetration of the phage into the bacterial cell
- Integration of phage DNA into the bacterial chromosome and the formation of prophage
- Formation of Lysogenesis

Transduction of resistance.



Bacteriophages infect only particular bacteria's, interacting with specific cell receptors.

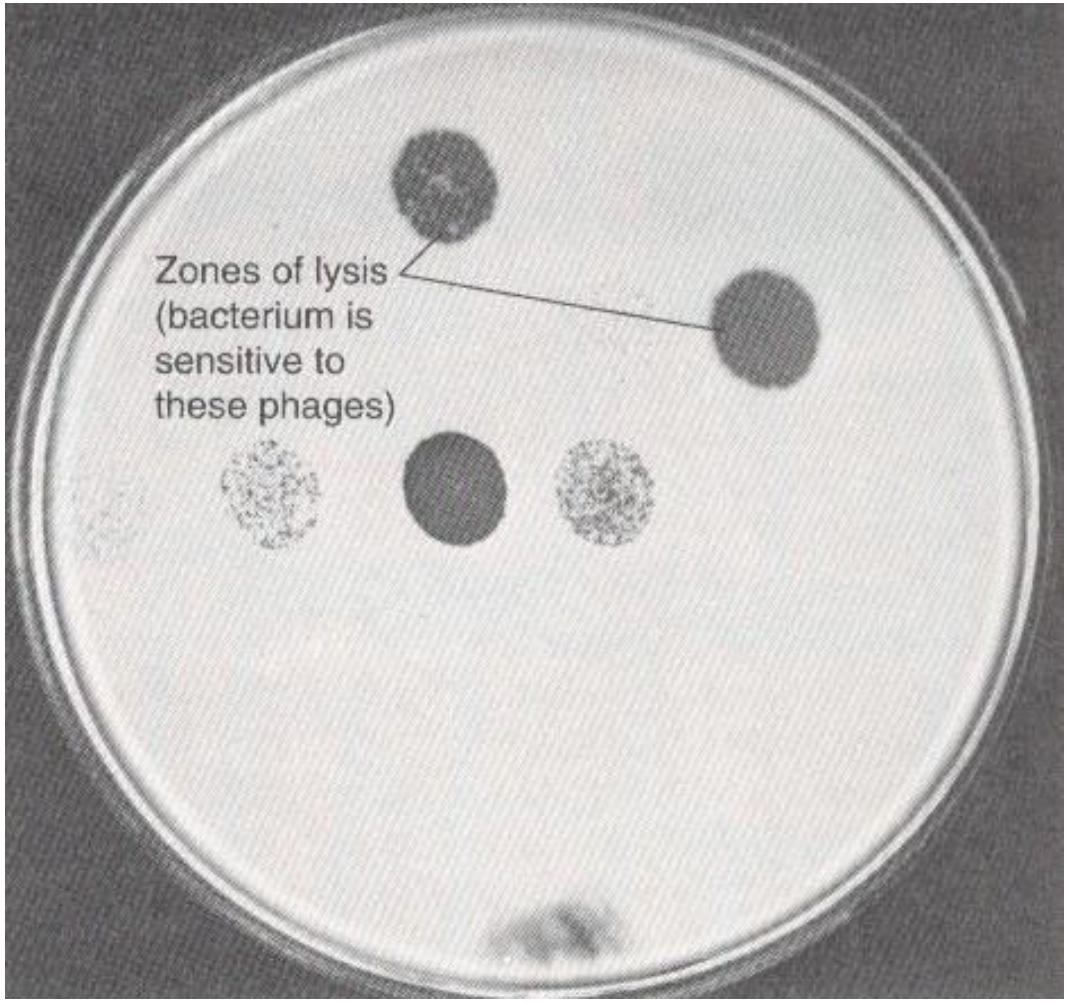
- By the spectrum of activity on bacteria, phages divide into:

  -polyvalent- lysed with familiar bacteria's.

  -monovalent- lysed with same gender bacteria's.

  -Type specific –lysing particular bacteria, bacteria's inside.

Phagodiagnosis.

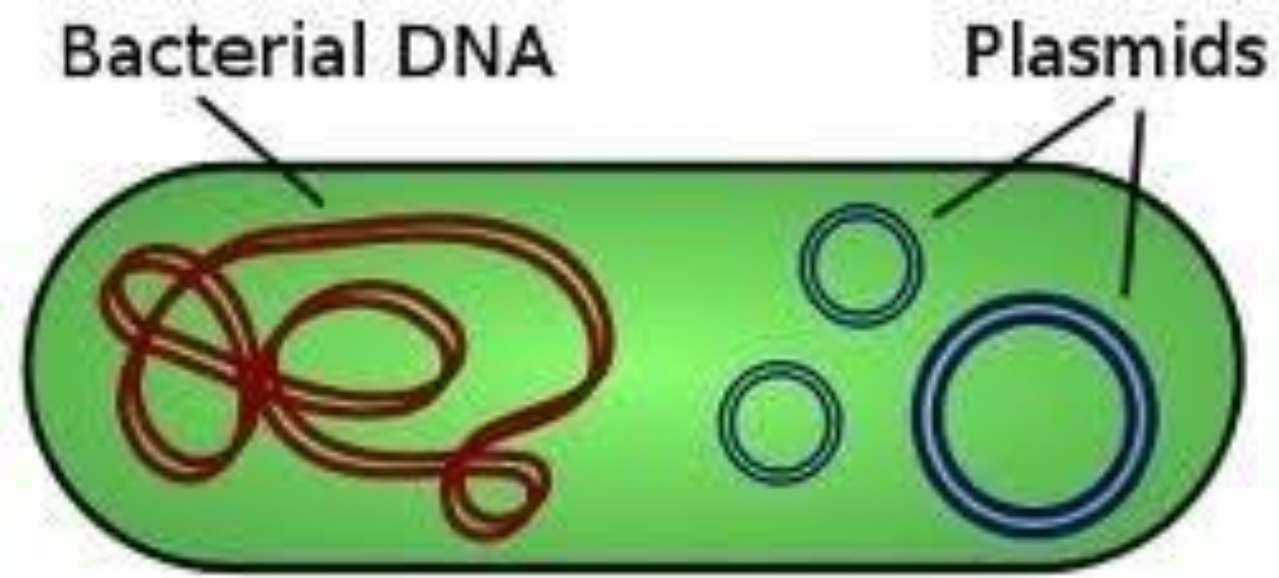


Защита и уход за кожей

Genetic appararus of bacteria

- Hereditary information in bacteria can exist in **nucleoid(chromosome), plasmids** – extrachromosomal structures, and in **migrating genetic elements**.
- The material basis of heredity is DNA. All features of organism are coded in DNA in form of nucleotide sequences.
- Only in some viruses (RNA viruses) the genetic information is coded by RNA.
- DNA molecule is formed by two spiral strands(chains). Each strand of the DNA is formed by nucleotides.

Bacterial genetic apparatus



Genes

- A part of DNA molecule responsible for synthesis of one protein is called gene. All organism features are coded by chromosomal genes.
- Structure and regulatory genes exist. ***Structural genes*** code information about protein, while ***regulatory genes*** regulate the activity of structure genes.

Genotype

- The whole set of cell genes comprises its genotype
- The genes responsible for synthesis of substance is named by initial letters of corresponding substance. For example, aminoacide arginine gene *arg*$^{+}$, lactase gene - *lac*$^{+}$
- Susceptibility to antibiotics and phages is denoted by **s** (*sensitive*), resistance – by **r** (*resistanse*). For exp., gene responsible for susceptibility to streptomycin is named as *str*^{s}, for resistance – as *str*^{r}.

Phenotype

- *Phenotype* refers to observable properties of an organism.
- In contrast to genotype phenotype can change. Manifestation of genitype in form of phenotype is called **expression**. However, genotype is not always expressed.
- Phenotype of bacteria is named as genotype (the first letter of phenotype name is written in capital).For example *arg*$^+$ *genotype corresponds to Arg*$^+$ phenotype, *lac*$^+$ - to *Lac*$^+$ phenotype.

Extrachromosomal genetic elements

- Some bacteria have extrachromosomal genetic elements – **plasmids and migrating genetic elements**.
- They are not of vital importance for bacteria, but support their variability and adaptation to environmental conditions.

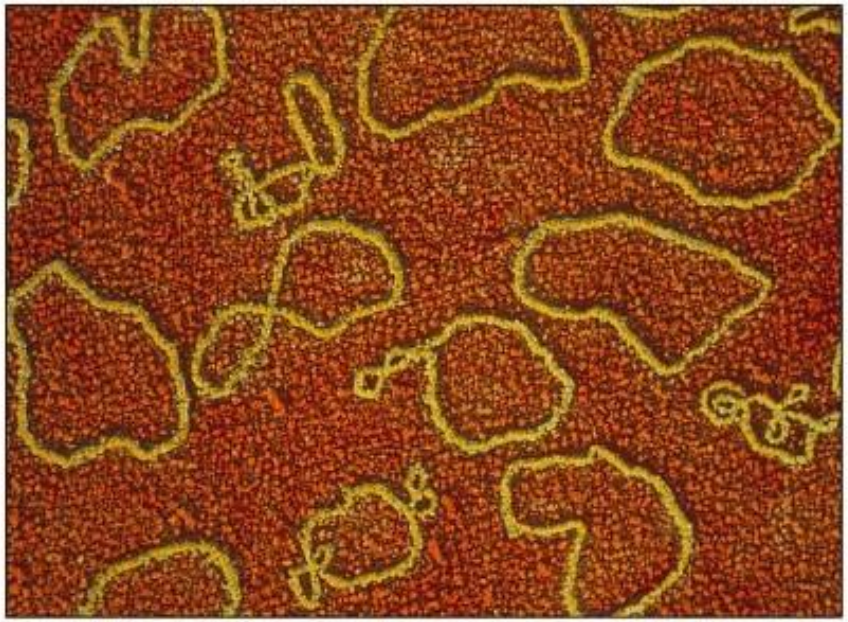
Plasmids

- **Plasmids are extrachromosomal DNA fragments consisiting of 40-50 genes.**
- Some circular plasmids are located in cytoplasma(**episomes**), some – integrated to chromosome(**integrated plasmids**).
Plasmids features:
- extrachromosomal DNA molecules;
- Multiply independently of chromosome;
- Can be transferred between bacteria;
- Exist in circular and linear forms;

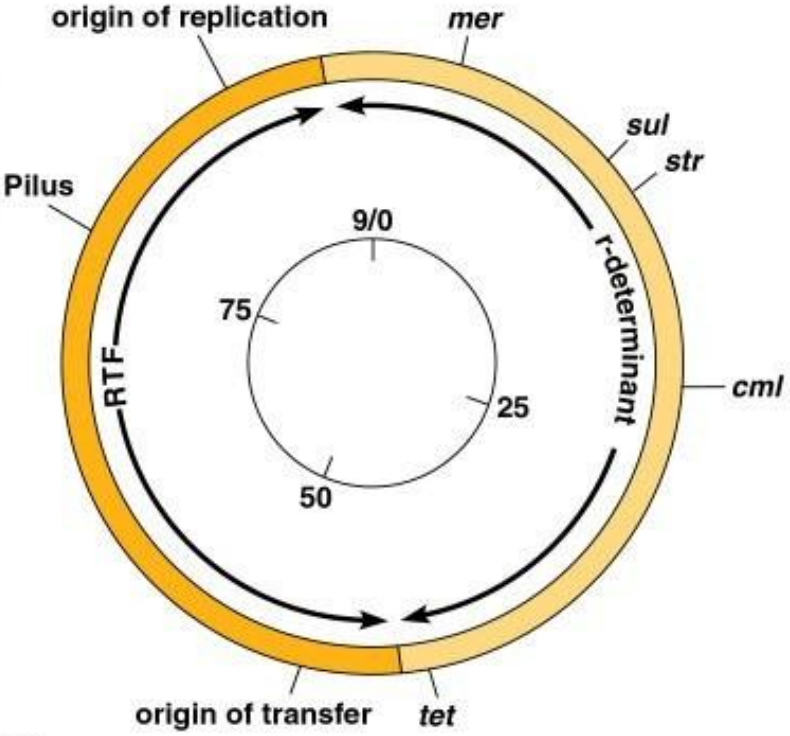
Plasmids

- Plasmids are a part of genetic apparatus of bacteria and responsible for antimicrobial resistance, toxin production, bacteriocin synthesis etc. Genes responsible for synthesis of these molecules are located in plasmids.
- ***F-plasmids*** (eng, *fertility*) – participate in conjugation
- ***R-plasmids*** (eng, *resistanse*) – antimicrobial resistance
- ***tox^{+}-plasmids***- synthesis of exotoxins (exp., diphtheria and botulism, prototoxins)
- ***Col^{+}-plasmidsr -*** synthesis of colicin and other bacteriocins by E.coli

Plasmids



(a)



(b)

Figure 8.29

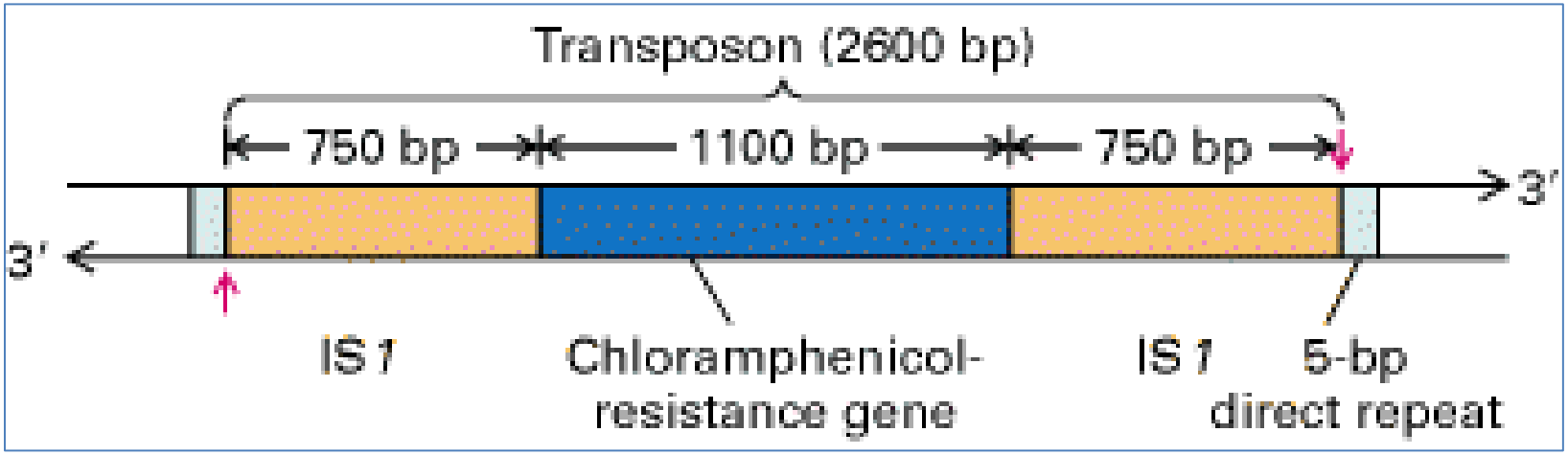
Migrating genetic elements

- Small DNA fragments are able to **migrate (transposition)** from one chromosome to another, from chromosome to plasmid, from plasmids to chromosome. This feature is due existence in migrating elements of enzyme – **transposase.**
- Migrating genetic elements

  - insertion sequences (IS-elements),
  - transposons(Tn-elements),
  - defective phages.

Transposons

- ***Transposons (Tn-elements).*** DNA fragments with 2000-25000 nucleotide pairs.
- Have specific structure gen and 2 IS-elements.
- Structure gene of transposon can transmit to bacteria special feature, for exp. Antimicrobial resistance, ability to produce toxin, bacteriocin etc.
- After entering bacterial cell they can cause duplication, deletion and inversion.



Types of genetic transfer

- **Nonhereditary variability(modification). It is also called phenotypic variability** as it is accompanied only by phenotypic changes.
- **Genetic variability**. Also called genotypic variability. In microorganisms genotypic variability occurs through **mutation** and **genetic recombination.**

Modification

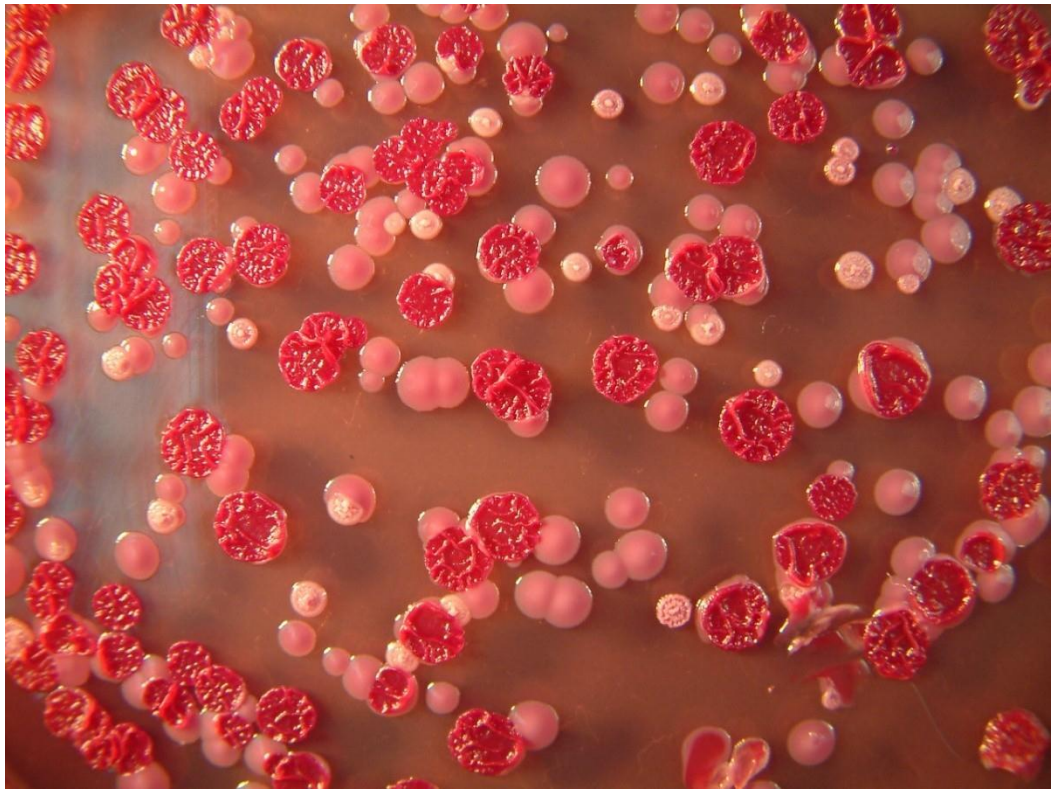
- Through modification microorganisms attain morphological, cultural, biochemical changes.
- Modification in ***morphological features*** is accompanied by changes in form and size of microorganisms***.***
- Modification can be represented by changes in***:***
- ***cultural features,***
- ***Biochemical features*** of microorganism
- Modification is manifested in microorganism population as **dissociattion** phenomenon.

Dissociation

- During dissociation some bacteria when cultivated in solid media form different types of colonies (2 or more types).
- Smooth ***S-colonies***, rough ***R-colonies***.
- Sometimes mucoid ***M-colonies, very small D-colonies*** (*dwarf) are formed*

R - S dissociation

- Under some circumstances S-colonies can change to R-colonies and vice versa. R-S dissociation is not frequently observed phenomenon
- Majority of human pathogens form S-colonies. Exceptions are *Mycobacterium tuberculosis, Yersinia pestis, Bacillus anthracis* etc.



Comparison of R- and S-colony forming microorganisms

S-colonies	R-colonies
Smooth, bright, convex	Irregular, turbid, wrinkled
Cause turbidity in broth	Sediment in broth
Motile species have flagella	Flagellalar olmaya bilər
Some species have capsule	Do not have capsule
High biochemical activity	Weak biochemical activity
High virulence	Weak virulence
Commonly isolated during active diseases	Commonly isolated during chronic diseases

Genetic variability

- As it is related to genotype it is called also genotypic variability.
- In microorganisms genotypic variability occurs through ***mutation*** and ***genetic recombinations***.

Mutation

- **Mutation** (lat, *mutatio* - change) – occurs in chromosomes and genes. As a result of mutation microorganism can obtain or loose some features. This variability is passed on future generations.
- In order to distinguish strains passed through mutation from ***wild strains*** they are called ***mutant strains.***

Mutations

- ***Spontaneous mutations***

  - ***reversible***

- ***inducible mutations***

  - ***mutagens*** (chemical substances, radiation– UV, ionizing, X-rays.)

- ***Point mutations***

  - ***frameshift mutations***

  - ***missens mutations*** –change in aminoacide

  - ***nonsens mutations***

- ***Chromosome mutations(deletion, inversion, duplication)***

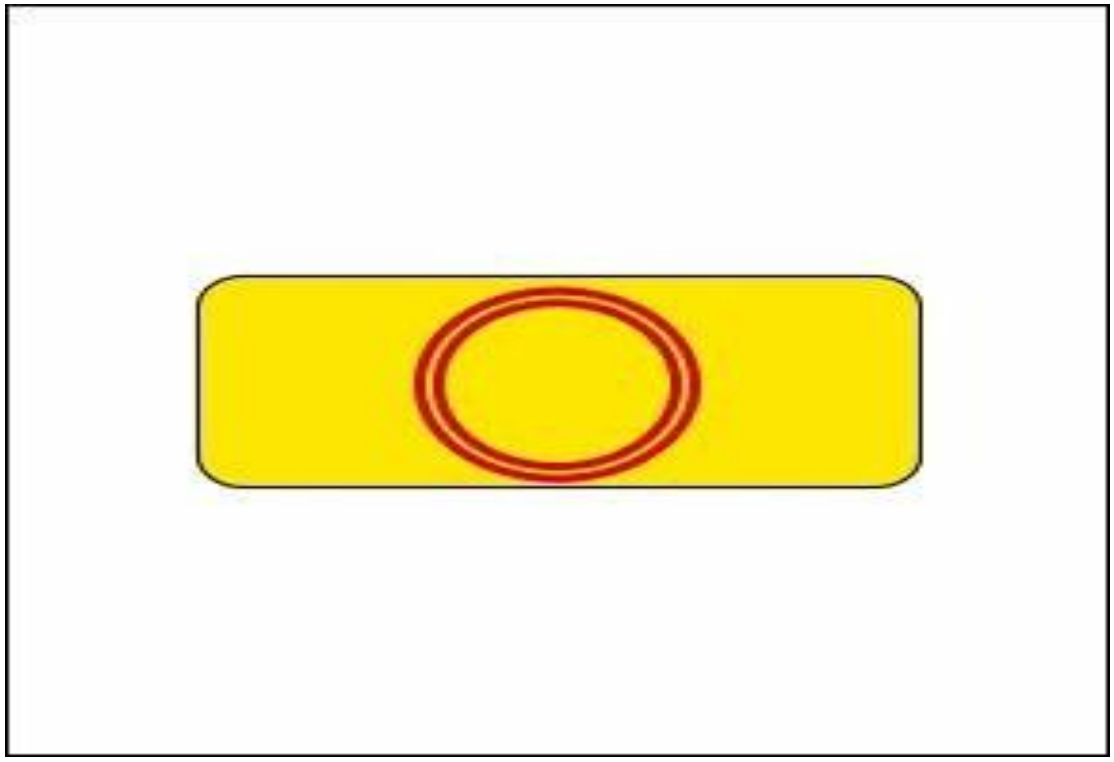
- ***According to phenotypic results- neutral mutations, conditional lethal, lethal mutations***

Genetic recombinations

- Exchange of genes occurs between two microorganisms. An isolate passing genetic material is called **donor**, while isolate receiving it – **recipient**.
- During recombination recipient cell receive a part of chromosome which leads to formation of noncomplete zygote – **merozygote**.
- After recombination from recipient cell **recombinant** cell is formed. Thus, recombinant cell posses recipient cell genotype and some genes of of donor.
- Transfer of genetic material in microorganisms occur through **transformation**, **transduction** and **conjugation**.

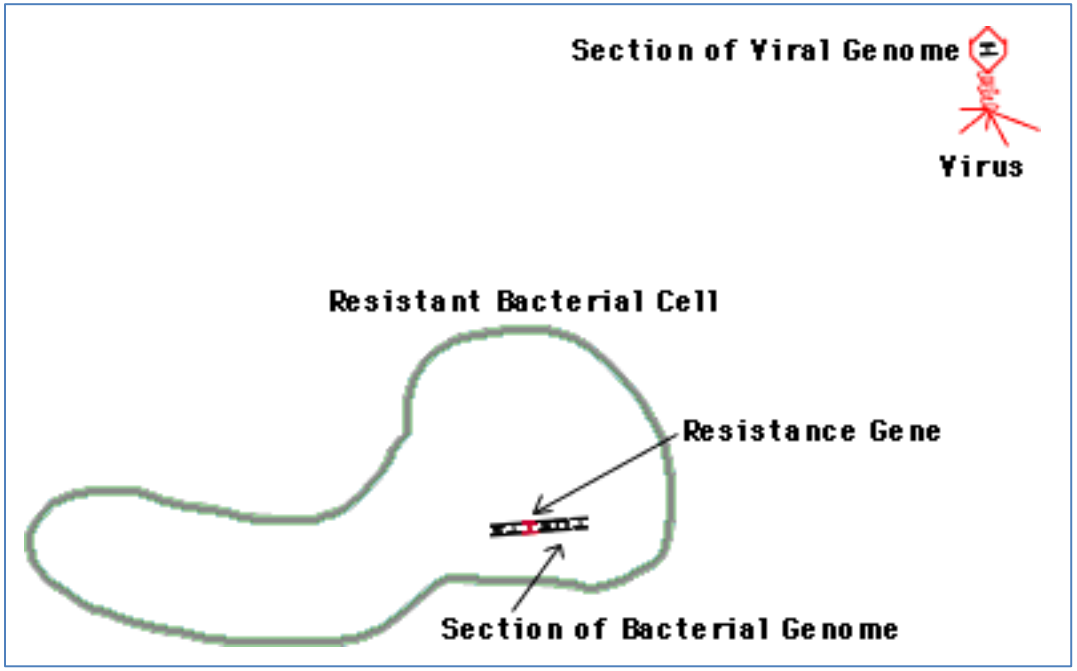
Transformation

Transformastion – direct transfer of genetic material (DNA)from donor to recipient



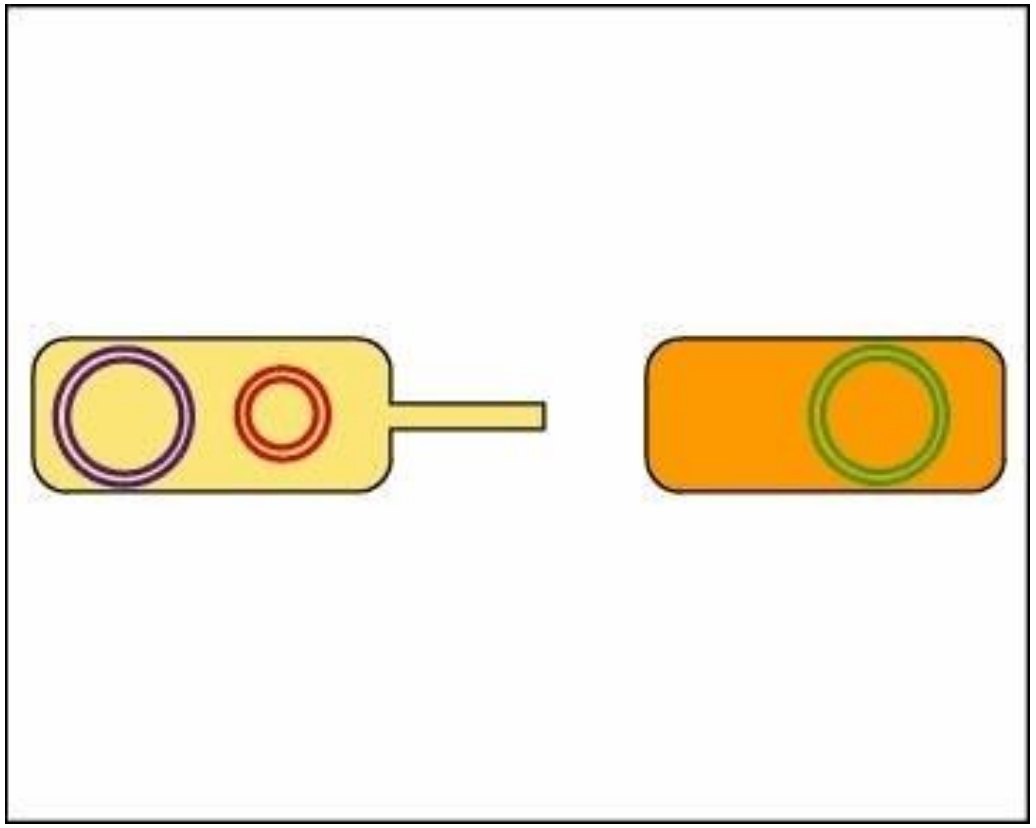
Transduction

Transduction – transfer of genetic material (part of a DNA molecule) from a donor to a recipient by bacteriophages

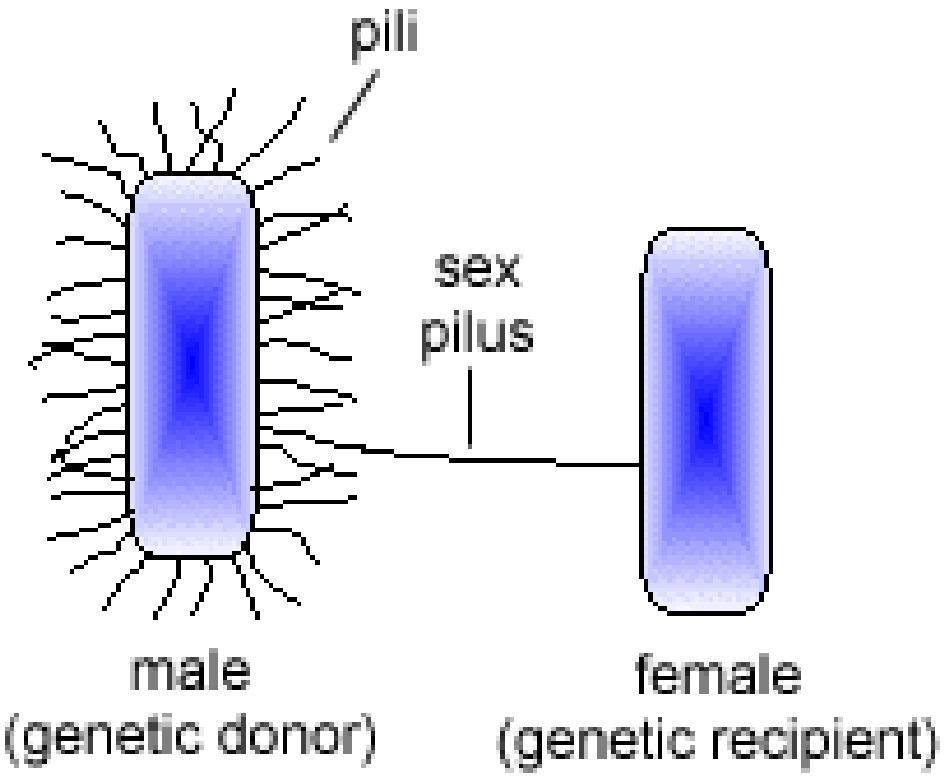


Conjugation

Conjugation- the most frequent mechanism of transfer of genetic material.
In this case, the genetic material is transferred from the donor to the recipient by direct contact.



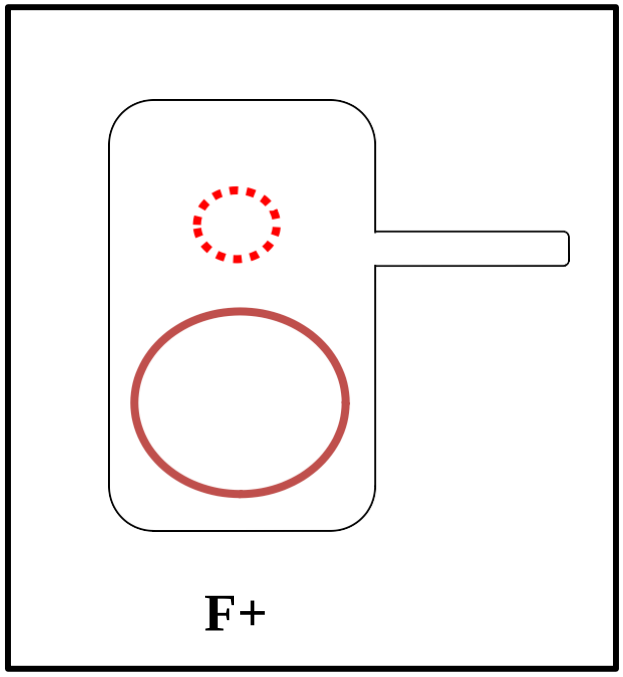
Conjugation



Conjugation

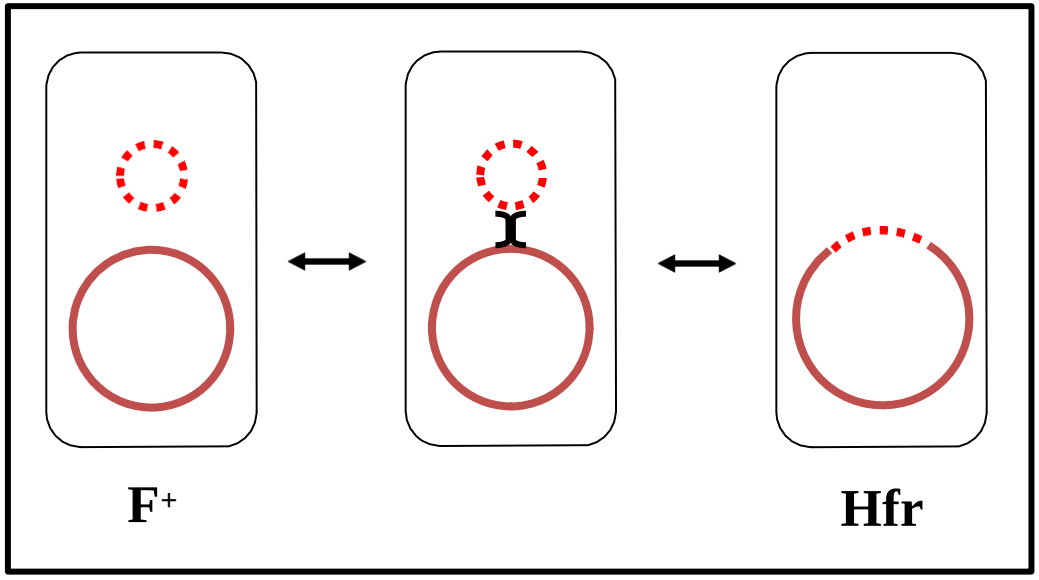
- As other recombination mechanism 2 cells participate in conjugation. The donor must have F-plasmid or F-factor (fertility), and called F + cell. Since this factor is not present in the recipient cell, it is referred to as F- cell.
- During conjugation the F-factor is transferred to the recipient cell in almost all cases, regardless of the donor chromosome.
- F-factor encodes conjugative pili (F-pili).
- After conjugation recipient cell becomes F^+-cell,which can transfer F-factor to other cells.

F^+ cell



Hfr-strains

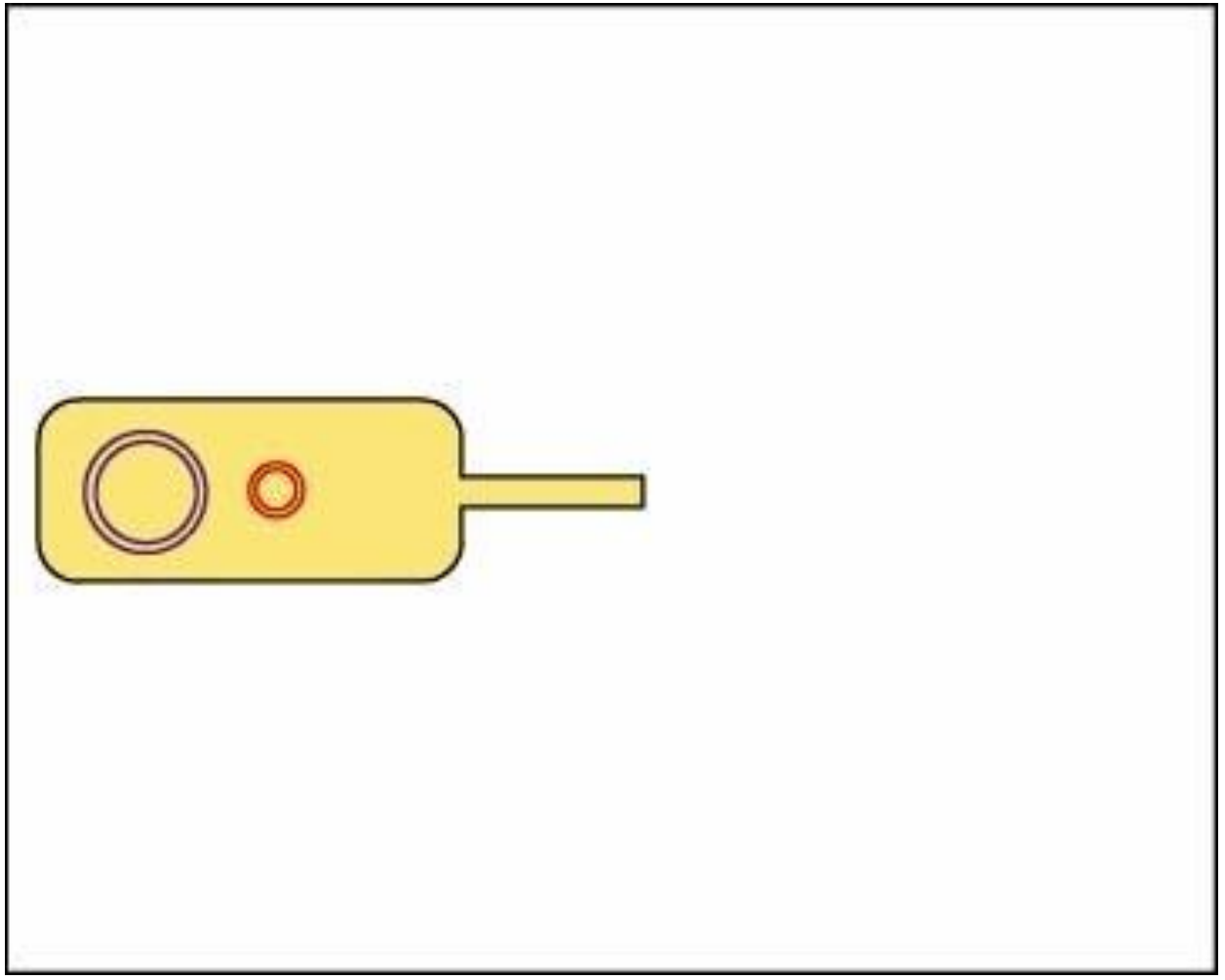
If F-plasmid integrates to cell chromosome it forms Hfr-cell (*high frequency of*). They are able to transfer chromosomal genes to recipient cells with high frequency



Conjugation between Hfr strain and *F*$^{-}$ cell

- During conjugation between Hfr-strain and F^{-} **cell** F-factor is not transferred, in contrast chromosome DNA is transferred with high frequency.
- After such conjugation, **the recipient still remains an F-cell**.
- During ***Hfr-conjugation*** chromosome DNA is replicated, as a result one strand of synthesized DNA copy is transferred to F^{-} cell. Thus, donor strain remains genetically stabile.

Conjugation between Hfr strain and *F*$^{-}$ cell



Characteristics of viral genome

- Viral genome consists of only one type nucleic acid - DNA or RNA;
- While the genome of other organisms consists of DNA, in viruses RNA also can play a genome role(RNA viruses);
- DNA viruses have 2-strand, nonsegmented genome with infectious properties (except *Poxvirus* and *Hepadnovirus* as their DNA strands have different lengths);
- Except Reoviruses and retroviruses majority of RNA viruses have single strand RNA;
- Genome of RNA viruses may be segmented(fragmented) or nonsegmented;
- Genome of positive (+RNA) viruses possess infectious properties;
- Genome negative (-RNA) viruses does not possess infectious properties

Types of variability in viruses

- **Modification**
- **Mutation**

  - ***Without phenotypic manifestation(neutral),***
  - ***with phenotypic manifestation***
    - ***lethal,***
    - ***conditional-lethal- temperature sensitive mutants*** (***ts-mutantlar***)
  - ***Increase of viral infectious spectrum***
  - ***resistance to antiviral drugs***

Genetic interactions between viruses

- When at the same time different viruses infect a cell they interact with each other during reproduction.
- ***Genetic recombination*** is exchange of genes between two or more viruses. It is common in DNA-containing viruses, resulting in the formation of recombinant viruses with two or more parental genes.
- ***Genetic reactivation*** occurs between to relative viruses with nonactive genes. After recombination these genes become activated (reactivation).

Nonspecific interaction between viruses

- ***Complementation*** – a protein encoded by genome of one virus supports reproduction of other virus. Complementation is observed between two defective viruses that cannot be reproduced separately, resulting in the reproduction of one or both of these viruses.
- **Phenotypic mixing** - when a susceptible cell is infected with two different viruses, sometimes one generation of the virus has the phenotypic characteristics of the both parental viruses.
- **Phenotypic masking** - the genome of one virus is surrounded by the capsid membrane of another virus, resulting in ***pseudotypes***.

Application of genetic methods in diagnostics

- Polymerase chain reaction
- Molecular hybridization
- Restriction analysis
- Sequenation

Antimicrobial drugs

- Non-selective actions - disinfectants and antiseptics that cause the death of most microbes, but are toxic to the body;
- Selective actions are chemotherapeutic agents.

Chemotherapy.

Chemotherapy is the treatment of people with infectious diseases using chemicals that act selectively on the pathogen in the human body without harming the patient's cells and organs.

Formation of Chemotherapy.

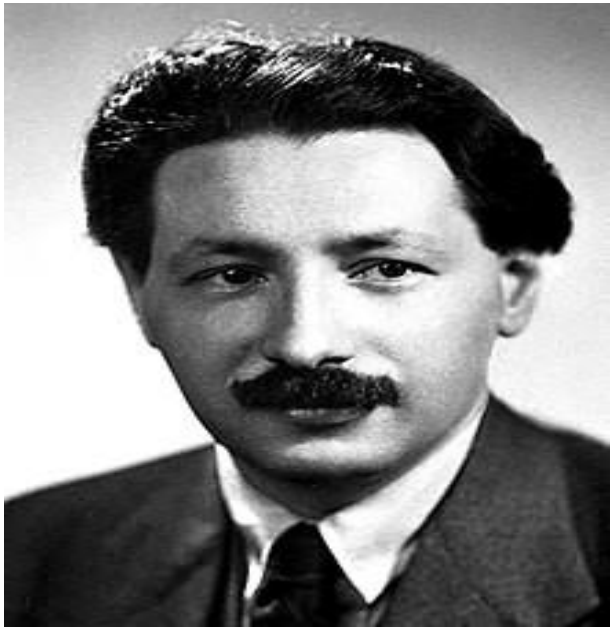
- 1885 - P. Ehrlich formulated the main idea of chemotherapy - the selectivity of the action of chemicals.
- 1886 - synthesis by P. Ehrlich and at the Pasteur Institute of antitrypanosomal drugs (trypan red and trypan blue)
- 1887-1888 - P. Ehrlich formulated the basic requirements for chemotherapeutic drugs and introduced the concept of a chemotherapeutic index

The idea of antibiotic therapy.

- 1871-1872 - V. Manassein and A. Polotebnov described the healing properties of green mold
- 1884 - L. Pasteur first observed microbial antagonism
- 1894 - I. Mechnikov discovered antagonism of lactic and putrefactive bacteria

Chemotherapy Development

- 1909-1910 - the synthesis of P. Ehrlich antispirochete drugs (salvarsan and neosarvarsan)
- 1920-1930 - synthesis in Germany and France of antimalarial drugs (plasmoquine)
- 1932 - synthesis in Germany by G. Domagk of an antibacterial drug - chrysoidine sulfamide (sulfanilamide)



Chain Ernst Boris (1906-1976)
English biochemical

Flory Howard Wolter
(1898-1968) Pathologist
Microbiologist

•In 1938 discovered penicillin in injection form.
•Became Nobel Laureate on physiology and medicine in 1945 along with Alexander Fleming for discovery and synthesis of penicillin.

History of antibiotics discovery.

- 1920-1929 - A. Fleming - the study of the
  - antibacterial properties of green mold and the
- discovery of penicillin
- •1940 - G. Flory, E. Cheyne and N. Heatley -
- received purified penicillin
  - 1942 - S. Waxman coined the term antibiotic
- (Greek anti-against, bios-life)
  - 1945 - A. Fleming, G. Flory and E. Cheyne - Nobel Prize for the discovery of penicillin

Basic requirements for chemotherapeutic drugs.

- Action specificity
- Maximum therapeutic activity
- Minimal toxicity to the body

Chemotherapy Index

- Minimum therapeutic dose
- Maximum Tolerated Dose
- Index must not be greater than 1

The range of action of chemotherapeutic drugs.

The spectrum of action distinguishes:

1. Acting on cell forms (antibacterial, antifungal, antiprotozoal)

- antibacterial can be wide and narrow spectrum of action
- 2. Acting on non-cellular forms (antiviral)
- 3. Suppressing tumor growth (antitumor)

Type of action of antimicrobial chemotherapy drugs.

- Microbicidal (bactericidal, fungicidal, etc.) chemotherapeutic agents - causing the death of microbes due to irreversible damage;
- Microbostatic chemotherapy - inhibiting the growth and reproduction of microbes

Antimicrobial chemotherapy.

- Antibiotics (act on the cellular forms of microbes and tumors);
- Synthetic chemotherapeutic agents (act on cellular and non-cellular forms of microbes, as well as on tumors).

Antibiotics.

Antibiotics are chemotherapeutic drugs made from chemical compounds of biological origin (as well as their semi-synthetic derivatives and synthetic analogues) that have a bactericidal and bacteriostatic effect on microorganisms and inhibit the growth and reproduction of some tumors.

Classification of antibiotics by source.

- Microbial origin
  - from actinomycetes (streptomycin, tetracycline)

  - from bacteria (polymyxin, gramicidin, etc.)
  - from mushrooms (penicillin, cephalosporins, etc.)

- Plant origin (volatile)
- Animal origin (interferon, ecmoline)

Classification of antibiotics by source.

- Biological synthesis (penicillin)
- Biosynthesis with subsequent chemical modifications (semi-synthetic antibiotics - benzylpenicillin, ampicillin, oxacillin)
- Chemical synthesis (synthetic analogues of natural antibiotics - chloramphenicol bicillin)

Classification of antibiotics by spectrum of activity and type of action.

- Broad spectrum (aminoglycosides and tetracyclines)
- Narrow Spectrum (Polymyxin)

•Bactericidal (fungicidal) –penicillin, cephalosporins
•Bacteriostatic (fungistatic) – tetracyclines, chloramphenicol

Classification of antibiotics by chemical structure (1)

Beta-lactams - the basis of the molecule is the beta-lactam ring

They act bactericidal. These include:

- penicillins: natural - benzylpenicillin, depot preparations - bicillin, acid-resistant - phenoxymethylpenicillin, penicillin-resistant - a narrow spectrum (methicillin and oxacillin) and a wide range (ampicillin and amoxicillin), which are antisine-free, combined with bicenitazin, biceniticillin, biceniticin, (amoxicillin + clavulanic acid, amoxicillin + sulbactam).
- cephalosporins - have a wide range, but are more active against gram-negative bacteria. There are 4 generations: 1st — more active against gram-positive bacteria and sensitive to beta-lactamases (cefazolin), 2nd — more active against gram-negative bacteria and more resistant to the enzyme (cefuroxime), 3rd — more active against gram-negative bacteria and highly resistant to the enzyme (cefotaxime), 4-y - act on gram-positive bacteria, some gram-negative and Pseudomonas aeruginosa, are resistant to beta-lactamases (cefipim).
- carbopenems - have the widest spectrum of action and are resistant to beta-lactamases
- monobactams - are active against gram-negative bacteria, including Pseudomonas aeruginosa, and are resistant to beta-lactamases (aztreona)

Cephalosporin's.

Cephalosporin antibiotics

1st Generation	2nd Generation	3rd Generation	4th Generation
• Cefadroxil • Cefazedone • Cefazolin • Cephalexin • Cephalothin • Cephradine • Cephaloridine • Cephapirin etc.	• Cefaclor • Cefamandole • Cefoxitin • Cefuroxime • Ceforanid • Cefonicid etc.	• cefixime • Cefoperazone • cefotaxime • cefpiramide • cefpodoxime • Ceftibuten • ceftizoxime • ceftriaxone etc.	• Cefepime • cefluprenam • Cefozopran • cefpirome • cefquinome etc.
Good against **Gram +**, Moderate against **Gram -**	**Good** against **Gram -**, Moderate against **Gram +**	**Good** against **Gram -**, Weak against **Gram +**	**Good** against **Gram -**, Extended activity against **Gram +**

Classification of antibiotics by chemical structure (2)

- Glycopeptides are large molecules that do not pass

  - through the pores of gram-negative bacteria, therefore they have a narrow spectrum of action (vancomycin)
- • Aminoglycosides - the molecule contains sugars, has a wide spectrum, bactericides (streptomycin, gentamicin)
- • Tetracyclines - are composed of 4 cyclic compounds, a wide spectrum (tetracycline, dioxicycline)
- • Macrolides and azolides - a family of large macrocyclic molecules with a wide spectrum of action, bacteriostatics (erythromycin, azithromycin, clarithromycin)
- • Lincosamides - bacteriostatic agents similar to macrolides are effective against anaerobes (lincomycin, clindamycin).

Classification of antibiotics by chemical structure (3)

- Chloramphenicol - have a nitrobenzene core, which gives them toxicity, bacteriostats (chloramphenicol / chloramphenicol).
- Rifampicins are broad-spectrum bactericides that are effective against mycobacterium tuberculosis (rifampicin)
- Polypeptides - narrow-spectrum bactericidal antibiotics, act only against gram-negative bacteria, toxic, applied externally (polymyxin)
- Polyenes are highly toxic antifungal drugs that are often used locally (nystatin, amphoterricin B).
- Other antibiotics (fusidic acid).

The main groups of synthetic chemotherapeutic drugs.

- Sulfanilamides - the basis of the drug is a para-amino group, which acts as an analog or antagonist of para-aminobenzoic acid, necessary for the synthesis of folic acid (co-trimoxazole, or biseptol).
- Quinolones - nalidixic acid has a limited spectrum of action.
- Fluoroquinolones - bactericidal effect, a wide range, high activity (ciprofloxacin, norfloxacin).
- Nitroimidazoles are bactericidal, especially active against anaerobic bacteria, as well as protozoa (metronidazole, trichopolum).
- Imidazoles - antifungal drugs that inhibit the synthesis of CPM (clotrimazole)
- Nitrofurans - used as uroseptics (furozalidone, furadonin, furagin)
- Oxyquinolines - nitroxoline

Additional groups of synthetic chemotherapeutic drugs.

- Arsenic preparations - novarsenol, osarsol, etc.
- Bismuth preparations - bismoverol, xoroform, etc.
- Antimony preparations - stibenil, syurmin, etc.
- Mercury preparations - salicylic, cyanide mercury, etc.
- Acridine preparations - rivanol, flavicide, etc.
- Anti-TB drugs – PASK, ethambutol, phtivazide, etc.
- Antimalarial drugs - Akrikhin, chloroquine, etc.
- Antineoplastic drugs - sarcolysin, vinblastine, dopan, etc.

Classification of antimicrobial chemotherapeutic agents according to the object of action.

- Antibacterial (most antibiotics, fluoroquinolones, nitroimidazoles, nitrofurans)
- Antifungal (nystatin, amphoterricin B, imidazoles)
- Antiparasitic (doxycycline, clindamycin, nitroimidazoles)
- Antiviral (reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, etc.
- Antineoplastic (rubomycin, olivomycin)

Classification of antibiotics by the mechanism of action.

- Synthesis of cell wall inhibitors.
- Synthesis of ribosome protein inhibitors.
- Synthesis of nucleotide acid inhibitors.
- Violation of CPM functioning.
- Disorder of exchange processes (synthesis of folic acid)
  sulfanilomides, trimethoprim, isoniazid

Mechanism actions of most antibiotics based on inhibitation of major life enymes.

The mechanism action of antimicrobial agents.

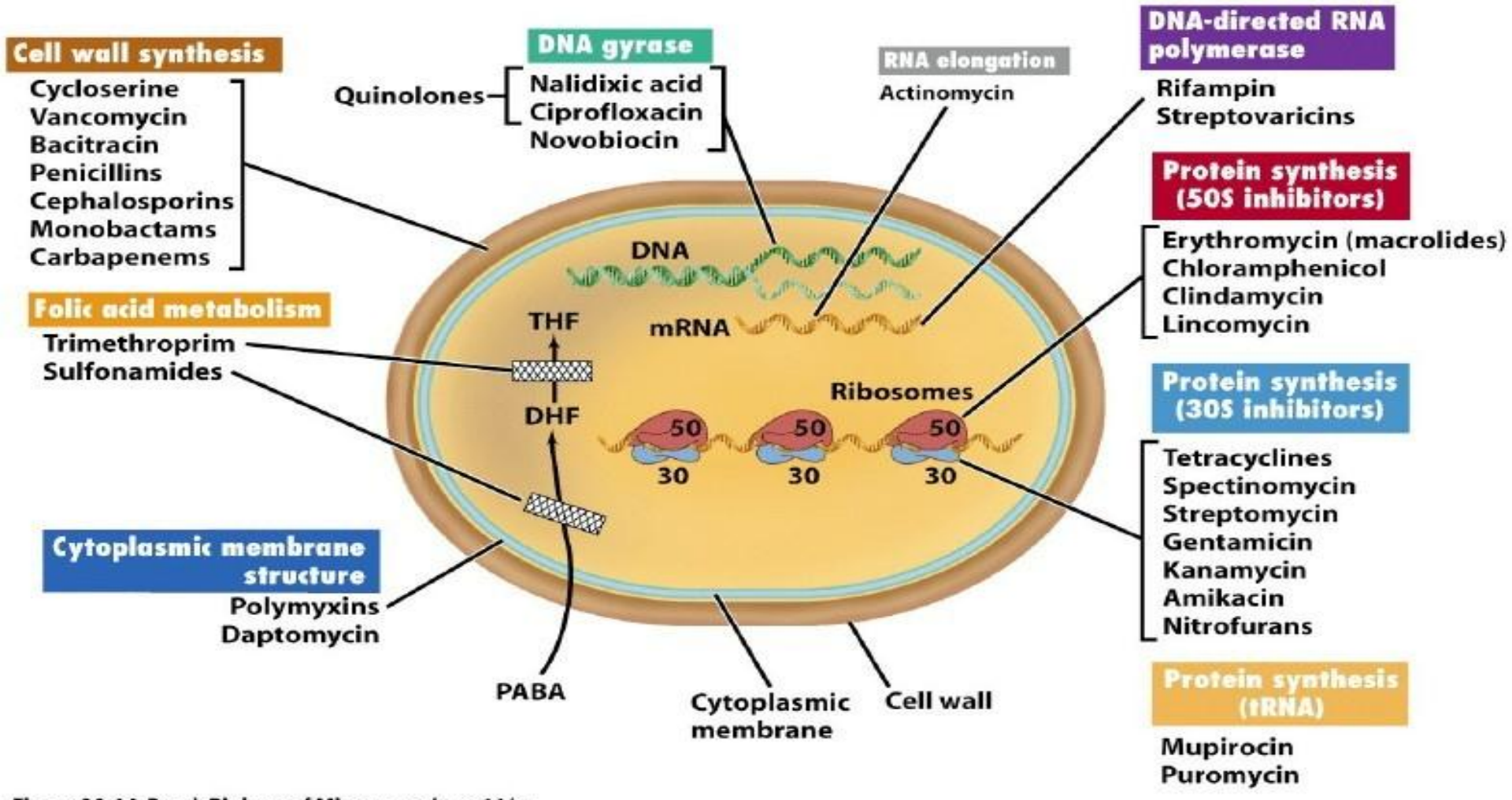
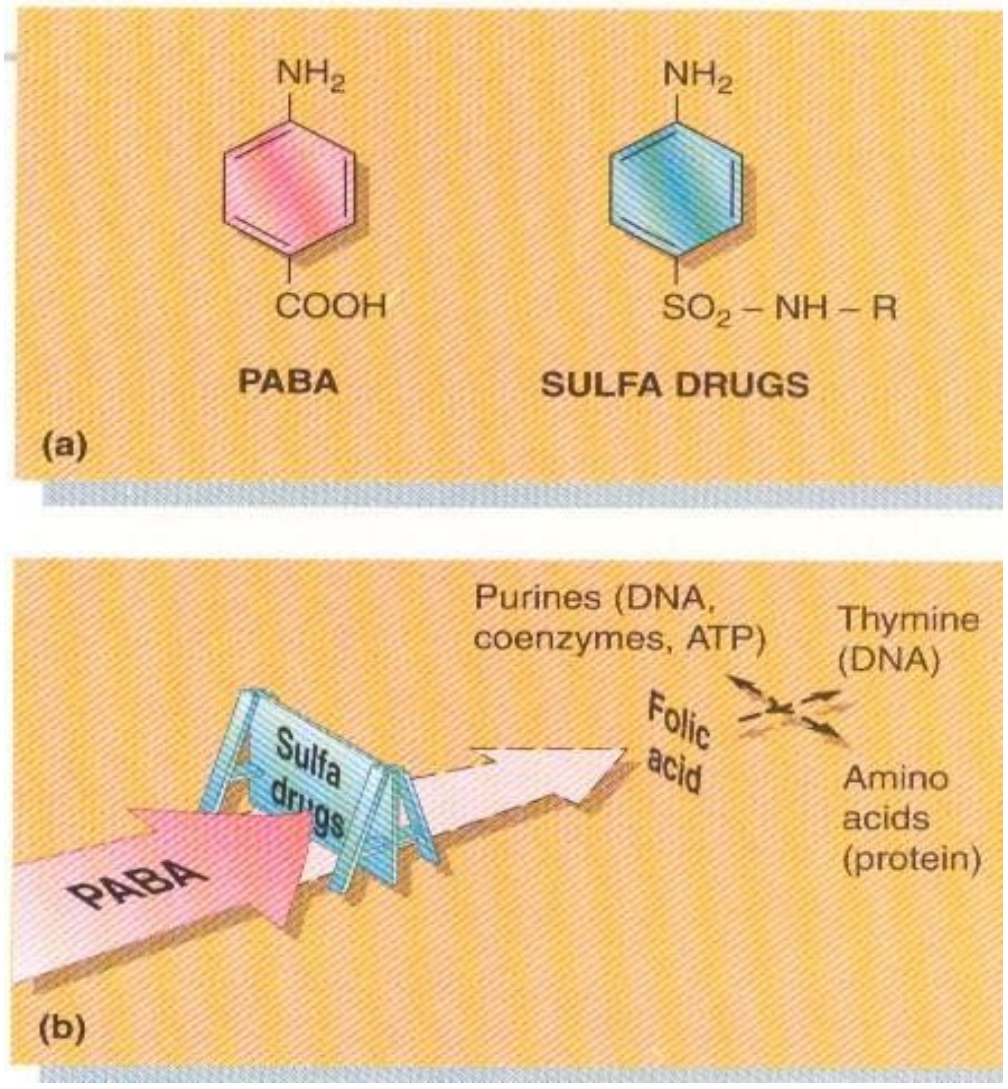


Figure 20-14 Brock Biology of Microorganisms 11/e

The mechanism of action of sulfa drugs.



Antiviral drugs.

- Interferons
- Inductors of endogenous interforons
- Synthetic chemotherapeutic drugs
- Immunomodulators

- Interferon – related to major defending proteins of immune system. Discovered in 1957 by A.Aiseek and J. Lindemaan by studying interference of viruses (lat. Inter- between and ferens-carrier) phenomenon, when animals and cell culture infected by one virus, they become insensitive to other viruses. It became known that interference is due to creation of protein, consisting of defensive antiviral features. This protein was called interferon. In nowadays interferon is studied good enough, structure and features, it is widely used in medicine as preventive, healing and therapeutic agent.

Interferon represents itself the family of glycoprotein proteins with the molecular mass from 15 till 70 kda, which synthesize with the cells of immune system and connecting tissues. Depending on which cells interferon synthesize, they create 3 main types: alfa, beta, y-interferons.

Types of interferons.

There are several types of interferons:

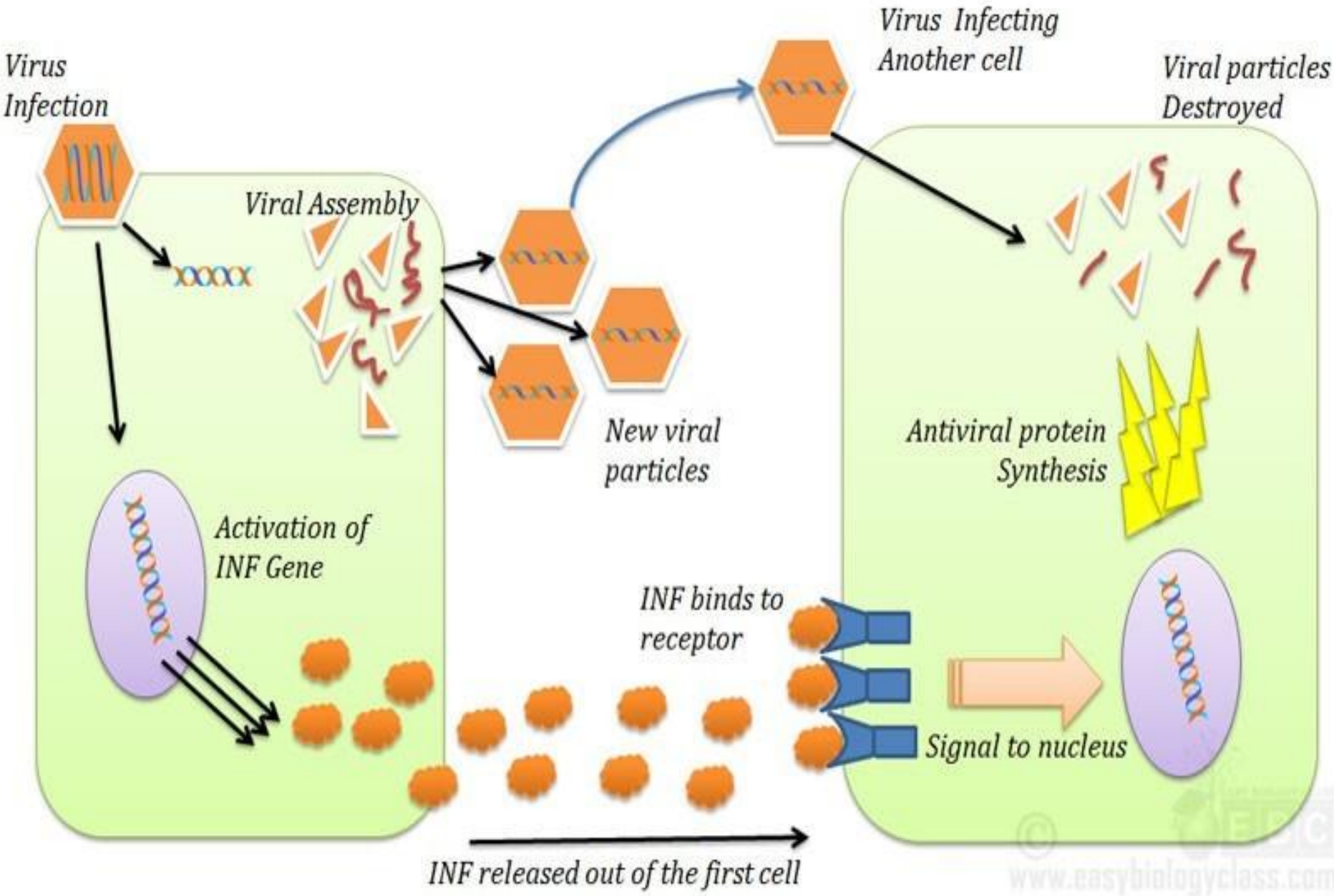
- - alpha-IFN - leukocyte (B-lymphocytes, macrophages);
- - beta IFN - fibroblast (fibroblasts, macrophages, endotheliocytes);
- - gamma IFN - immune (T-lymphocytes, production is enhanced with the participation of macrophages and NK cells);

- - omega-IFN - has common features with alpha-IFN;
- - tau-IFN - found in sheep, cows and birds;
- -lambda-IFN - discovered in 2003 and originally assigned to interleukins. They have their own receptor and are classified as type III interferons. Appointment - protection of the skin, lungs and gastrointestinal tract from the action of viruses (rotaviruses).

Interferon actions.

- Antiviral effect (inhibits the process of reproduction of the virus at the stage of protein synthesis)
- Antiproliferative effect (delays the growth of tumor cells
- Immunoregulatory effect (stimulation of macrophage, EKK, antibody production, complement formation, IL-1 and IL-2, expression of MHC class II antigens)

ANTIVIRAL ACTION OF INTERFERON (INF)



Synthetic antiviral drugs.

- Drugs that inhibit the adsorption of the virus on the cell and its deproteinization inside the cell (amantadine and remantadine)
- Inhibitors of virus-specific DNA polymerase (iodoxyuridine)
- Nucleoside analogues (acyclovir, ribavirin)
- Reverse transcriptase inhibitors (azidothymidine)
- Viral protease inhibitors (saquinavir)

Complications during antimicrobial therapy.

1. From the macroorganism:
- Toxic effect of drugs
- Dysbiosis (dysbiosis)
- Immune system dysfunctions (allergies, depression)
- Endotoxic shock
- Negative interactions when combining drugs.

2. On the part of microorganisms:
- The formation of L-forms and persistent forms of microbes
- The formation of antibiotic dependence
- The formation of drug resistance, including antibiotic resistance

Microbial drug resistance.

- Natural sustainability:

- lack of target
- bacterial impermeability for this drug

- Acquired Sustainability:

-Mutations under the influence of antibiotics and selection of antibiotic-resistant mutants
- Stability due to R-plasmids
- Resistance associated with transposons carrying r-genes

Principles of rational antibiotic therapy.

- The microbiological principle is to establish the causative agent of the disease and determine its individual sensitivity to the antibiotic.
- Pharmacological principle - consider pharmacokinetics and pharmacodynamics.
- The clinical principle is to take into account the individual characteristics of the patient's condition.
- The epidemiological principle is to take into account the state of resistance of microbial strains circulating in a given region
- The pharmaceutical principle is to take into account the expiration date and follow the storage rules of the drug.