V lecture

Ecology of microorganisms. Microbiota of Biosphere. Normal microbiota of human organism. Affect of environmental factors (physical and chemical) on microorganisms. Genetics of microorganisms, types of genetic variability. Biotecnology and genetic engineering

Ecology of microorganisms
Microbiota of biosphere

Ecology of microorganisms

- Microorganisms are widely spread inenvironment-soil, air, water, human, plant and animal organisms.
- Ecology of microorganisms (greek, *eikos* – household, home, place to live) – study interaction rules of microorganisms with environment, each other.

Ecosystem and its components

- The main object of study in Ecology is ***ecosystem*** consisting of biotic and abiotic components.
- **Biotic** components form biocenosis – consists of microbial populations with different species and numbers of microorganisms.
- **Abiotic components –**physical and chemical factors of environment.

Ecosystem microorganisms

- 2 types of microorganisms exist in ecosystem– autochtone and allochtone.
- ***Autochtone microorganisms*** are permanent representatives of ecosystem (exp., soil, intestine). These ecosystems have all growth requirements for microorganisms.
- ***Allochtone (zymogen) microorganisms*** are transient representatives of ecosystem and can be isolated only in presence of special growth conditions.
- For exp., bifidobacteria are permanent (autochtone) microorganisms of intestinal tract while *Candida* species are allochtone representatives of intestine.

Mutual interactions between microorganisms

- In environment and host organisms microbes live in form of ***biocenosis***. Association between 2 ore organisms is called ***symbiosis*** and organisms living in symbiosis – symbionts.
- Depending on mutual relationship between symbionts three types of symbiosis are known:
- ***mutualism***
- ***antagonism***
- ***neutralism***

Mutualism

- **Mutualism** is beneficial for both organisms-organisms supply each other with nutrients. Examples are lichens – symbiosis of fungi and blue green algae (cyanobacteria).

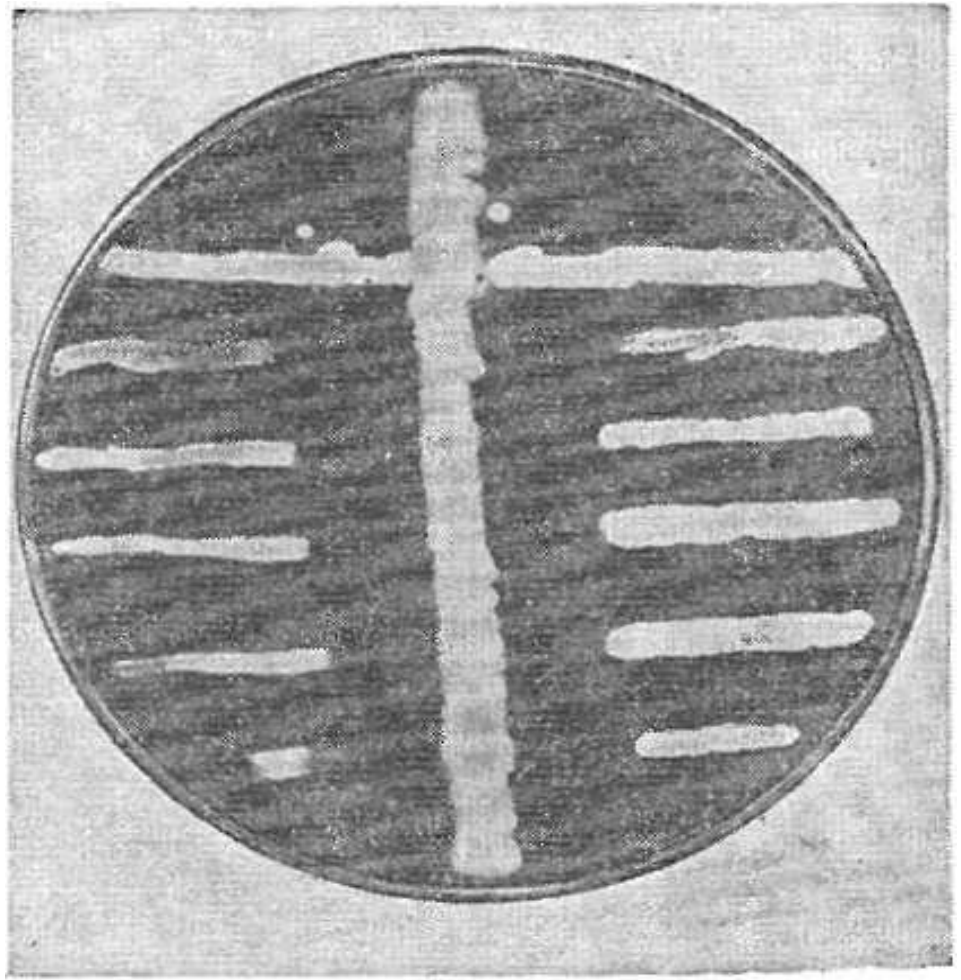
  There variants of mutualistic symbiosis:

- ***Metabiosis*** – microorganism use its symbiont metabolites as nutrients
- ***Commensalism*** – one organism obtain benefits from other without harming the latter.
- ***Satellism*** – growth of one species is bir növ mikroorqazimin təsiri ilə digər növün inkişafı stimullaşır

Antagonism

- During **antagonism** one organism harms other organism sometimes causing death of latter.
- One of the most common form of antagonism is production of antibiotics by microorganisms which inhibits growth of other microorganisms.
- ***Bacteriocins*** released by bacteria act on genetically close bacteria.
- Antagonism can exist in form of competition for nutrients when one microorganism use nutrients depleting them and inhibiting growth of other.
- Sometimes one organism digests another as ***predator.***
- The process when one microorganism uses another as a food source is called parasitism.

Test for detection of antagonism between bacteria



Microorganisms and environment. Basics of sanitary microbiology

- ***Sanitary microbiology*** is a study of microorganisms living in environment (soil, water, air, food etc.) and processes caused by them.
- ***The main aim of sanitary microbiology is*** detection of infectious disease agents in environment and conduction of measures preventing contamination of environment by microbes thus preventing spread of infectious diseases.

Sanitary indicative microorganisms

- Detection of microorganisms in environment is difficult process.
- Thus, contamination of environment by microorganism is detected by indirect methods – by detection of ***sanitary indicative microorganisms***. Each object of environment has its own sanitary indicative microorganism detection of which helps to evaluate sanitary condition of object.
- ***These microorganisms are normal flora of human and animal organism and released to environment.***
- ***Their ability to live in environment is similar to that of of pathogenic microorganisms – they cannot grow in environment.***

Soil microbiota

- **Microbe count of soil**. Many pathogenic and opportunistic bacteria can be released in environment by human and animal excrements.
- **Diseases spread by soil**
- **Sanitary indicative microorganisms of soil** *Escherichia coli* and *Clostridium perfringens*.
- **Evaluated during sanitary microbiological analysis of soil:**

  - General number of microorganisms in 1 g of soil;

  - titer of sanitary indicative microorganisms (*E.coli* and *C.perfringens*);

  - number of termophyle bacteria in 1 g soil;

  - pathogenic microorganisms during epidemiological conditions (Salmonella, Shigella, C.tetani, C. botulinum, some viruses).

Water microflora

- **Microbe count of water**
- Ability of microorganism to live in water and process of self-clearance of water
- Pathogenic microorganisms living in water and water borne pathogens
- Sanitary indicative microorganisms of water (*E.coli*)
- Evaluated during sanitary microbiological analysis of water.

  - general number of bacteria in 1 ml of water, ***general microbe count***

  - ***Coli-titer the lowest amount of water (count in ml)*** bağırsaq çöpü rast gəlinən suyun ml-lə ən az miqdarına deyilir.

  - ***Coli-index is amount of E.coli in 1 liter of water***.

  - in the presence of epidemiological indications, pathogenic microorganisms are identified in the water.e
- The coli-titer of tap water should not be less than 300, the coli-index should not exceed 3, and the microbial number should not exceed 100, and pathogenic microorganisms should not be detected.
- The problem of water neutralization

Microbiota of air

- **Microbial composition of open and closed environment**
- **Ability of microorganisms to live in air**
- **Pathogenic microorganisms in air and airborne diseases**
- **Sanitary indicative microorganisms of air -** hemolytic streptococci and *Staphylococcus aureus*
- **Principles of sanitary microbiological investigation of air**
- **Sanitary microbiological investigation of air is performed in treatment and childcare facilities**:

  - general number of bacteria in 1 m^3 of air;

  - number of hemolytic streptococci and *Staphylococcus aureus in* 1 m^3 of air

  - detection of pathogenic and opportunistic microorganisms in 1 m^3 of air.
- **Air clearance**

Role of microorganisms in environment

- Macroorganism can not live without microbiota.
- The same role possess microorganisms living in environment.They participate in geochemical cycle.
- During ***geochemical cycle*** organic compounds are made from inorganic and eventually disintegrate again to inorganic compounds.

Role of microorganisms in environment(Nitrogen cycle)

- In nature, there are processes of constant decomposition of nitrogenous organic compounds and the re-formation of organic matter from the products of decomposition.
- During this process called ammonification organic matter in the presence of microorganisms is first converted to ammonium compounds and ammonia, which is called ammonification.
- In anaerobic condition ammonification results in formation of a number of substances with unpleasant odors - indole, skatol, hydrogen sulfide, etc.. In aerobic ammonification proteins break down into smaller molecules - ***decomposition***..
- In the next stage, ammonia is oxidized to nitrites (NO2) and then to nitrates (NO3) - ***nitrification***. Bacteria of the genus Nitrosomonas and Nitrobacter are involved in this process.
- Some microorganisms reduce nitrates to free nitrogen - ***denitrification***. In this case, nitrates are reduced to nitrites, nitrites to ammonia, and the latter to free nitrogen. This process is performed by Chromobacter, Achromobacter, E. coli, etc.

Role of microorganisms in environment(Carbon cycle)

- During photosynthesis***, carbon dioxide (CO2)*** in the air is converted into ***organic matter***. Along with plants, cyanobacteria and algae are also involved in this process.
- Breakdown of ***organic matter with formation of CO2*** occurs mainly in animal and human organisms. Microorganisms take an active part in this process.
- Anaerobic breakdown of nitrogen-free organic matter by microorganisms - fermentation processes have been described above. Under aerobic conditions, breakdown products are water and carbon dioxide.

Role of microorganisms in environment(Sulfur cycle)

- ***It begins with breakdown of organic matter to hydrogen sulfide (H2S).*** Microorganisms, especially Desulfovibrio and Desulfotomaculum, play an important role in this process.
- ***Conversion of hydrogen sulfide to free sulfur.***
- ***Oxidation of free sulfur to sulfates (SO4)***
- ***Re-synthesis of organic matter from sulfates*** - this process involves microorganisms, as well as other organisms.

Normal microbiota of human organism, its role

Normal microbiota of human organism

- Most representatives of the normal microbiota are saprophytes - commencal microorganisms, i.e. they do not have a harmful effect on the body.
- In general, the normal microbiota is found in the skin and mucous membranes - the upper respiratory tract, gastrointestinal tract, as well as the urogenital tract.
- The normal microbiota of mucous membranes resides with a peculiar regularity. Thus, the distal parts of the mucous membranes, which are in close contact with the environment, are rich with microorganisms.
- Tissues and organs of human organism which do not have direct contact with the environment are sterile - have no microorganisms. These include blood, lymph, internal organs, brain, cerebrospinal fluid, etc.

Normal microbiota of human organism

- The normal microbiota of the organism can be divided into two groups - obligate and facultative microbiota.
- ***Obligatory microbiota is also called permanent, residual, indigenous or autochthonous microflora. The obligate microflora*** is adapted to live in the macroorganism and is found here permanently, consisting of saprophytes and opportunistic microorganisms.
- ***Facultative, or transient, allochthonous microbiota*** resides in organism for a certain period of time, temporarily. These microorganisms usually enter the body from the environment and leave it after a certain period of time.

Microbiota of skin

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

The normal microbiota of outer ear skin and conjunctiva.

Anatomic area	Microorganism	Morphology
outer ear	***Staphylococcus epidermidis*** ***Staphylococcus aureus*** **alpha-hemolytic Streptococci** ***Corinebacterium* (diphteroids)** ***Mycobacterium***	Gram + cocci in grape clusters Gram + cocci in grape clusters Gram + cocci in chains Gram + pleomorphic rods Acid fast Gram + rods
conjunctiva	***Branhamella catarrhalis*** ***Haemophilus*** ***Corinebacterium* (diphteroids)** ***Neisseria*** ***Staphylococcus epidermidis*** **alpha-hemolytic Streptococci**	Gram + **coccobacteria** Gram - pleomorphic rods Gram + pleomorphic rods Gram – diplococci Gram + cocci in grape clusters Gram + cocci in chains

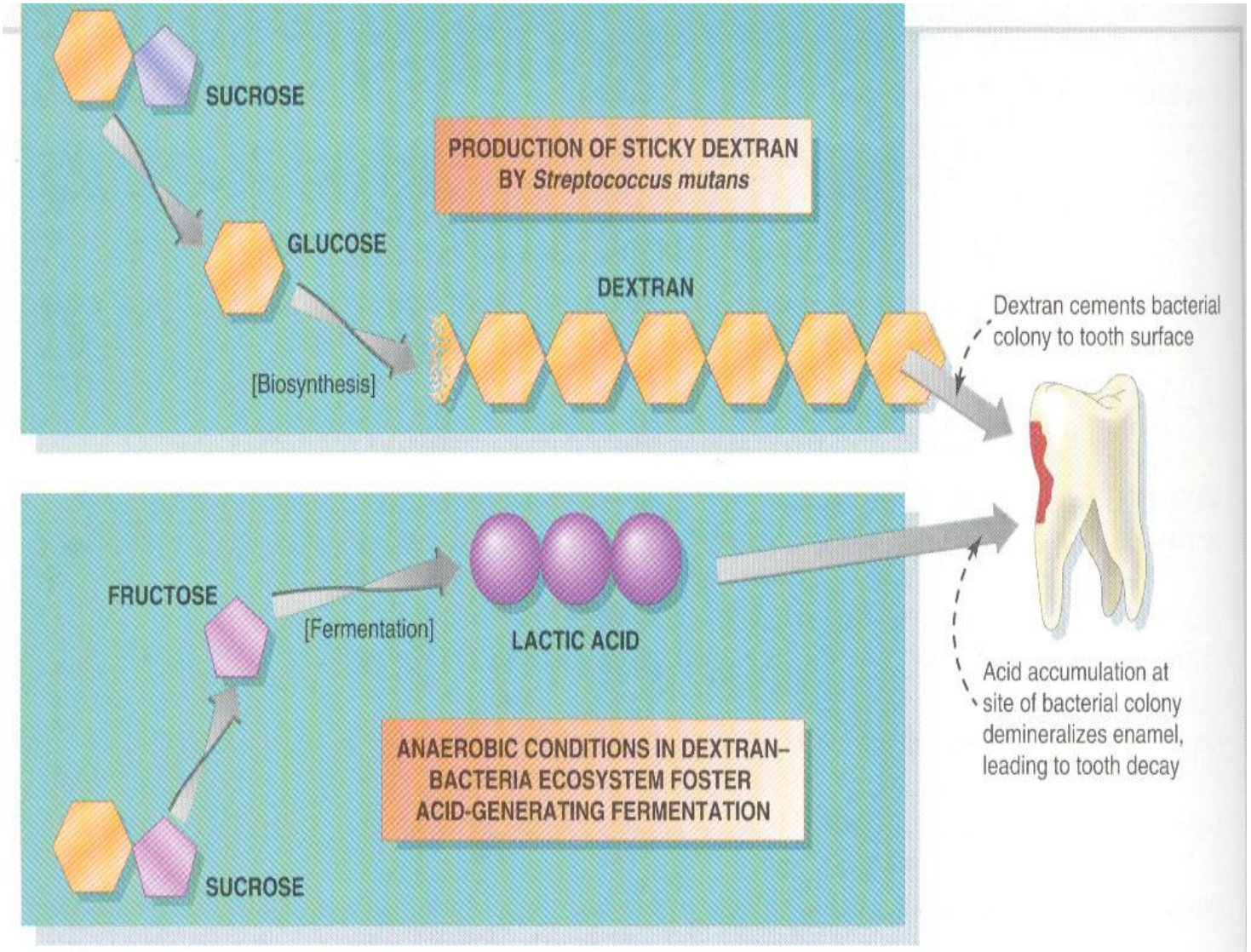
Normal microbiota of respiratory tract

Anatomik nahiyyə	Mikroorqanizm	Morfoloji xüsusiyyətləri
Upper respiratory tract(nasal cavity and naso-pharynx)	Staphylococcus epidermidis Staphylococcus aureus Alpha hemolytic Streptococci Streptococcus pneumoniae Branhamella catarrhalis Corynebacterium (diphteroids) Haemophilus Bacteroides Actinomyces	Gram positive cocci in grapelike clusters Gram positive cocci in grapelike clusters Gram positive cocci in chain clusters Gram positive diplococci Gram positive coccobacterial Gram positive pleomorphic rods Gram negative pleomorphic rods Gram negative pleomorphic rods Gram+ rods, or mycelium
Lower respiratory tract (trachea, bronchi, bronchioles, lungs)	Sterile	

Normal microbiota of digestive system

Anatomic part	Microorganism	Morphological features
Oral cavity		
Saliva and teeth	Streptococcus	Gram+ cocci in chain clusters
	Lactobacillus	Gram+ rods
	Veilonella	Gram- diplococci
	Bacteroides	Gram- pleomorphic rods
	Fusobacteria	Gram- rods
	Actinomyces	Gram+ rods, or mycelium
Pharynx(tonsils)	Streptococcus	Gram+ cocci in chain clusters
	Branhamella catarrhalis	Gram- cocci
	Corynebacterium (diphteroids)	Gram+ pleomorphic rods
	Staphylococcus	Gram positive cocci in grapelike clusters
Esophagus	Microorganisms of food and saliva	
Stomach	Lactobacillus	Gram+ rods
	Corynebacterium (diphteroids)	Gram+ pleomorphic rods
	Candida	Yeastlike fungi

Etiological role of microorganisms in formation of dental plaque



Normal microbiota of intestinal tract

Anatomik nahiyyə	Mikroorqanizm	Morfoloji xüsusiyyətləri
Small intestine	Lactobacillus Enterococcus Bacteroides Candida	Gram positive rods Gram positive diplococci Gram negative pleomorphic rods Yeastlike fungi
Large intestine	Bacteroides Bifidobacterium Enterobacteriaceae Enterococcus Clostridium Fusobacteria Lactobacillus Staphylococcus Peptostreptococcus Candida 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 in grapelike clusters Gram positive cocci in chain clusters Yeastlike fungi Protozoa Protozoa

Large intestine microbiota

- Large intestine is extremely rich with microbiota. In upper parts of large intestine - cecum and colon the number of bacteria is approximately 10^8-10^{10} /g.
- The number of microbes reaches the maximum in distal part of large intestine. 20-30% of faeces consists of microorganisms, the number of bacteria is approximately 10^{11}/g.
- The normal microbiota of large intestine consists of up to 500 species of microorganisms and considered as microbial reservoir of human organism.

Large intestine microbiota

- Large proportion of ***obligate microbiota*** (96-99%) of large intestine is composed of anaerobic bacteria.
- The anaerobic microflora number is 1000-folds higher than other bacteria. *Bacteroides, Bifidobacterium,* anaerobic lactobacteria are dominant species here.
- 1-4% of obligate flora is composed of other representatives such as *E.coli*, *Enterococcus, Lactobacillus and*
- ***Facultative microflora*** (*Enterobacteriaceae family, Clostridium*, *Fusobacterium, Staphylococcus*, *Peptostreptococcus* genus, *Candida* etc.).

Mucous membrane microbiota
Lumen microbiota

- Mucous membrane and mucus covering it has their own specific microbiota called mucous microbiota. Microbiota of mucus plays an important role in prevention of invasion of mucosa by pathogenic microorganisms. Mucous microbiota is stabile.
- In contrast intestinal ***lumen microbiota*** is more variable. Affect of different factors can change the number and content of this microbiota resulting in disbiosis and disbacteriosis.

Mucous membrane as portal of entry of infections

- Intestine mucosa serves as semipermeable membrane.
- Sometimes, microorganisms can pass mucosa and enter the blood and lympha causing transient bacteriemia.
- Many pathogens can pass mucosa and cause disease. In these cases intestine mucosa plays role of entry portal for microorganisms.

Large intestine and age

- ***Intestine of newborns is sterile***, microbiota of human organism is formed from first hours of life through food.
- ***In breastfeeding*** children it is mainly composed of Streptococci and Lactobacteria***.***
- In contrast, ***children not receiving breastfeeding*** has more complex microbiota with low amount of Lactobacteria.
- At the end of the first year of life the normal microflora of healthy children is the same as in adults.

Normal microbiota of genito-urinary tract

Anatomic part	Microorganism	Morphological features
Urinary tract (lower 1/3 part)	Micrococcus Staphylococcus epidermidis Streptococcus Mycobacterium smegmatis Corynebacterium (diphteroids) Bacteroides Neisseria Enterobacteriaceae	Gram+ cocci Gram + cocci in grapelike clusters Gram + cocci in chain clusters Gram + cocci acid fast rods Gram + pleomorphic rods Gram - pleomorphic rods Gram - diplococci Gram – rods
Kidneys, ureters, bladder, urethra upper 2/3 part	Sterile	
Vagina	Lactobacillus Corynebacterium (diphteroids) Streptococcus Staphylococcus Enterobacteriaceae Candida Trichomonas vaginalis	Gram + rods Gram + pleomorphic rods Gram + cocci in chain clusters Gram + cocci in grapelike clusters Gram - rods Yeastlike fungi Protozoa
Fallopian tubes, ovaries	Sterile	

Importance of normal microbiota

- Most members of the normal microbiota, especially the obligate microorganism, have ***antagonistic effect*** against pathogenic and opportunistic microorganisms.
- They produce organic acids (lactic acid, acetic acid, etc.), antibiotics, bacteriocins, etc.
- The normal microbiota prevents the ***settlement (colonization) of mucous membranes*** by pathogenic microorganisms.
- Therefore, the normal microbiota can be considered as one of the factors of non-specific resistance of the organism.

Importance of normal microbiota

- Representatives of the normal microbiota play an important role in the formation of ***natural immunity***, acting as antigens for the organism immune system.
- The baseline level antibodies in the blood serum is induced by the normal microbiota.
- Intestinal microflora is involved in the process of digestion, metabolism, as well as the ***synthesis*** of some biologically active substances, ***vitamins (vitamin K, B vitamins).***

Importance of normal microbiota

- The importance of normal microbiota is observed in animals which do not have microbiota(gnotobiont animals).
- These animals do not have microorganisms and are kept in special sterile conditions.
- As gnotobionts have poorly developed lymphoid tissue and they are very vulnerable to infections and usually cannot survive under normal conditions.

Life in sterile conditions

- The main difference between gnotobionts and ordinary animals is that they are not decomposed after death and have different defense mechanisms.
- Gnotobionts are not decomposed after death as they don’t have microbiota.
- Gnotobionts never have contact with microorganisms, thus activity of the immune system is weak, they have low white blood cell count and lymphoid tissue, and virtually no antibodies.
- They are supplied with vitamins, even without the presence of bacteria (previously it was thought that bacteria are needed for the synthesis of some vitamins). The weight of their excrement is the same as in ordinary animals (50% of the excrement consists of decomposed substances).

Life in sterile conditions

- Because there is no risk of infection, gnotobionts die only from organ disorders.
- Thus, they are considered a good model for studying organ dysfunction, tissue aging and other medical problems of old age.
- Under similar circumstances, researchers may try to answer another interesting question: how long can life be extended?
- Notre Dame scientists in cooperation with the University of Chicago study caries, viral infections, heart disease, cancer, nutrition, vitamins etc.

Disbiosis and disbacteriosis

- There is a dynamic balance between obligate and facultative microbiota.
- This balance is supported by antagonistic relationship between obligate and facultative microbiota.
- Violation of this balance by different factors may end with ***dysbacteriosis*** and ***dysbiosis***.

Factors causing dysbacteriosis and dysbiosis

- Wide and irrational use of antimicrobial drugs.
- Concomitant (underlying) diseases, especially intestinal infections, helminth and parasite invasions, hormonal and chemotherapy, stress, etc. factors.
- Worsening of ecology is accompanied with increase of dysbacteriosis

Development mechanism of dysbiosis and dysbacteriosis

- The development of dysbacteriosis is associated with a decrease in the amount of ***obligate microflora***.
- It results in increase of opportunistic pathogens – staphylococci, *Proteus*, *Pseudomonas, Candida* etc. and diseases associated with them.
- By etiological origin: fungal, staphylococcal, Proteus etc. dysbiosis.
- Dysbiosis is also classified according to its location (oral cavity, intestines, uterus, etc.).

Dysbiosis and dysbacteriosis associated diseases

- Long term changes in composition and function of normal microbiota results in development of symptoms.
- These include diarrhea, constipation, colitis, malignant tumors, allergies, hypovitaminosis, hypo- and hypercholesterolemia, hypo- and hypertension, caries, arthritis, various pathologies of the liver, etc.

The following criteria are taken into account in the diagnosis of intestinal dysbiosis and dysbacteriosis:

- Total number of Escherichia coli in 1 g of feces sample;
- Relative amount of hemolytic Escherichia coli;
- presence and relative amount of opportunistic pathogens, including Proteus and Candida
- The amount of Bifidobacteria, Lactobacteria and Bacteroides.

Correction of dysbiosis and dysbacteriosis

- First, factors causing dysbiosis should be detected and eliminated.
- Elimination of opportunistic microbiota is also important (***selective decontamination***).
- Probiotics (eubiotics) are used to restore the microbiota.
- Eubiotics are mainly obligate representatives of the normal intestinal microflora - bifidobacteria, lactobacilli, E.coli, enterococci, etc.
- For this purpose, bacterial preparations are used in the form of lyophilized dry powder, pills,extracts

The impact of environmental factors in microorganisms

The impact of environmental factors on microorganisms

- The vital activity of microorganisms, their development, reproduction and destruction depend on environmental factors.
- Factors that can affect microorganisms can be divided into three groups: ***physical***, ***chemical*** and ***biological***.
- The effect of these factors varies depending on both their nature and the nature of the microorganism. Thus, each of these factors can have a lethal effect on microorganisms, as well as beneficial for their development.

The effect of physical factors on microorganisms

- **Temperature.** All microorganisms are divided into three groups according to temperature:
- ***Psychrophilic*** (Greek, psychros-cold, phileo-love) microorganisms
- - minimum temperature– 0^0C, optimal – 6-20^0C, maximum– 30^0C
- ***Mesophilic (Greek, mesos-middle) microorganisms***
- - minimum temperature – 10^0C, optimal – 34-37^0C, maximum– 45^0C
- ***Thermofilic*** (***Greek***, *termos*-hot), , or heat-loving microorganisms grow at relatively high temperatures, usually above 55^0C

  - minimum temperature – 30^0C, optimal – 50-60^0C, maximum– 70-75^0C
- ***The effect of low and high temperatures***

The effect of physical factors on microorganisms(drying)

- ***Dryness*** - results in dehydration of the cytoplasm of microbial cells and disruption of the permeability of the cytoplasmic membrane, which leads to disruption and destruction of their nutrition.
  Some microorganisms, such as meningococci, gonococci, leptospira, the causative agent of syphilis, etc. die within a minute or two of drying. then they are destroyed. The causative agent of cholera can last for 2 days, the causative agent of typhoid fever for 2 months, and the causative agent of tuberculosis can last up to 3 months.
  Lyophilic drying or ***lyophilization*** is widely used in the storage of microbial cultures and preparations made from them, as well as many biological preparations. To do this, the drugs are first frozen and then dried under vacuum. In this case, the microbial cells become anabiotic and retain their biological properties for a long time.

The effect of physical factors on microorganisms (light radiation)

- **Radiation energy.** Under natural conditions, microorganisms are mainly exposed to light rays.
- Direct exposure light rays to bacteria, especially pathogenic bacteria, has a destructive effect.
- The destructive effect of light on microorganisms is due to its ***ultraviolet rays (UVR)*** with a wavelength of 254-300 nm. UVS inactivates enzymes in microbial cells and causes changes in the DNA molecule.
- Other rays, such as ***X-rays,*** as well as ***alpha-, beta- and gamma-rays***, have a destructive effect on microorganisms only in large doses. As a rule, doses of 44,000 r and more of these rays have a lethal effect on microbes.
- The bactericidal effect of ionizing radiation is sometimes used for canning food, ***sterilization*** of biological products (serum, vaccines, etc.).

The effect of physical factors on microorganisms(*Ultrasound)*

- ***Ultrasound***. Sound waves with a frequency greater than 20,000 Hz are called ultrasound. Ultrasound waves cause some effects when passing through the environment. The most important of these is the **cavitation effect** (Latin, cavitum - space).
- Under the influence of ***ultrasound*** waves cavitation with high pressure of 10,000 atm. are formed in the cytoplasm of microorganisms, which results in the breakdown of the microbial cell.
- Ultrasound waves are also used to sterilize some foods (milk, fruit juices, etc.) and drinking water.
- **High pressure.** High atmospheric pressure is harmless to most microorganisms. Some microorganisms withstand a pressure of 3000-5000 atm, and bacterial spores withstand a pressure of even 20,000 atm. Interestingly, saturated water vapor under high pressure has a destructive effect on all microorganisms and their spores. Sterilization of materials in autoclaves is based on this principle.

The effect of physical factors on microorganisms **(high pressure)**

- **High pressure.** High atmospheric pressure is harmless to most microorganisms. Some microorganisms withstand a pressure of 3000-5000 atm, and bacterial spores withstand a pressure of even 20,000 atm.
- Interestingly, saturated water vapor under high pressure has a destructive effect on all microorganisms and their spores. Sterilization of materials in autoclaves is based on this principle.

Sterilization

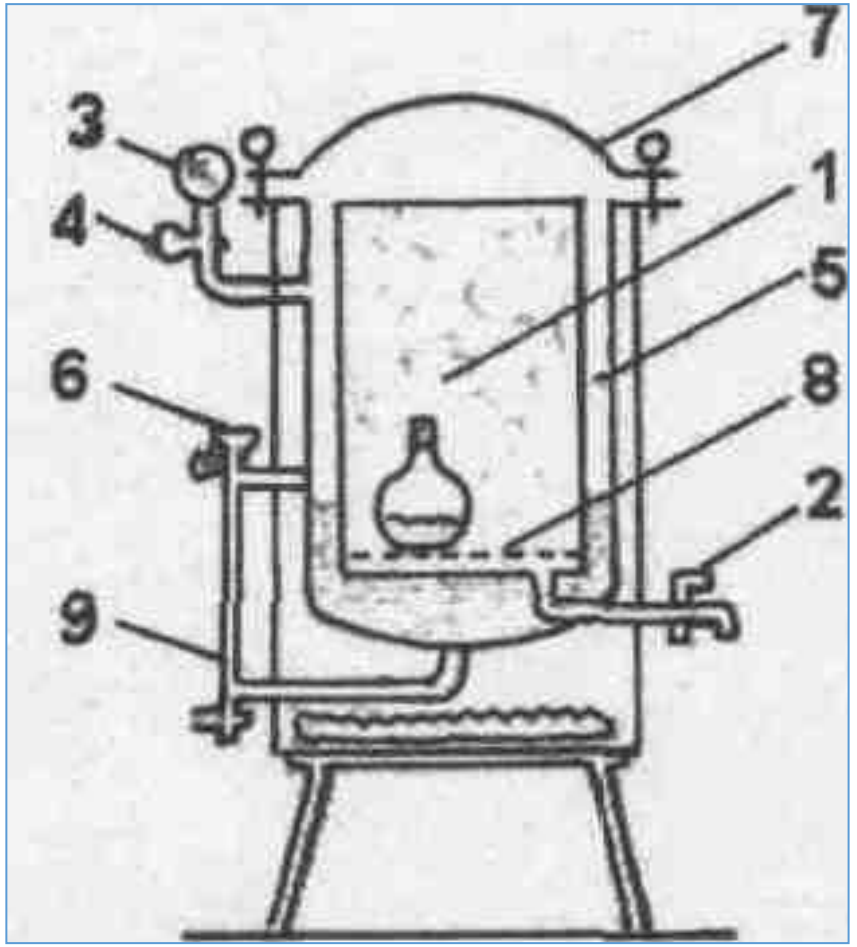
- It is the complete destruction of microorganisms, as well as their spores, in various objects.
- Sterilization is carried out in different ways:
- ***Physical methods*** (under the influence of high temperatures and various rays);
- ***Chemical methods*** (under the influence of various disinfectants and antiseptics, as well as antibiotics);
- ***Mechanical methods*** (application of bacterial filters)

Physical sterilization (thermal sterilization)

- **Burning and boiling sterilization** is one of the simplest and most convenient methods of heat sterilization
- Dry heat and high pressure saturated water vapor are mainly used for thermal sterilization.
- **Dry hot sterilization** is carried out in pasteurization furnaces (air sterilizers). The most common mode is sterilization at 165-170^{0}C for 1 hour, in which case all microorganisms, as well as their spores are completely destroyed.
- High pressure saturated water vapor is used to sterilize materials that change their properties and quality at high temperatures. Autoclaves (steam sterilizers) are used for this purpose. The most common mode of operation is sterilization at 2 atm at 121^{0}C for 30 minutes, in which case all microorganisms, as well as their spores, are completely destroyed.
- ***Pasteurization*** can be considered conventional sterilization. 1-hour exposure at 65^0-70^{0}C allows to destroy vegetative forms of microorganisms in food (milk, wine, beer, fruit juices, etc.).

Air sterilizer

Autoclave and its working principle

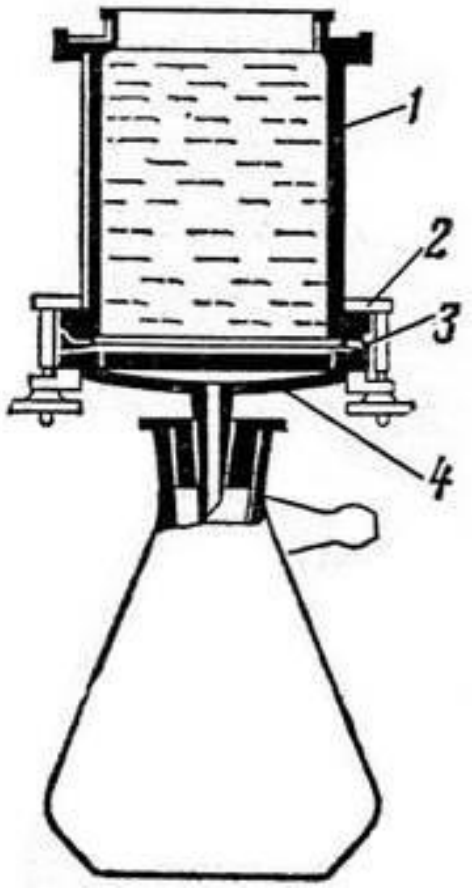


Physical sterilization (radiation sterilization)

- It is used for sterilization of thermolabile materials.
- The sterilizing effect of ultraviolet rays is limited by its low permeability and high absorption when passing through water and glass.
- Although gamma and X-rays have effective sterilizing properties, their application requires strict adherence to safety regulations. Biological preparations (serum, vaccines, etc.), disposable syringes, petri dishes, surgical sutures are sterilized by these rays.
- In some cases, microwave radiation and ultrasound are also used for sterilization.

Mechanical sterilization (mechanical)

- ***Sterilization by filtration*** through bacterial filters is used for sterilization of thermolabile liquid solutions.
- In microbiological practice are widely used ***Zeitz*** filters made from a mixture of asbestos and cellulose, ***membrane filters*** made from nitrocellulose**,** ***Chamberlain and Berkfeld*** filters made from a mixture of kaolin sand and quartz.
- Filters allow to get release from most microorganisms and sometimes viruses protein-rich media, blood serum and various drugs



Chemical sterilization

- Antimicrobials, disinfectants and antiseptics, as well as selective antibiotics and synthetic antimicrobials are used for **chemical sterilization** for the destruction of all microorganisms (see below).
- In some cases, toxic gases, such as ethylene oxide, are used for this purpose.

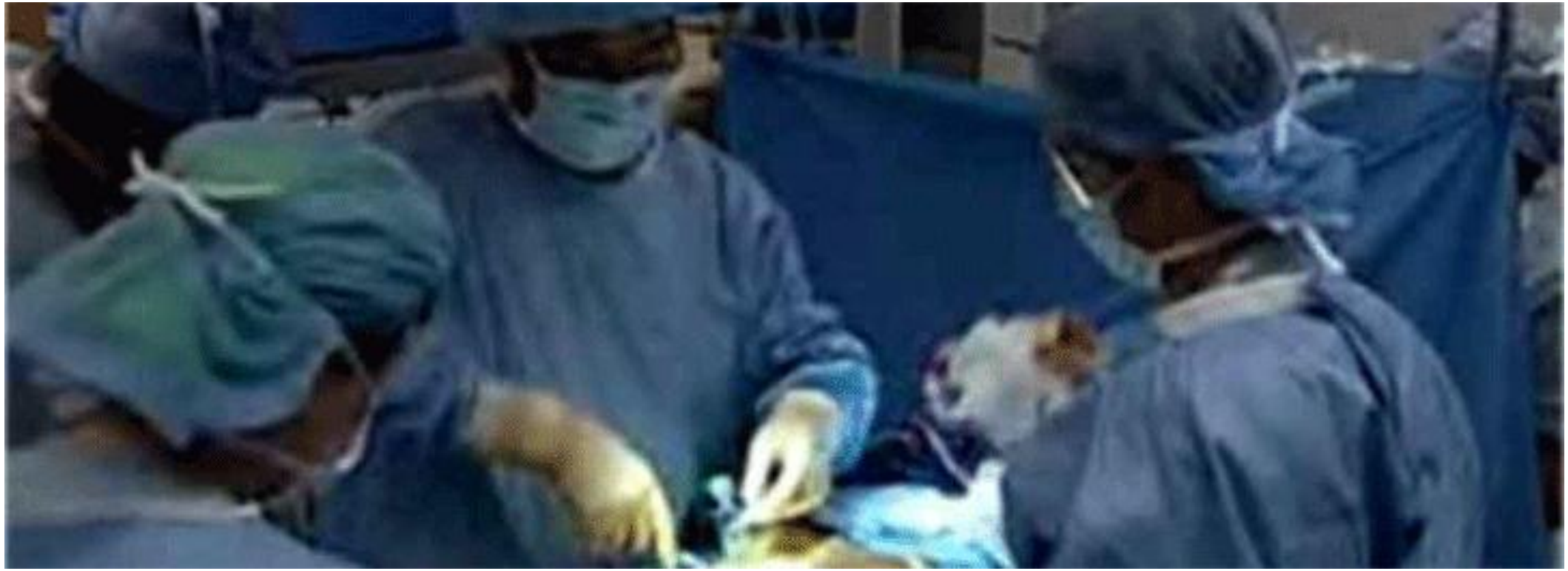
Action of chemical factors on microorganisms

- **Disinfection** (*des* – deny) is killing of pathogenic microorganisms in environmental objects.
- Chemical substances used for this purpose are called disinfectants.
- Similar substances that are not harmful to the human body are used to remove microorganisms from the skin and mucous membranes, wounds. In such cases, these substances are called antiseptics and are used for antiseptic purposes.

Aseptics and antiseptics

- ***Aseptics*** is a set of measures taken to prevent contamination of various objects (various areas of the body, skin and mucous membranes, including wounds) with microorganisms.
- A set of measures applied to remove microorganisms from different parts of the human body, as well as from wounds, is called ***antiseptics***.

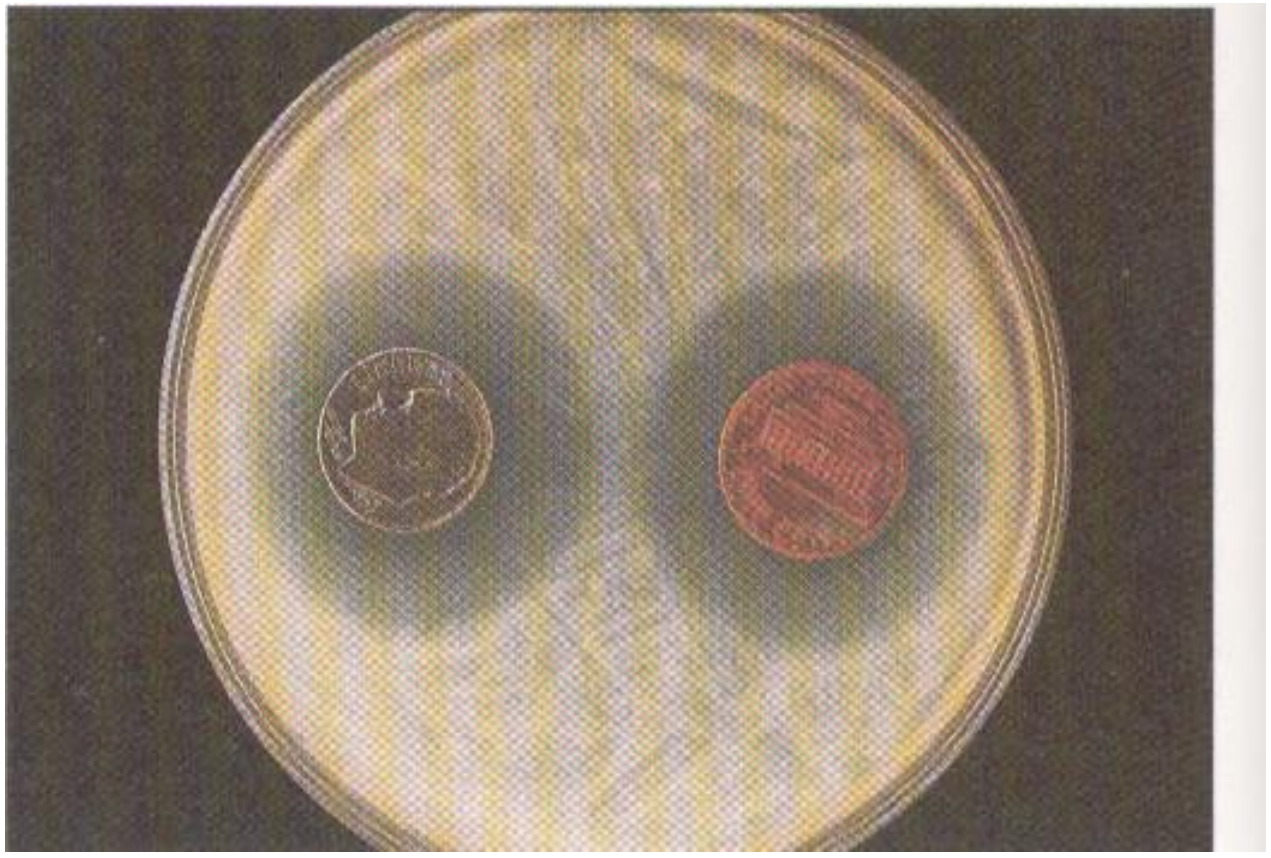
Aseptics and antiseptics



Disinfectants and antiseptic preparations

- ***Surfactants*** **-** soaps and detergents (decamine, chlorhexidine, etc.)
- ***Phenol*** and its derivatives (tricresol, phenylresorcinol, phenylsalicylate)
- ***Oxidizers*** (hydrogen peroxide, potassium permanganate, etc.)
- ***Halogens*** (alcoholic solution of iodine, lugol's solution, iodoform, iodinol), chlorine (chlorinated lime, chloramines, pantoside)
- ***Alcohols*** (ethyl alcohol, etc.)
- ***Acids, their salts*** (boric, salicylic, benzoic, acetic acids) and alkalis (ammonia and its salts, zinc);
- ***Aldehydes*** (formaldehyde and its 40% solution - formalin, hexamethylenetetramine - urotropin, glutar aldehyde, etc.)
- ***Heavy metal salts*** (mercury dichloride, silver nitrate, copper sulfate, etc.).
- ***Dyes*** (diamond green, methylene blue, ethacridine lactate - rivanol, etc.)

Experiments showing the antimicrobial effect of heavy metals (silver and copper)

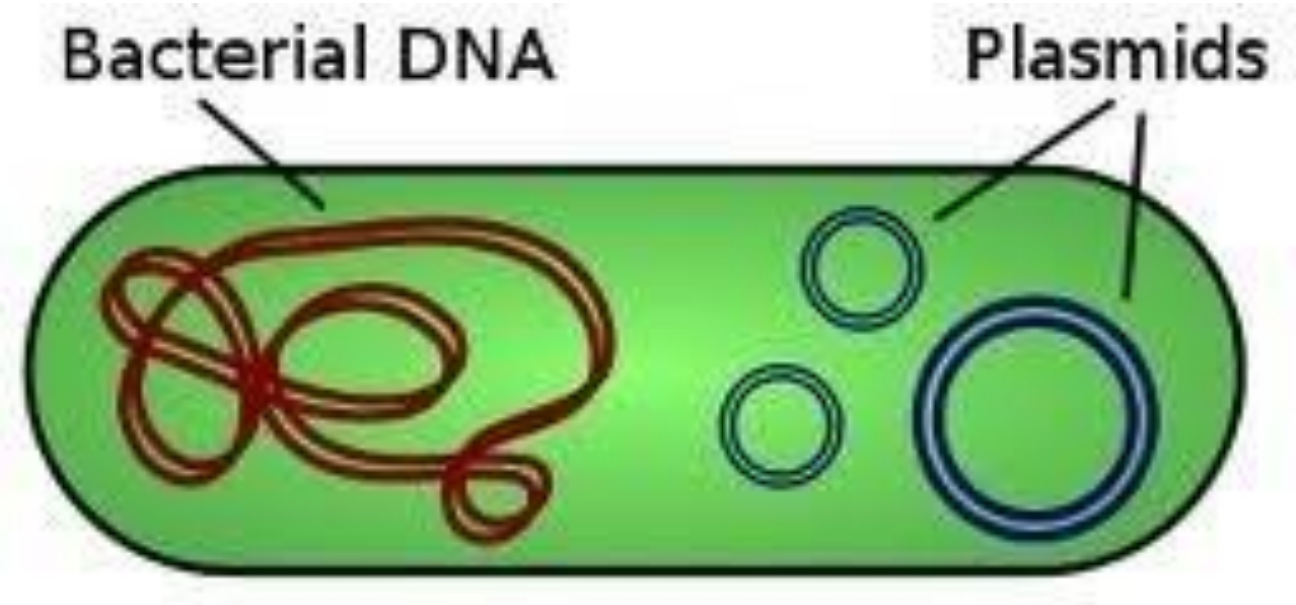


Genetics of microorganisms

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



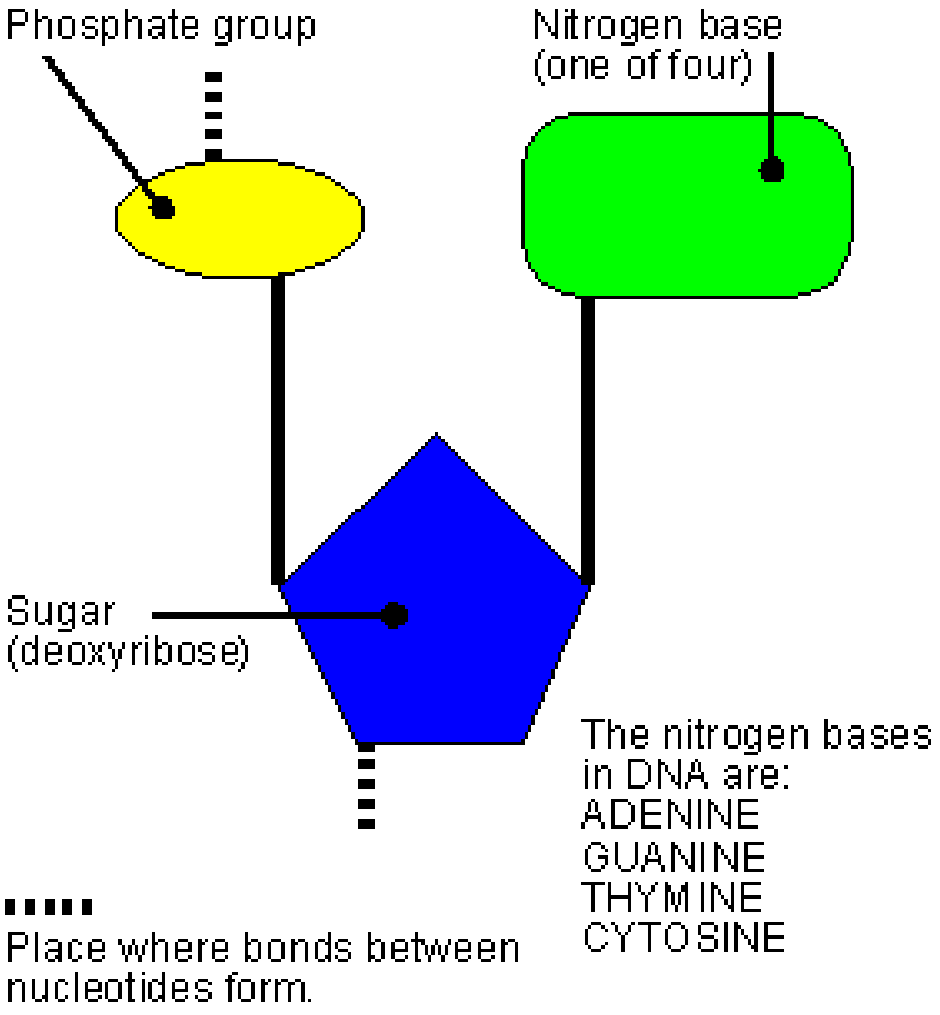
Bakteriya nukleoidi

- Bakteriyalarda ***nukleoid*** 4000-ə qədər gendən ibarət bir həlqəvi xromosomdan ibarətdir, yəni bakteriya hüceyrəsi ***haploiddir***, xromosomun ikiləşməsi bütün hallarda onun bölünməsini müşaiyət edir.
- Inkişaf etməkdə (bölünməkdə) olan bakteriya hüceyrələrində xromosomların miqdarı adətən 2-4, bəzən isə hətta 10-15-ə qədər ola bilər.
- Bakteriya hüceyrəsinin adi xromosomunun molekulu təqribən $5x10^6$ nukleotid cütündən ibarətdir (müqayisə üçün, insan genomu $2,9x10^9$ nukleotid cütündən ibarətdir).
- Bakteriya hüceyrəsinin (*Escherichia coli*) xromosomunun uzunluğu açılmış vəziyyətdə təqribən 1mm-ə qədər olur.

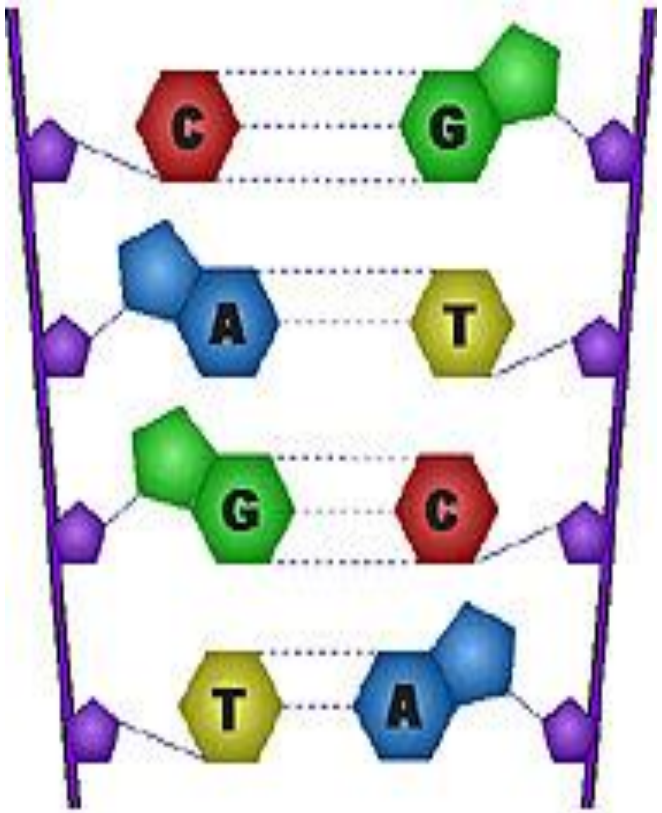
Bacterial nucleoid

- Nucleoid consists of one circular chromosome(haploid) with approximately 4000 genes. Duplication of chromosome is always associated with cell multiplication.
- Multiplicating bacterial cell has 2-4, even 10-15 chromosomes.
- Single chromosome of bacteria consists of $5x10^6$ nucleotide pairs (if compare human genome consists of $2,9x10^9$ nucleotide pairs).
- The length of the chromosome of a bacterial cell (Escherichia coli) is about 1 mm

DNA chemical structure



DNA molecule



Genes

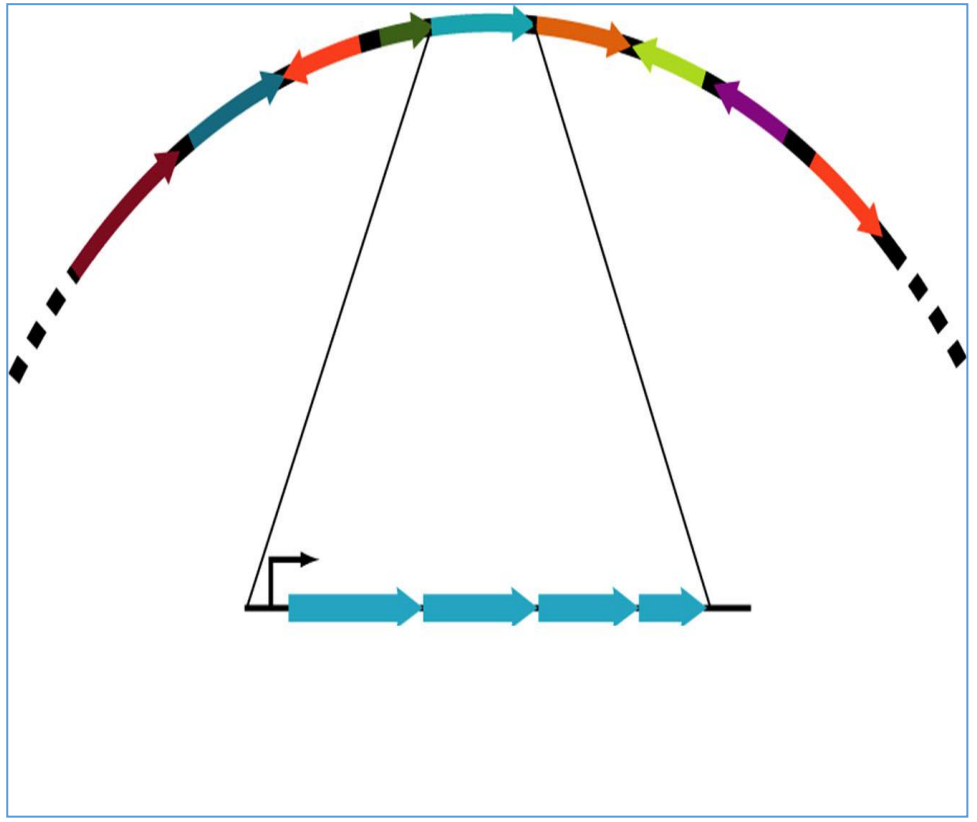
A part of DNA molecule responsible for synthesis of one protein is called gene.
All organism features are coded by chromosomal genes.

Structure and regulatory genes exist.

Structural genes code information about protein, while

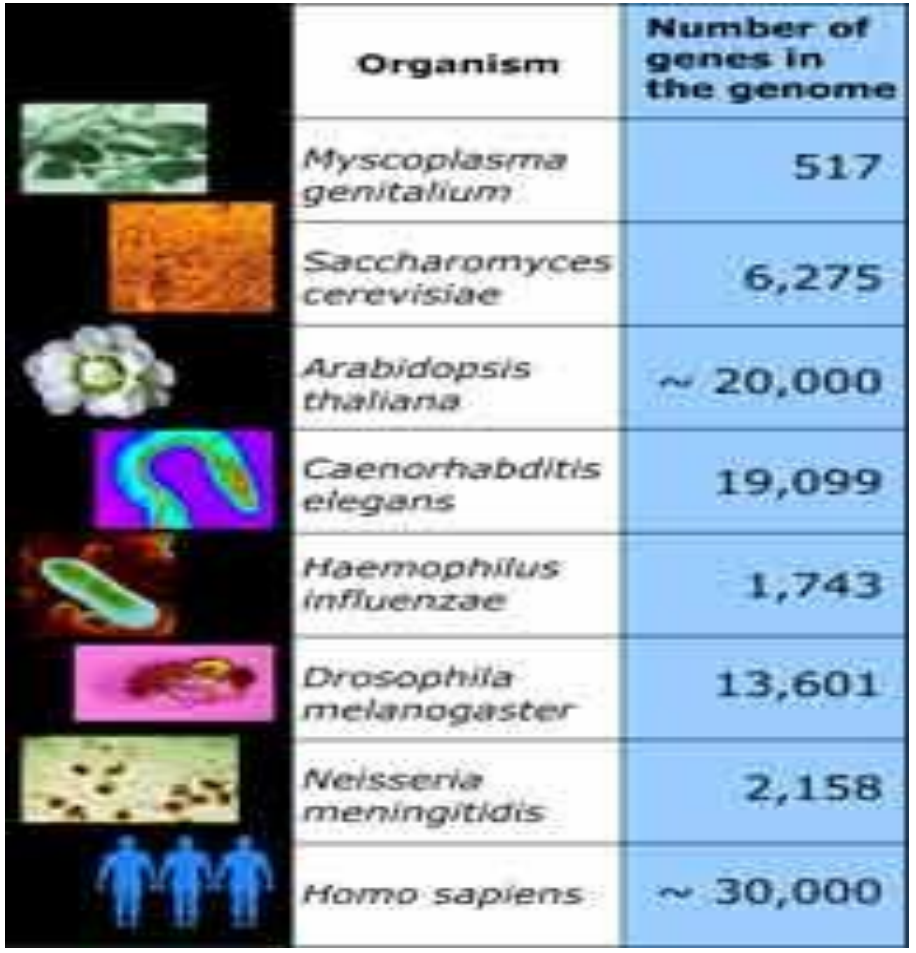
regulatory genes regulate the activity of structure genes.

A part of DNA molecule coding synthesis of one protein is called gene



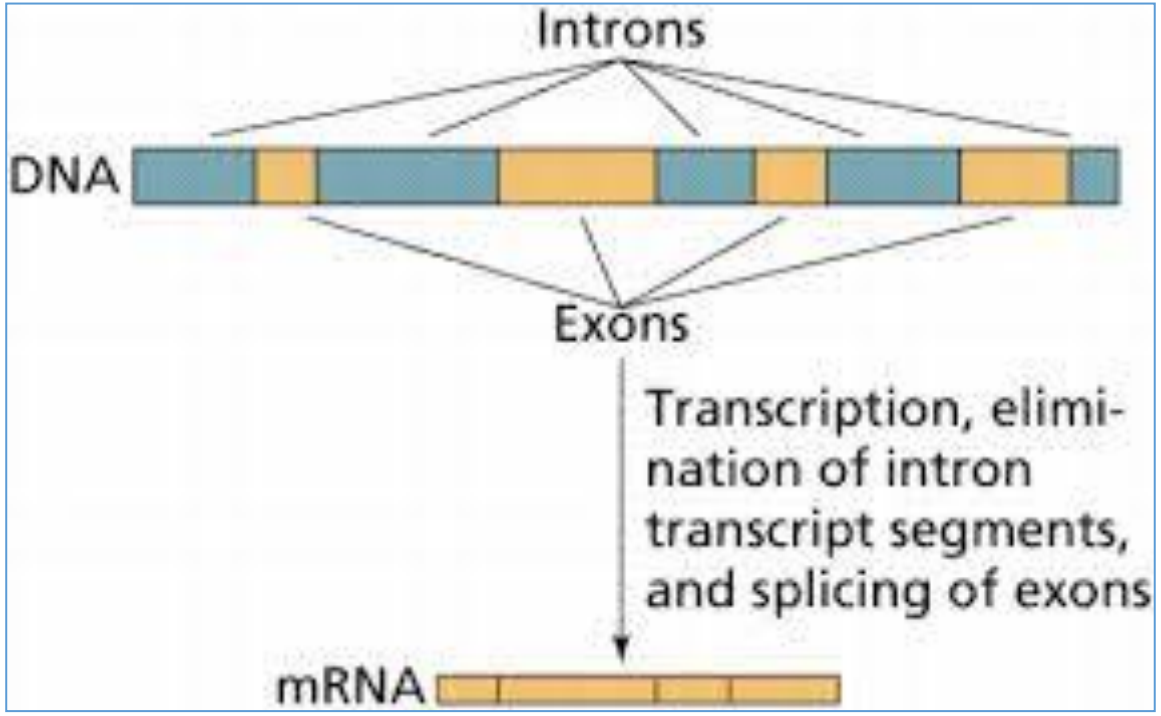
The number of genes in different organisms

• The number of genes in chromosome (DNA molecule) varies widely. Exp., *Mycoplasma genitalium has* 517 genes, *Neisseria meningitidis*- 2158.
• Human chromosome consists of approximately 30 000 genes.



Organism	Number of genes in the genome
Myscoplasma genitalium	517
Saccharomyces cerevisiae	6,275
Arabidopsis thaliana	~ 20,000
Caenorhabditis elegans	19,099
Haemophilus influenzae	1,743
Drosophila melanogaster	13,601
Neisseria meningitidis	2,158
Homo sapiens	~ 30,000

Prokaryotes in contrast with eukaryotes don’t have introns between coding genes



Operon consception

- According to current understanding genes activilty is regulated by ***operon***.
- Operon conception suggests that one gene or gene group expression is regulated by operon, in the true sense of the word, the operon supports "working“ of genes.

Operon structure

- Operon consists of regulatory gene, promotor, operator and structural genes.
- ***Regulatory gene codes repressor protein with high affinity to operon DNA***.

  - Repressor protein can bind to DNA.

  - Repressor protein binds and blocks transcription of gene.

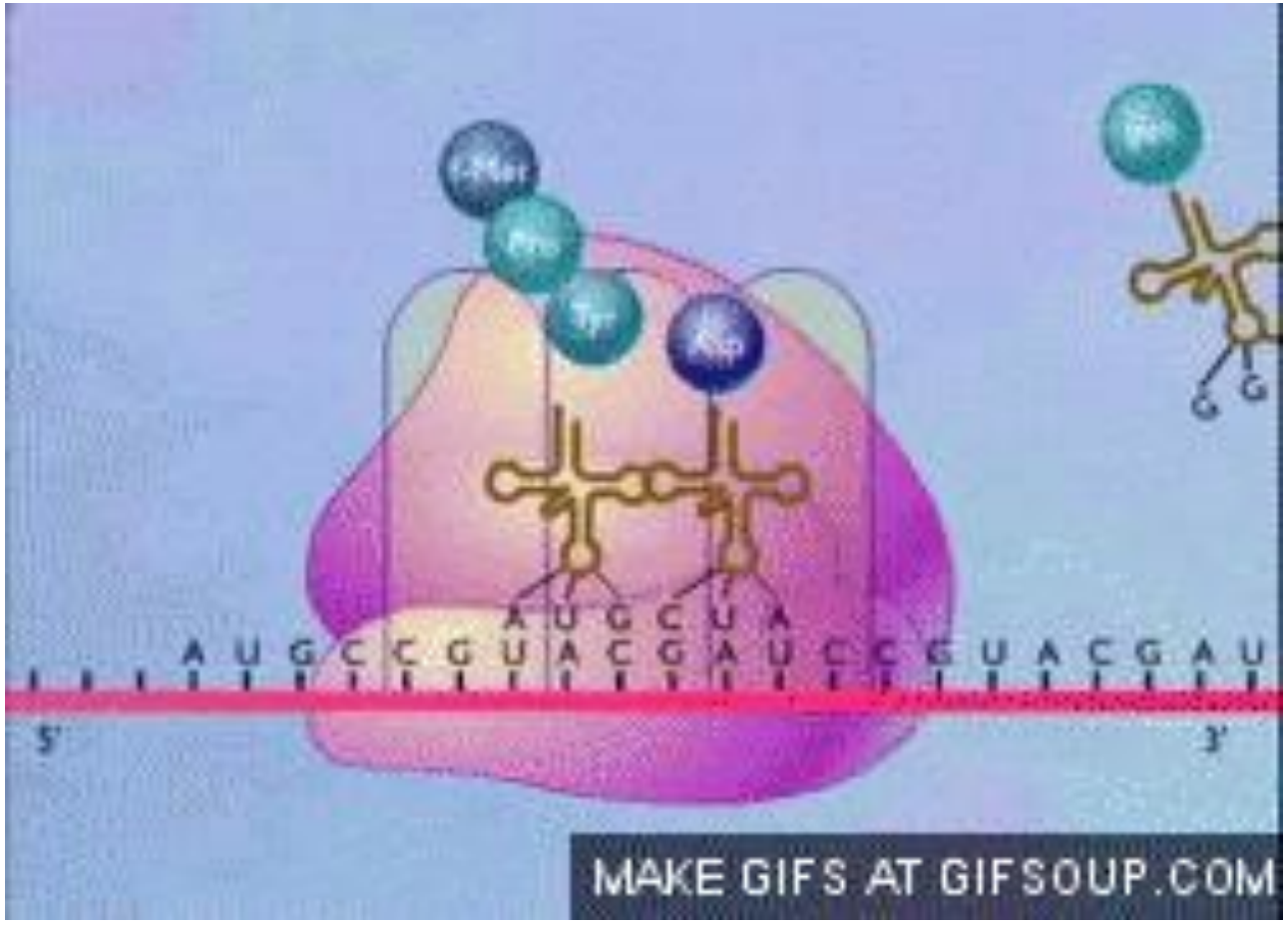
- ***Promotor consists of nucleotide sequences recognized by RNA-polymerase***. Its S-factor provides a specific connection with the promoter.
- ***Operator*** *is area for repressor protein binding and located between promoter and structural genes.*

How does gene “work”?

- When the repressor binds to the protein, the gene is blocked
- Removal of the repressor protein enables the synthesis of mRNA on the structural gene by RNA polymerase.

Transcription

Translation



Genotype

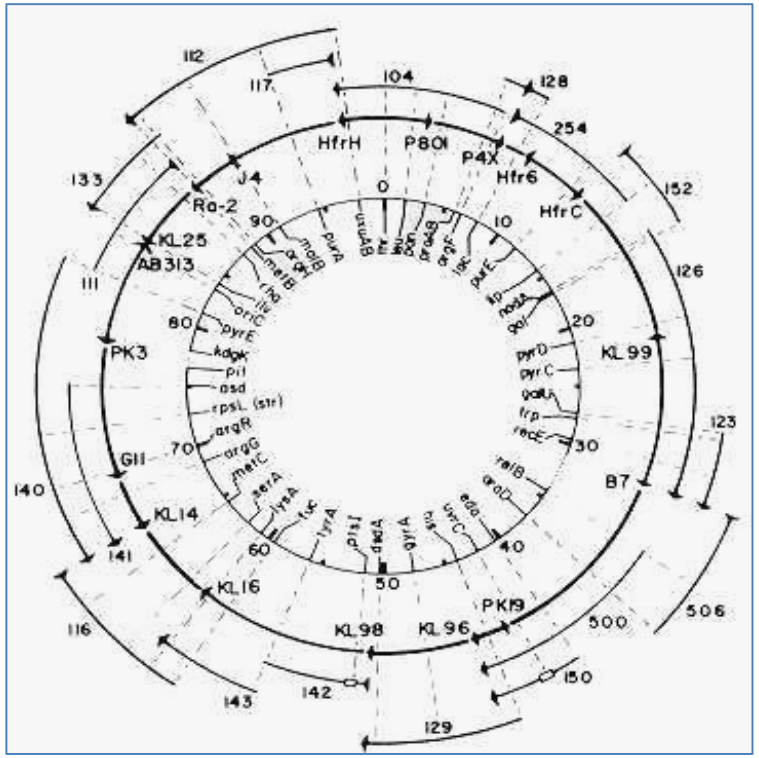
- The whole set of cell genes comprises its genotype
- The genes responsible for synthesis of substance is named by initial letters of corresponding substance. For example, aminoacide arginine gene *arg*$^{+}$, lactase gene - *lac*$^{+}$
- Susceptibility to antibiotics and phages is denoted by **s**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

Genetic map

- Location of genes in chromosomes can be determined by genetic analysis and on this basis **genetic map** is prepared.
- In genetic map circular chromosome with genes is represented.



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.

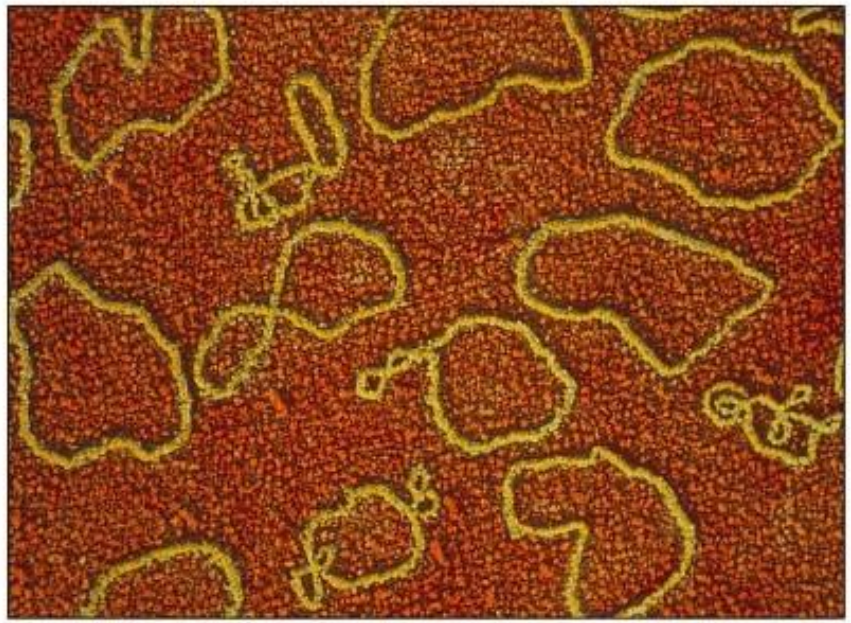
Plasmids

- **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;

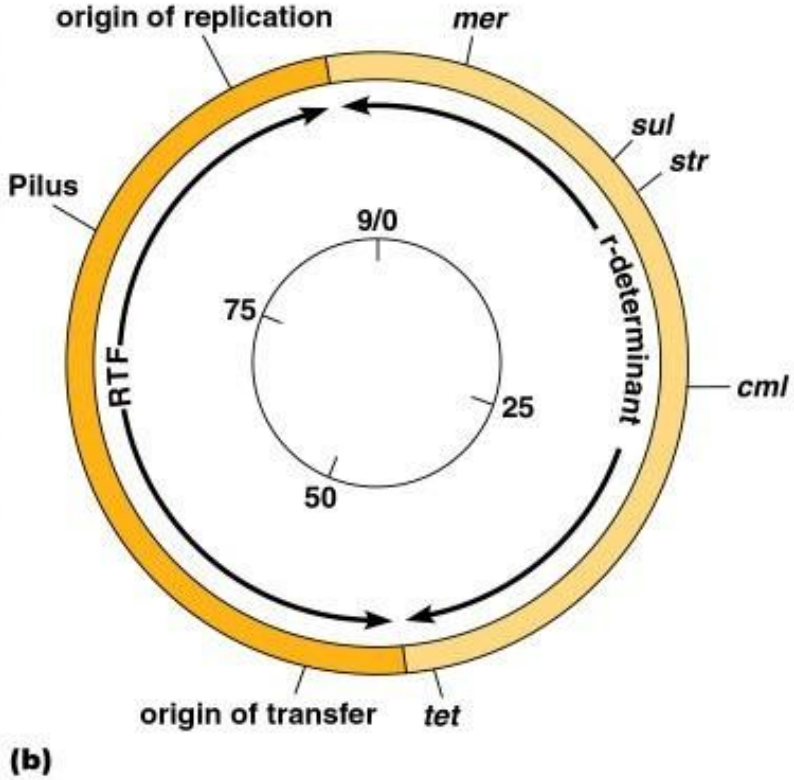
Plasmids

- 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),

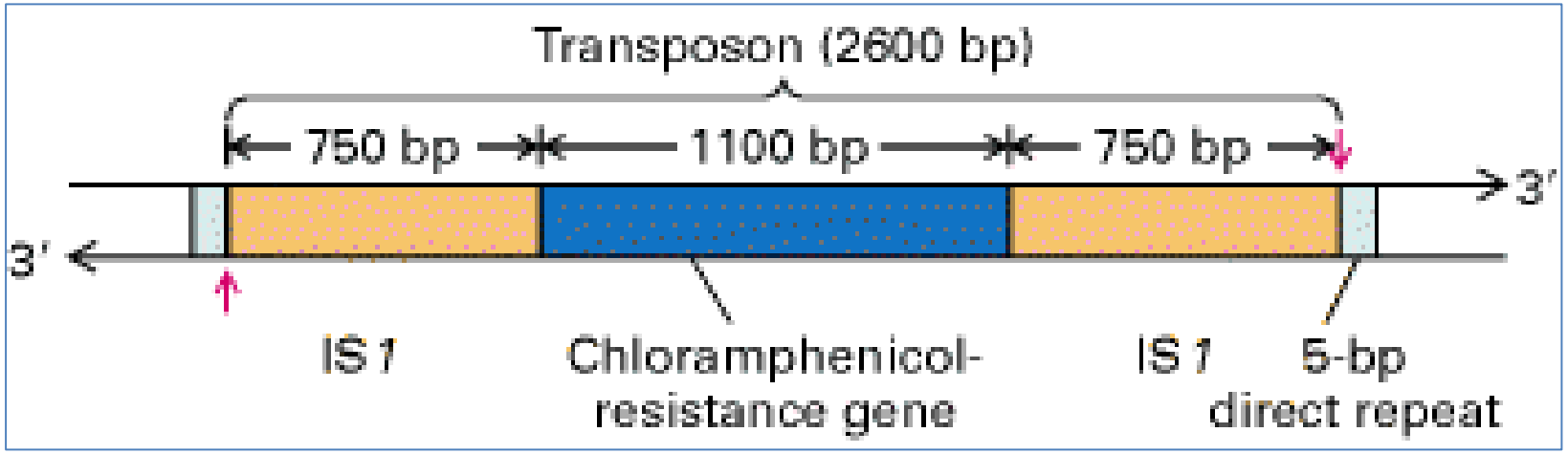
  - defective phages.

IS elements

- Insertion sequences or IS-elements are the simplest migrating elements.
- They consist of approximately 1500 nucleotide pairs and can migrate from one region to another.
- They include only genes responsible for transfer and are not able to reproduce independently.

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

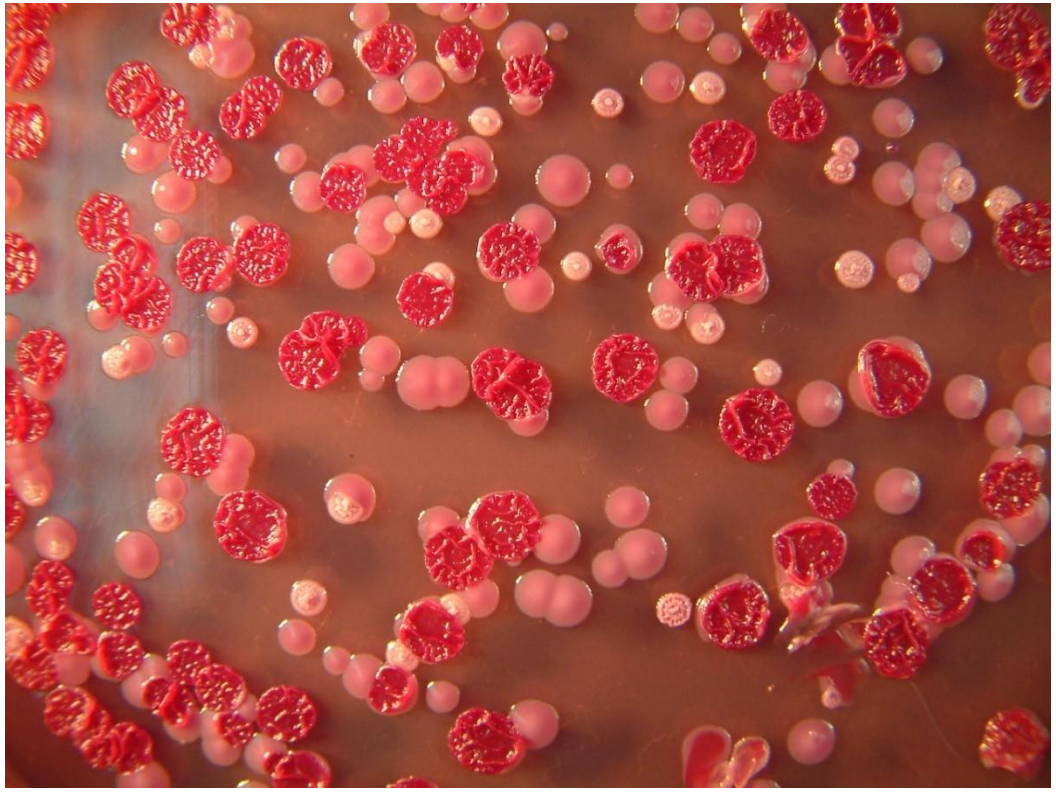
- Through modification microorganisms attain morphological, cultural, biochemical changes.
- Modification in ***morphological features*** is accompanied by changes in form and size of microorganisms***.***
- Modification can be represented by changes in***:***
- ***cultural features,***
- ***Biochemical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

Auxotrophic mutants

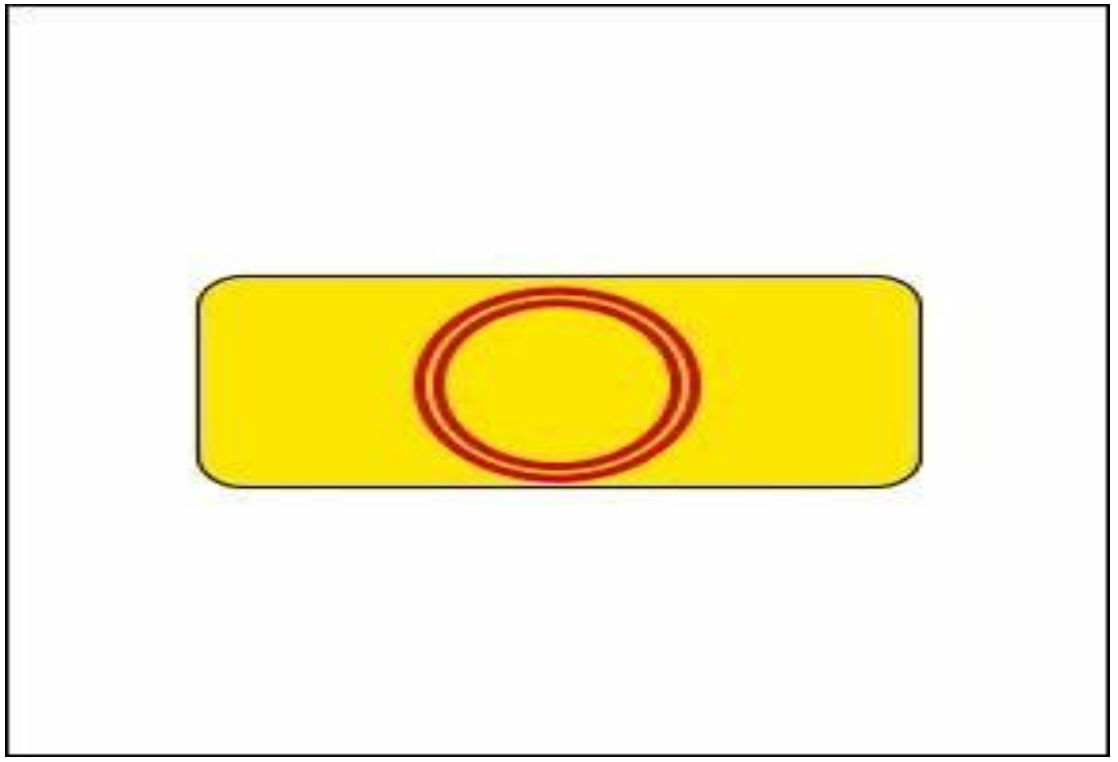
- After mutation microorganisms can lose ability to produce enzymes, amioacids, growth factors etc.
- These microbes are called **auxotrophic mutants** and grow only in presence of additional substrates.

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**.

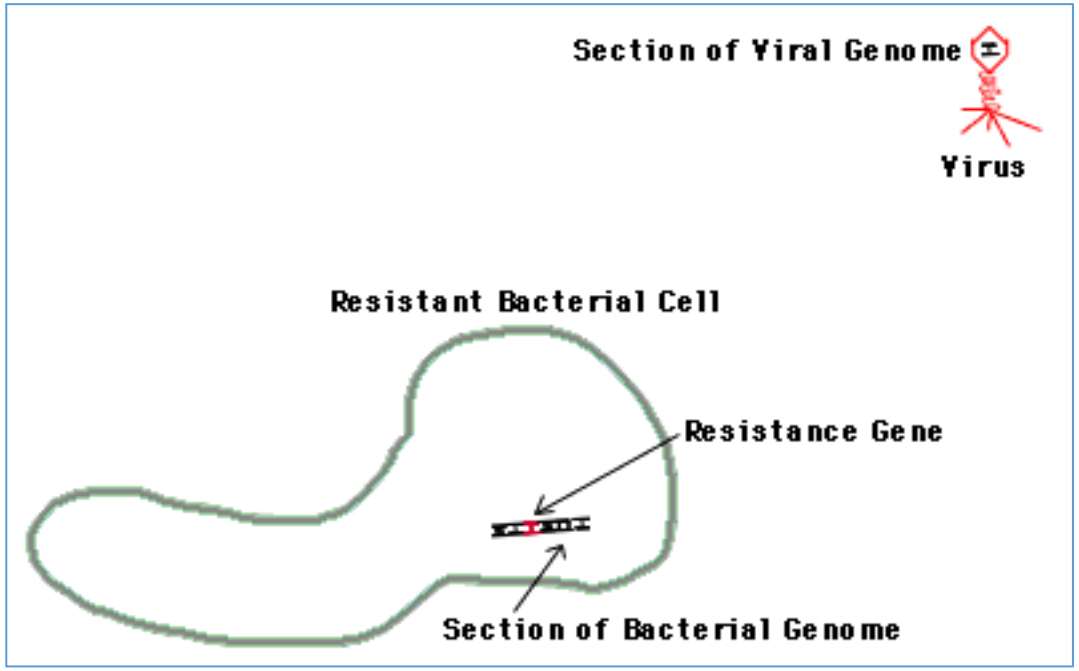
Transformation

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



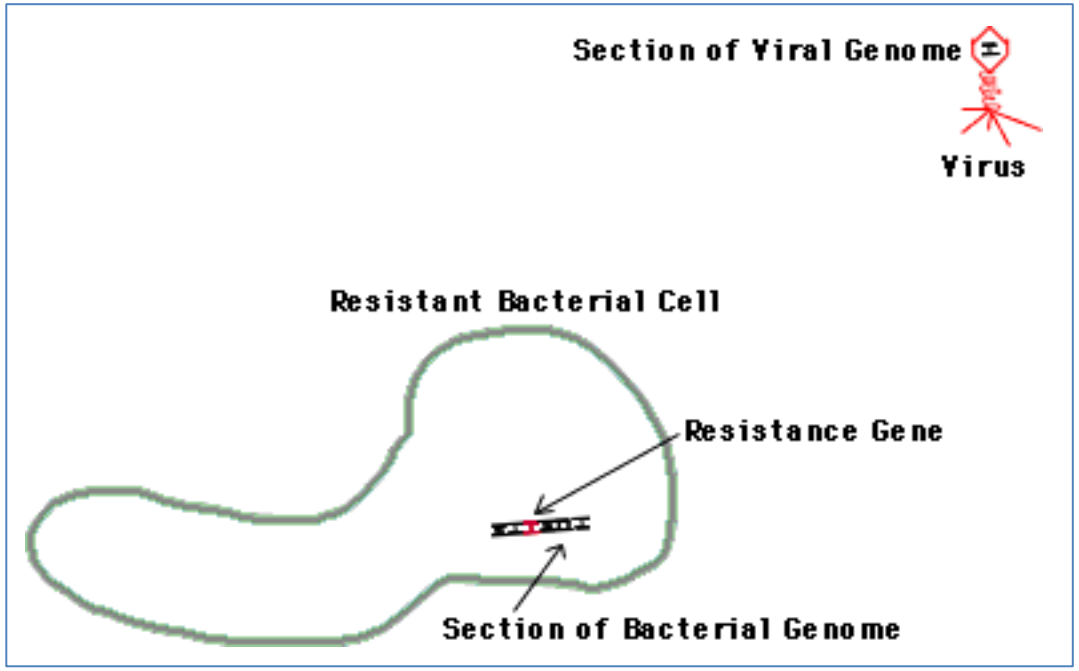
Transduкsiya

Transduкsiya – genetiк materialın (DNT moleкulunun bir hissəsinin) baкteriofaqlar vasitəsilə donor hüceyrəsindən resipientə ötürülməsinə deyilir



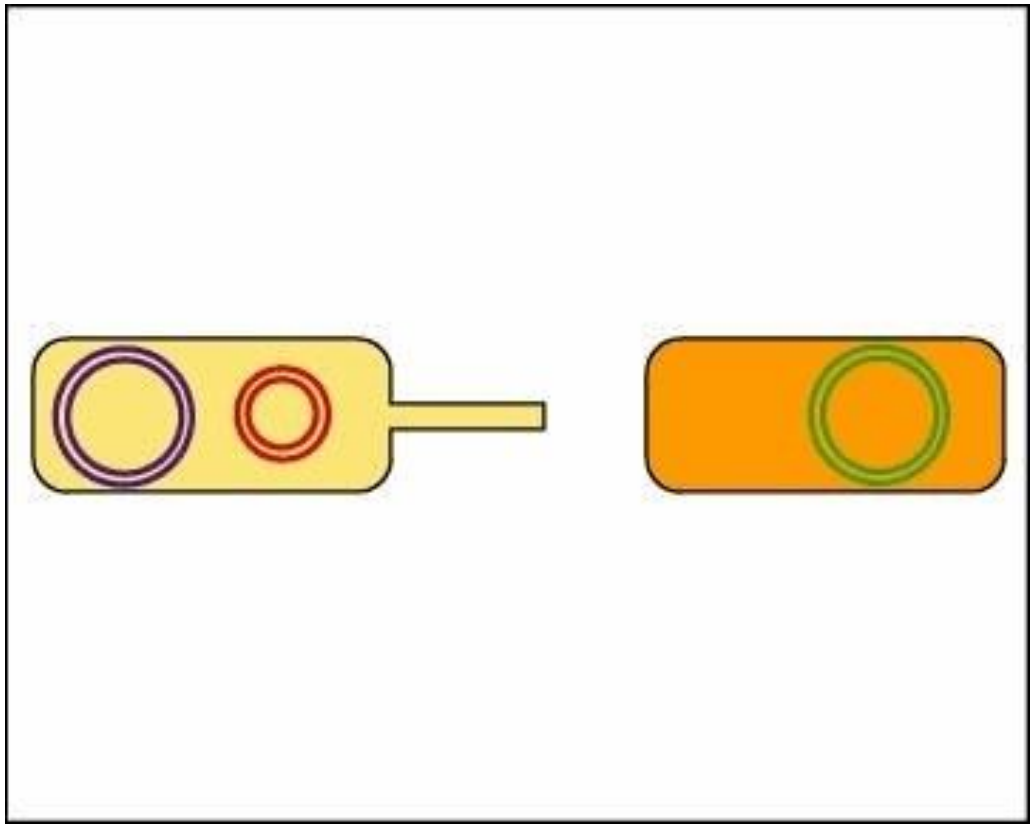
Transduction

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

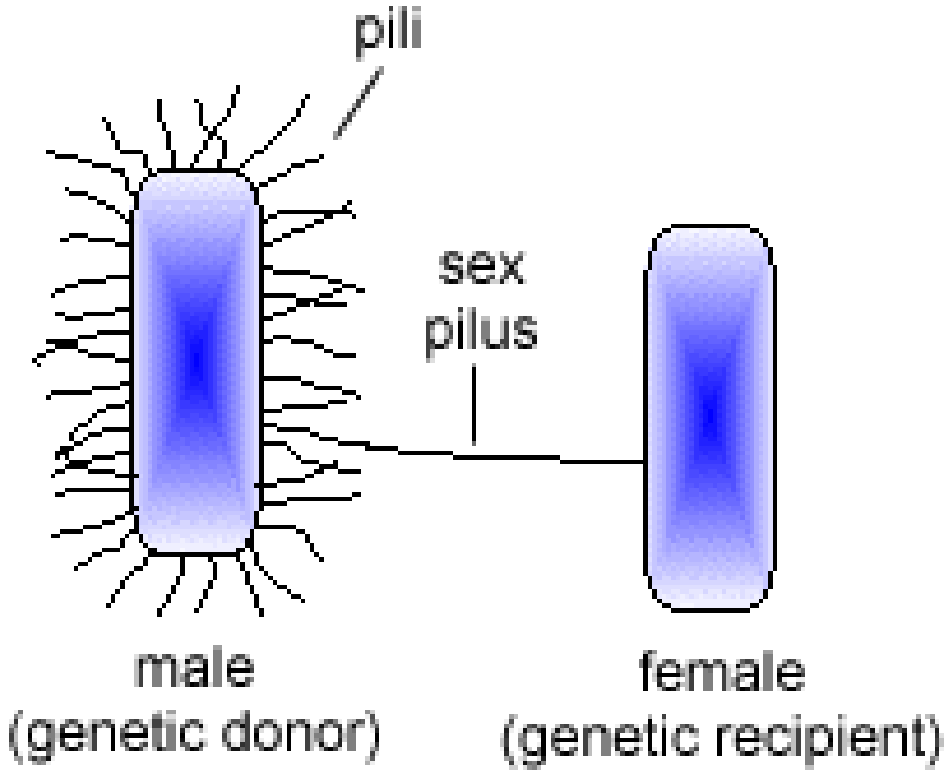


Conjugation

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.



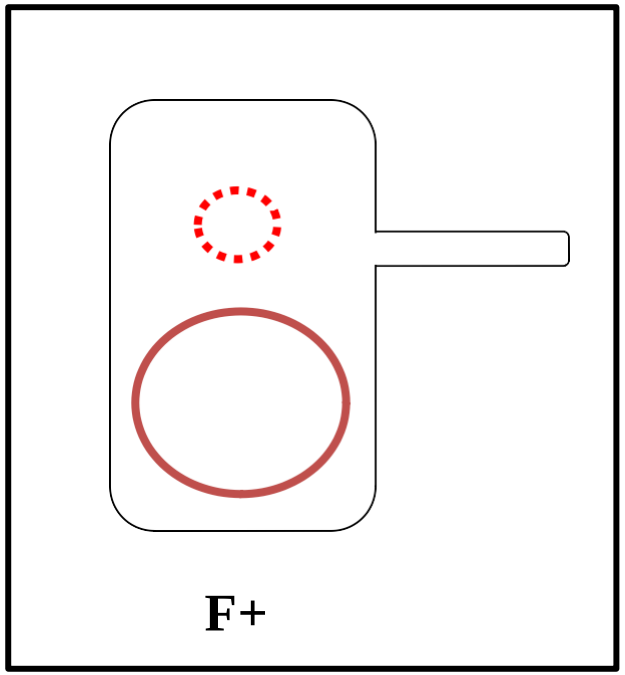
Conjugation



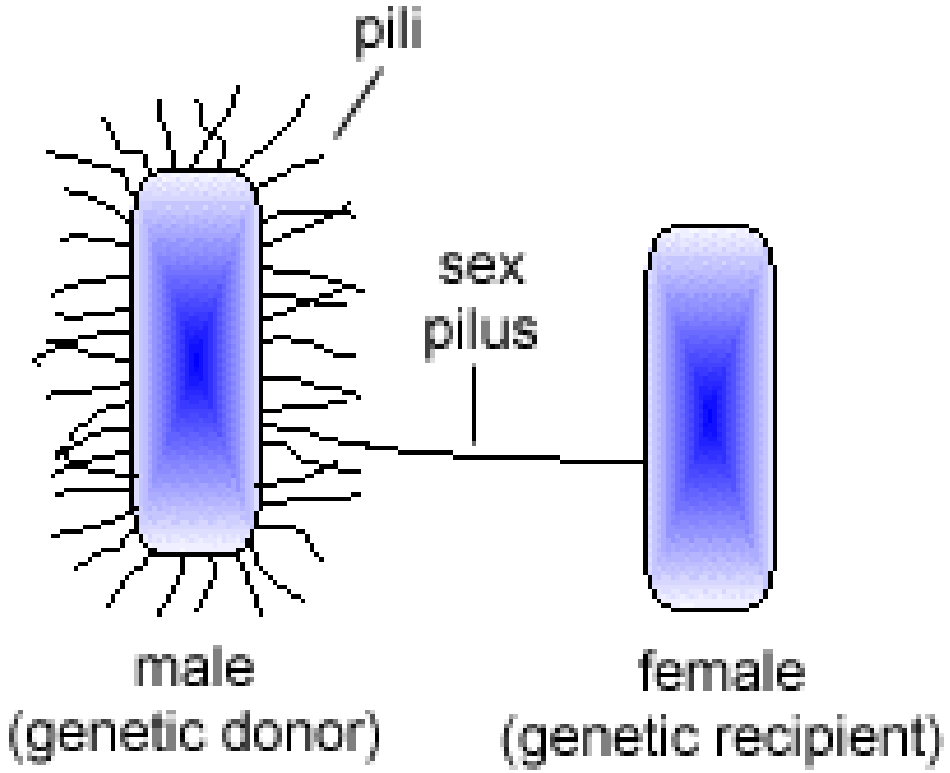
Conjugation

- 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

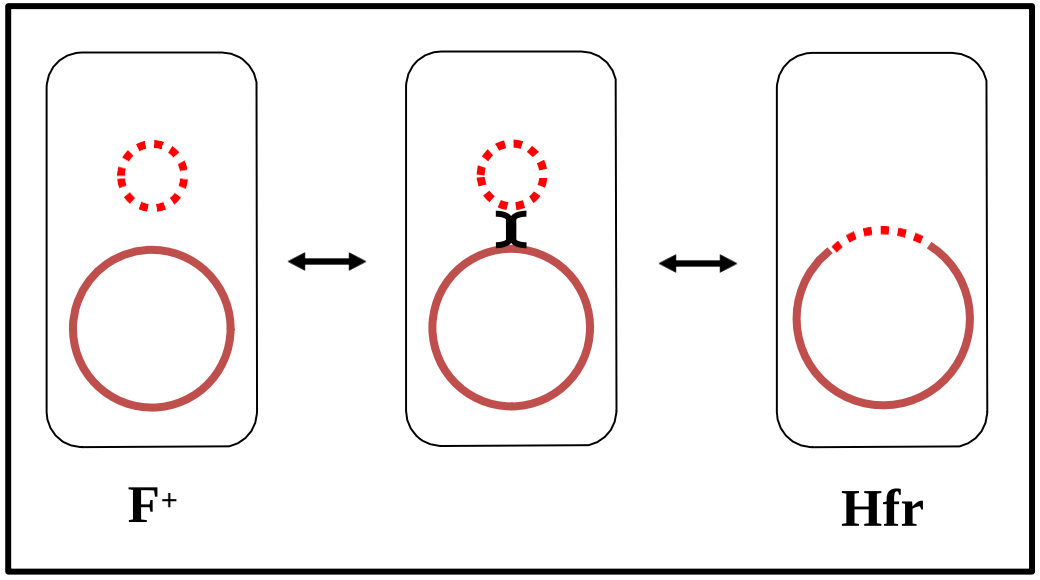


Konyuqasiya



Hfr-strains

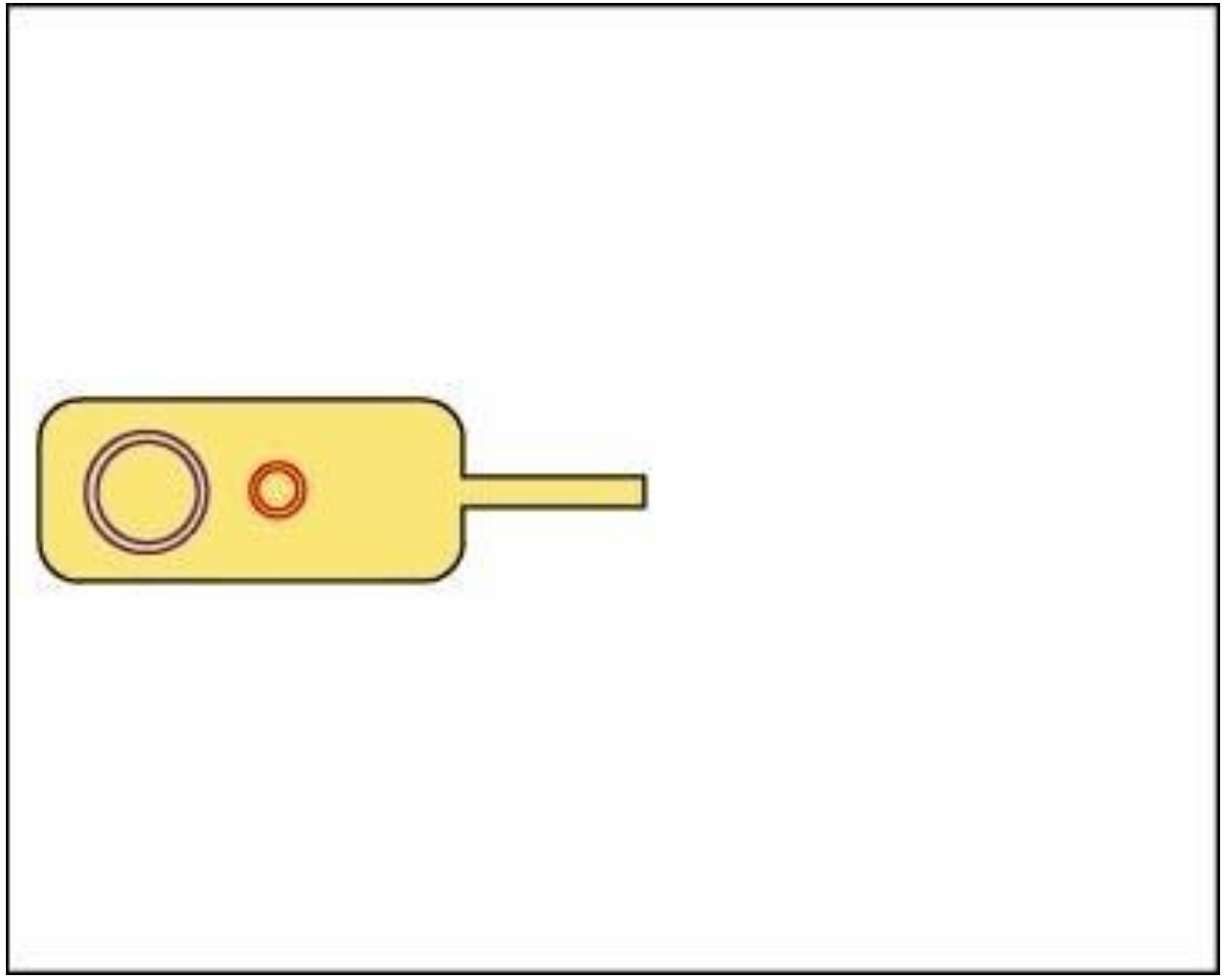
If F-plasmid integrates to cell chromosome it forms Hfr-cell (*high frequency of*). They are able to transfer chromosomal genes to recipient cells with high frequency



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



Genetics of viruses

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***.

Genetic engineering

Genetic engineering

- Single nucleotides produced after DNA breakdown can be transferred to prokariotic and eukaryotic cells. Resulting cell – **hybrids** possess and express foreign genes.
- The main goal of genetic engineering is ability to obtain product of special gene in recipient organism.

Genetic engineering(obtaining genes)

- First a gene (DNA molecule) encoding the product or feature is obtained or synthesized. The DNA molecule is then broken down into fragments using enzymes called restriction enzymes. This enzyme belongs to the endonucleases and has the ability to break down the DNA molecule only in certain places.
- Fragments of DNA molecules obtained by the action of restriction enzymes are called restricts. If necessary, it is possible to combine the ends of the restricts with **DNA ligases**.

Genetic engineering(transfer of gene)

- DNA fragments are bound to vectors. **Vector** is agent transferring foreign DNA fragments to recipients.
- **Plasmids** and **phages** or their combinations – **cosmids** and **phasmids** are used as vectors.
- Recombinant DNA is transferred by vector to recipient via **transformation, transfection** or **microinjection.**

Genetic engineering(gene transfer)

- *By **native transformation** r-DNA* can be transferred to *Bacillus subtilis*, *Streptococcus pneumoniae* and *E.coli strains*.
- Transfer of r-DNA to prokaryotic or eukorayotic cells by phage is called **transfection**. In some cases eukaryotic cell is infected by vector virus (polioma andSV-40 viruses).
- ***During microinjection DNA or r-DNA is transferred to animal and plant cells via glass microneedles.***
- R-DNA can be transferred to recipients via liposomes as well. Lipososmes are prepared from equal mixture of phosphatidylserine and cholesterol. A mixture of rDNA and liposome is treated with ultrasound, and then incubated with the recipient cell.

Genetic engineering(obtaining the final product)

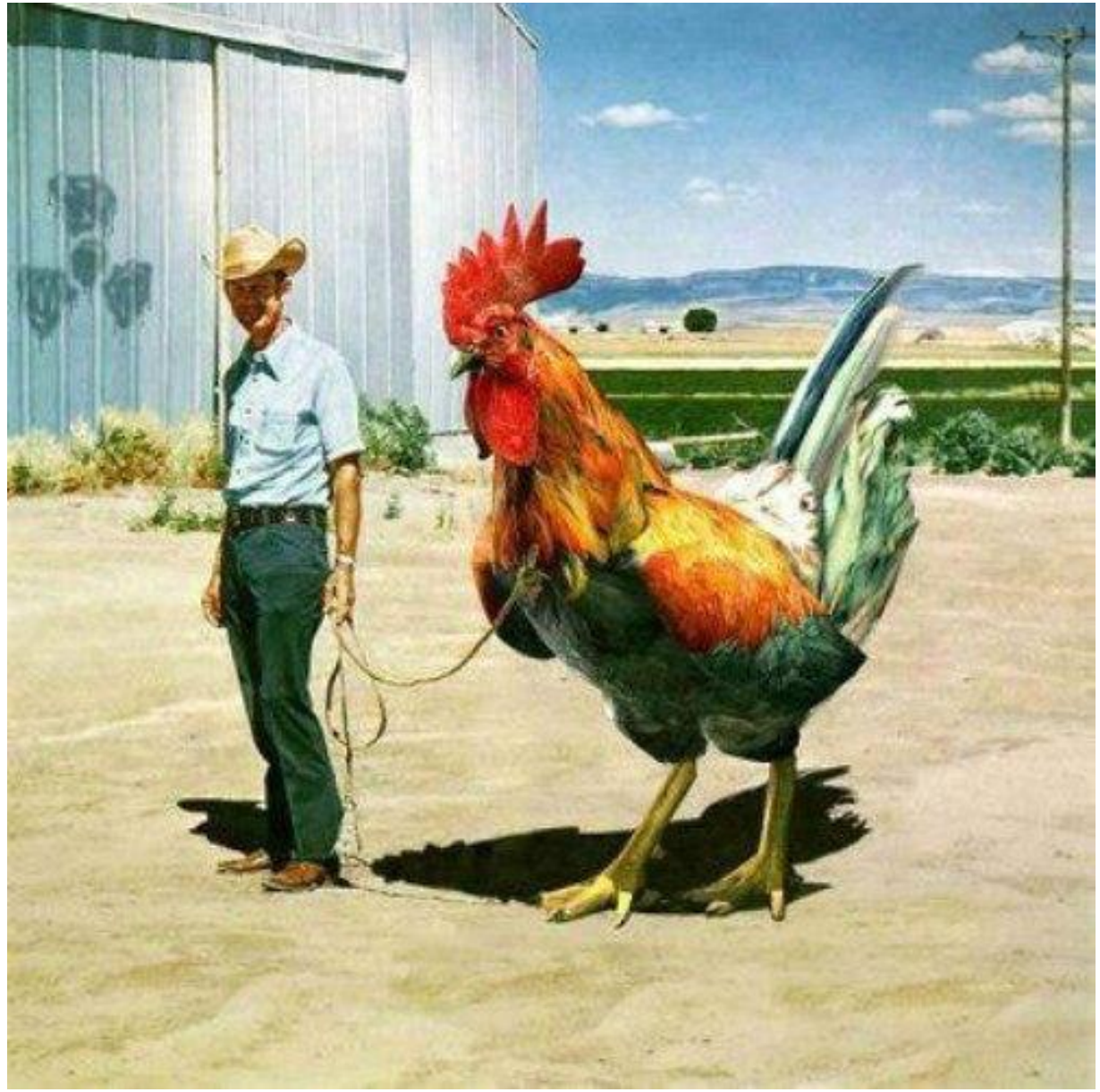
- rDNA is transferred to recipient cells called **permissive cells**. In these cells transferred r-DNA is not broken down and can be expressed.
- Prokaryotic *E.coli, B.subtilis*, eukaryotic *Saccharomyces cerevisiae* are often used in genetic engineering
- Nowadays, insuline, somatotrope hormone, interferons etc. synthesizing bacterial and fungal strains are obtained and used in biotechnology.

Modern opportunities of genetic engineering

- Microinjection of r-DNA in animal embryo cells made possible obtaining of **transgenic animals.**
- Via same the method phytopathogen, cold resistant **transgenic plants are obtained.** By transferring of immune dominant antigen genes of microorganisms to plants “vaccine” containing fruits and carrots were obtained.
- One of the recent successes of genetics is the creation of a genetic clone. The genetic clone was first created at the end of the last century by Scottish scientists Jan Welhmut and Ken Campbell.

Mouse with human gene

Dog possessing human genes



Application of genetic methods in diagnostics

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