AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 14.

Ecology of microorganisms. Microflora of soil, water, air and human body. Genetics of microorganisms

FACULTY: General Medicine
SUBJECT: Medical microbiology - 1

Discussed questions:

• Ecology of microorganisms.
• The interactions types between microorganisms. Symbiosis and its forms.
• The spreading of microorganisms in environment (autochthonous, alloxton microbiota), the role of microorganisms in environment.
• Sanitary indicator microorganisms and their determination.
• The microflora of soil, soil as a source of infection, its sanitary-indicating microorganisms (*E.coli, enterococci, C.perfringens*, thermophilic bacteria)
• Sanitary microbiological examination of soil (a) determination of the total number of bacteria, b) determination of the titer of sanitary indicator bacteria, c) determination of pathogenic microorganisms (salmonella, shigella, *B.anthracis, C.perfringens, C.tetani*).
• The microflora of water (polysaprobic, mesosaprobic and oligosaprobic zones), water as a source of infection, sanitary-indicating microorganisms (*E.coli*, enterococci, *C.perfrengens*, etc.).
• Sanitary microbiological examination of water a) determination of total microbial count, b) determination of titer and index of sanitary-indicating microorganisms: membrane filters and two-phase brodil method, c) determination of pathogenic microorganism (*V.cholera*, legionella, salmonella, shigella).
• The microflora of air, air as a transmitter of infectious diseases. Sanitary-indicative microorganisms of air (S.aureus, hemolytic streptococci).
• The sanitary microbiological examination methods of air: a) sedimentation method (Khoch method), b) aspiration method (Krotov method). Determination of the total number of microbes in the air. Determination of airborne microorganisms (S.aureus, hemolytic streptococci).
• Human normal microbiota of the (skin, respiratory tract, digestive tract, urogenital tract, etc.), its importance, detection by qualitative and quantitative methods of microbiota. Sterile organs.
• Dysbiosis and dysbacteriosis.
• Genetics of microorganisms.
• Organization of the hereditary apparatus of bacteria (chromosomes and plasmids).
•The variability kinds in bacteria:
• Modification (non-hereditary) variability (morphological, cultural, biochemical).
• Hereditary (genotypic) variability.
•a) mutation and its types (spontaneous mutation, inductive mutation; point (gene) mutations, chromosome mutations).
•b) genetic recombination: transformation, transduction and conjugation.
• Genetics of viruses. Modifications, mutations, genetic and non-genetic interactions between viruses.

Purpose of the lesson:

- To inform students about the microflora of the environment and the normal microflora of the human body, their role in medicine. Explain sanitary-indicating microbes and their importance. Explain to students the genetics of microorganisms, the organization of the hereditary apparatus of bacteria, the types and mechanisms of variability in bacteria. To provide information about the genetics of viruses.

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**

The role of microorganisms in environment

- Microorganisms participate in **nutrient cycle**
- **Nutrient cycle -**organic substances are formed from inorganic substances, and after a certain period of time these substances break down again with the formation of inorganic substances.

Environmetal role of microorganisms (nitrogen cycle)

- Nitrogen compounds are continuously broken down from which new organic substances are formed.
- During this process organic substances are converted to ammonia and ammoniac compounds by microorganisms9ammonification). If ammonification process takes place under anaerobic conditions, a number of unpleasant-smelling substances - indole, skatol, hydrogen sulfide, etc. is formed. If this process takes place under aerobic conditions, proteins break down into smaller molecules (decay).
- During next step ammonia is converted to nitrites (NO_2) and nitrates (NO_3) through oxidation (nitrification). This process is carried out by *Nitrosomonas* and *Nitrobacter genus bacteria*.
- Some microorganisms convert nitrates to free nitrogen (denitrification). During this process nitrates are converted to nitrites, nitrites – to ammonia, and the latter – to nitrogen. *Chromobacter, Achromobacter, E.coli* etc..participate in this process.

Environmetal role of microorganisms (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.
- ***Utilization of organic substances with formation of CO_2 occurs in human and animal organisms. Microorganisms participate in this process as well.***
- Anaerobic degradation of nitrogen-free organic substances by microorganisms – fermentation is described above. Under aerobic conditions, decomposition products consist mainly of water and carbon dioxide.

Environmetal role of microorganisms (sulfurcycle)

- ***Begins with decomposition of organic substances which results in production of hydrogen sulfide(H_2S) by*** *Desulfovibrio* and *Desulfotomaculum*.
- ***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.***

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.
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Indicator microorganisms

- Direct detection of pathogenic microorganisms in environment is difficult as they are rarely found in environment objects.
- Thus, contamination of environment is evaluated indirectly- by detection of indicator microorgansims. Each environmental object has special indicator microorganisms. Investigation of number of these microorganisms help to examine sanitary condition of environmental objects.
- ***These microorganisms are part of human and animal bormal flora and excreted to environment.***
- ***Like pathogenic microorganisms they can survive in the environment and do not reproduce there***

Microflora of soil

◙ Soil is the most superficial layer of the earth, it is the main reservoir and natural environment for various microbes.

■ In the soil - the abundance of substances, the presence of humidity ensures that it is a favorable habitat for microbes.

■ In 1 g of soil - several billion microbial cells are found.

■ As the soil is exposed to sunlight and drying, there are few microbes on its surface, more at a depth of 10-20 cm, minimum at a depth of 1 m, no microbes at a depth of 3-4 m.

■ 4.8-5.2 billion microbes are found in 1 g of fertilized and cultivated soils, 2-3 billion in forest soils, 0.9-1.2 billion in desert soils.

Microflora of soil

- **Microbial flora of soil**. Various pathogenic and opportunistic pathogenic microorganism are excreted in the environment by human and animals.
- **Soil-borne diseases**
- **The sanitary indicator microorganisms of soil are** *Escherichia coli and Clostridium perfringens*-dir.
- **During sanitary microbiological investigation of soil:**

- the total number of bacteria in 1 g of soil;
- the titer of sanitary microorganisms (E.coli and C.perfringens);
- thermophilic bacteria in 1 g of soil;
- If there are epidemiological indications, pathogenic microorganisms (salmonella, shigella, tetanus, botulism and some viruses) are detected

Soil sampling

■ Depending on the purpose of the examination - a sanitary doctor or bacteriologist selects a certain place for taking soil samples and divides it into 2 areas of 25 m2 (5x5 m).

● One of these areas should be close to the source of pollution (waste heap, trash cans, cesspools, etc.),

● Other area - should be in a place far from them (control area).

● Samples are taken from different depths (20-25 cm) and 5 points ("envelope method" - 1 center, 4 corners) in a rectangular area (about 200-300 g) following aseptic rules.

► Preparation of samples for examination

■ In the laboratory - soil samples are thoroughly mixed, cleaned of large particles (stones, glass, plant roots, etc.).

● 200-300 g is separated from the middle part - it is poured into a bowl, pounded in a mortar and made into a pomegranate, and then it is sifted through a filter paper.

● 30 g of filtered soil is taken - poured into a 500 ml flask, 270 ml of sterile water is added to it (1:10).

● Take 1 ml of this dilution - make serial tenfold dilutions (102, 103, 104, 105) in test tubes.

● In the examination of clean soils - the first 3-4 dilutions are sufficient, in the examination of contaminated soils - more dilutions (105, 106, 107, etc.) are used.

► Soil testing methods

■ In the sanitary-microbiological examination of the soil:

♦ total number of microbes in 1 g of soil (TNM);

♦ titer of sanitary-indicative microbes (E.coli and Clostridium perfringens);

♦ amount of thermophilic and nitrifying bacteria in 1 g of soil;

♦ when there is an epidemic indication:

▪ pathogenic bacteria;

▪ ratio of the number of spore-forming bacteria in percentage,

▪ actinomycetes, fungi,

▪ Cellulose-decomposing and ammonifying bacteria are prescribed.


ГЕОМИР

► Determination of the total number of microbes

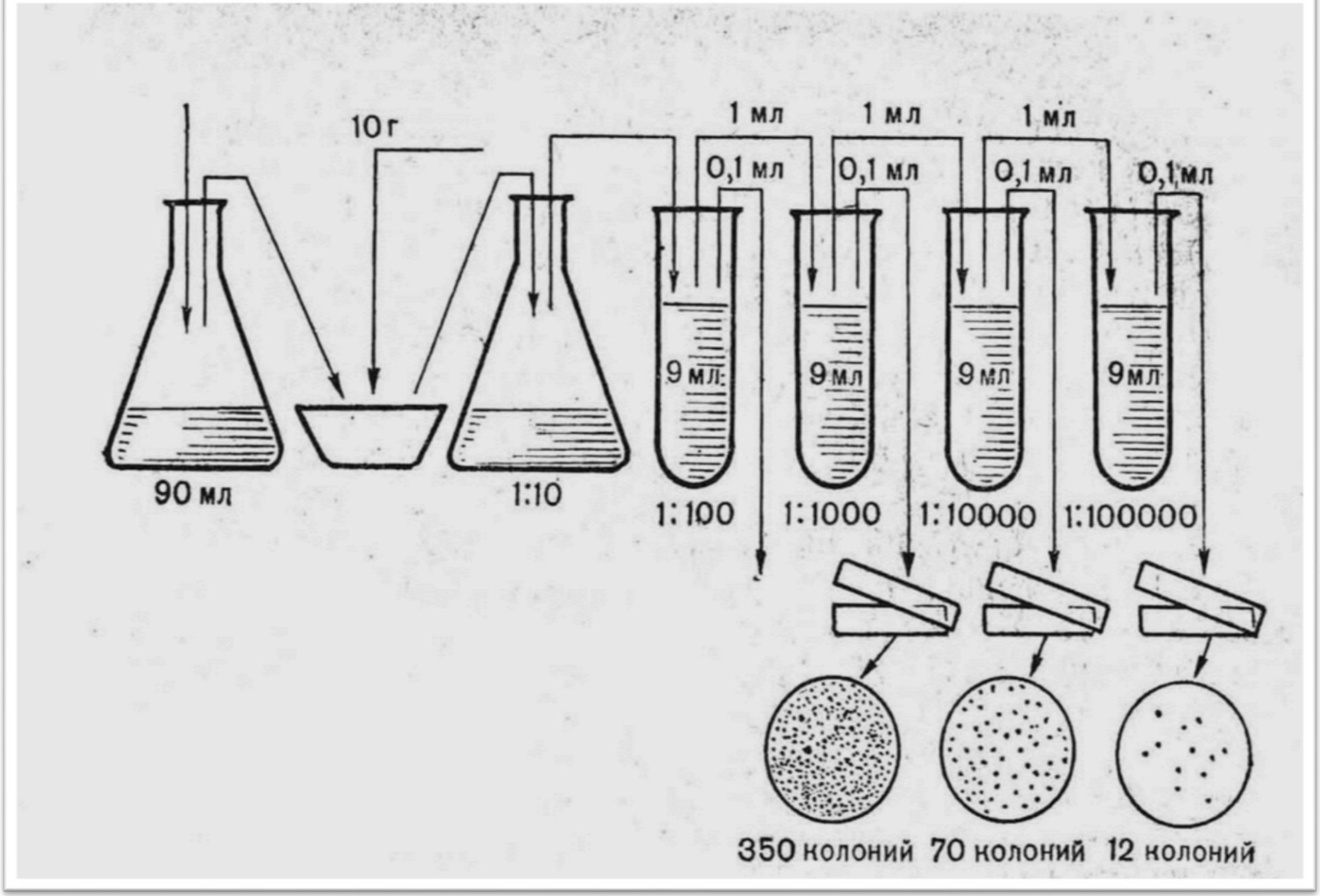
■ The determination of the total number of microbes in the soil is carried out in the same way as the determination of the total number of microbes in water.

● Depending on the degree of contamination - 1 ml of each of at least 2 dilutions prepared from soil samples is taken and inoculated into solid nutrient media (Endo media).

● It is incubated in a thermostat - 370C for 24 hours.

● Colonies grown in the nutrient medium are counted - the average number is calculated (as in water) and the TNM (CFU/ml) is recorded.

● Total microbial count (CFU/ml) - should not exceed 10,000 in 1 g of clean soil!


10 г
90 мл
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1 мл
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9 мл
1:10000
0,1 мл
9 мл
1:100000
350 колоний
70 колоний
12 колоний

Sanitary microbiological examination of soil

- 5 soil samples are taken from 5x5 m area (“envelope method”). 1 kg soil sample is taken aseptically from 20-25 cm depth.
- The total number of microorganisms is detected by inoculation of 10-fould diluted sample onto solid medium. In case of weak fecal contamination E.coli number is determined by fermentation or membrane filter methods.
- In case of high fecal contamination 10-fould diluted soil suspension is directly inoculated onto Endo medium..

Sanitary microbiological examination of soil

- An important criterion of soil sanitary condition and self-cleaning ability is its C.perfingens-titre (minimum amount of soil containing C.perfringens). E.coli is not detected in soil after 4-5 weeks of contamination while Clostridia can be detected in 0.01 g titer.
- C.perfringens titer is detected by inoculation of 10-fould diluted sample onto Wilson-Blair medium.
- Thermophilic bacteria are detected by 24-hour incubation of bacteria at 60^0C.
- The titer of nitrifying bacteria is detected by inoculation of 10-fould diluted soil suspension into a synthetic liquid Vinogradsky medium.

Microflora of water

- **Microbial composition of water**
- **The viability of microorganisms in water and its self-purifying process.**
- **Disease causing microorganisms living in water and water-borne diseases.**
- **Sanitary indicator microorganisms of water (*E.coli*)**
- **During sanitary microbiological investigation of water** .

  - the total number of bacteria in 1 ml of water

  - Coli-titer – the lowest amount of water in which E.coli is detected

  - Coli-index – the number of E.coli in 1 l of water

  - In case of epidemiological indications pathogenic microorganisms are detecteded.
- The coli-titer of tap water should not be less than 300, the coli-index should not be more than 3,the number of microbes should not exceed 100, and pathogenic microorganisms should not be detected.
- **The problem of water sterilization**

Determination of total microbial count of water

- 1 ml tap water and 1.0; 0.1; 0.01 ml spring water are taken for examination.
- Examined water is poured into Petri dish and 45-50^{0}C cooled nutrition medium is added.
- After incubation 24 hours inoculation at 37 ^{0}C, it is stored at room temperature for another 24 hours.
- Grown colonies are counted and the arithmetic mean number of bacteria, yeasts and molds colonies are calculated in CFU/ml.

Detection of water coli-titer

- The coli-titer and coli-index of water are determined by the membrane filtration or titration method.
- ***Membrane filtration method***. Three samples (100 ml) of examined water are filtered through nitrocellulose membrane filters. These filters are placed on Endo medium and incubated at 37^0C for 24 hours. After incubation the number of lactose-positive colonies is detected.

Microflora of air

- **Microbial composition of indoor and outdoor air**
- **The viability of microorganisms in the air**
- **Air microorganism and airborne diseases**
- **Sanitary indicator microorganism of air** - hemolytic streptococci and *Staphylococcus aureus*
- **The principles of air sanitary-microbiological investigation of air**
- Sanitary microbiological examination of the air is carried out mainly in medical and child-care institutions:

  - The total number of bacteria in 1 m^3 air;

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

  - The number of pathogenic and opportunistic bacteria 1 m^3 in 1 m^3 air.
- **Air sterilisation**

Microbiological investigation of air

- During ***aspiration method*** air is passed through nutrition media using aspirating devices. It enables determination of number and the microbial composition of air.
- Krotov device is used for this purpose. The air aspirated through hole in device and sedimented on surface of rotating medium.
- The number of colonies(CFU) in volume of air is counted after incubation and contamination of air is evaluated.

Microbiological investigation of air

- ***Sedimentation method is based on mechanical sedimentation of microorganisms on surface of nutrient agar. This method is used for evaluation of microbial composition of air.***
- Opened Petri dish with nutrient agar is placed on table. Sonra kasanı bağlayıb inkubasiya edirlər. Contamination of air can be evaluated using Omelianski equation: bacteria from 10 dm^3 air are sedimented on 100 cm^2 agar surface for 5 min.

Omelianski equation: $$X=\frac{A\ x1\ 00\ x1\ 00\ x\ 5}{B\ x\ 10\ xt}$$

X – the number of microorganims in 1 m^3
A – the number of colonies in Petri dish
B – the area of Petri dish (πr^2)
T – the time when plate remained opened
5 – Omelyanski time
10 – air volume sedimented in 5 minutes
100 – sedimentation area (cm^2)
1000 – examined air volume(liter)

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

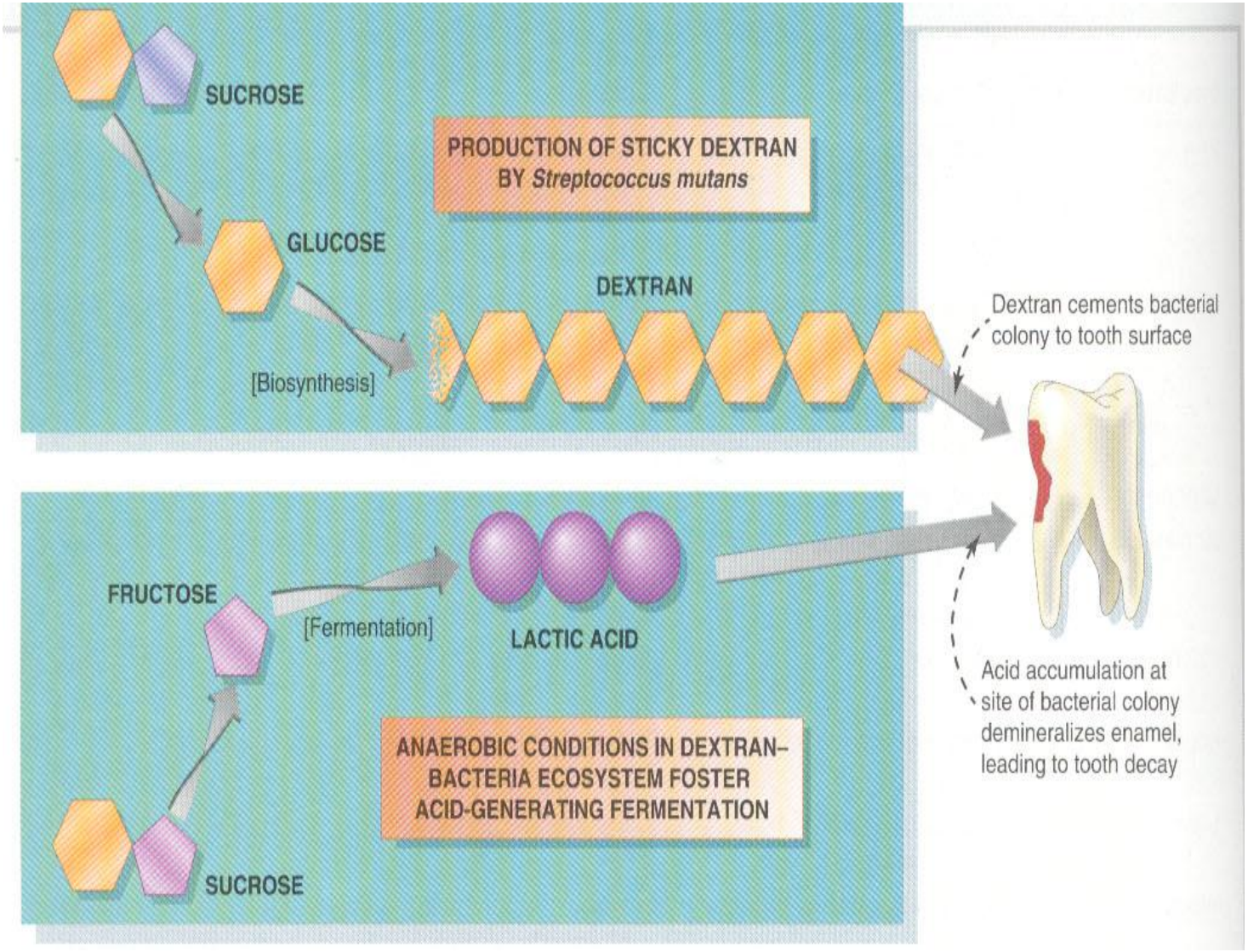
Microflora of respiratory airways

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

The intestinal mucosa – infection entry

- Intestinal wall plays a role of special semiconducting membrane
- At some circumstances microorganisms penetrate intestinal wall, spread through lympha and blood causing bacteriemia
- Pathogenic microorganisms are able to invade the organism through intestinal wall. In this case intestinal tract plays a role of infection entry portal.

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.

Life in sterile condition

- The main distinctive features of gnotobionts – they are not decomposed after death and have different defense mechanism against infections.
- As gnotobionts do not have bacteria they are not decomposed after death.
- Gnotobionts have weak defense system, low leucocyte number, weak lymphoid tissue, practically no antibodies.
- They are supplied with vitamins, even without the bacteria (previously it was thought that bacteria are needed for the synthesis of some vitamins). The weight of their excrement is the same as that of ordinary animals (it is still believed that 50% of the excrement consists of decomposed substances).

Life in sterile condition

- As there is no risk of infectios gnotobionts die from organ disfunctions.
- Thus, they are considered as convenient model to study organ disorders, tissue aging and other problems of aging.
- Under similar circumstances, researchers try to answer another interesting question: **how long can life be extended**?
- Notre Dame scientists in collaboration with Chicago University study caries, viral infections, heart, oncological diseases, etc.).

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.

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

- The total number of E.coli in 1 gr of faeces;
- Number of hemolytic E.coli;
- Number of opportunistic pathogens (*Proteus* spp. and *Candida* spp.):
- Bifidobacteria, lactobacteria and bacteroides number.

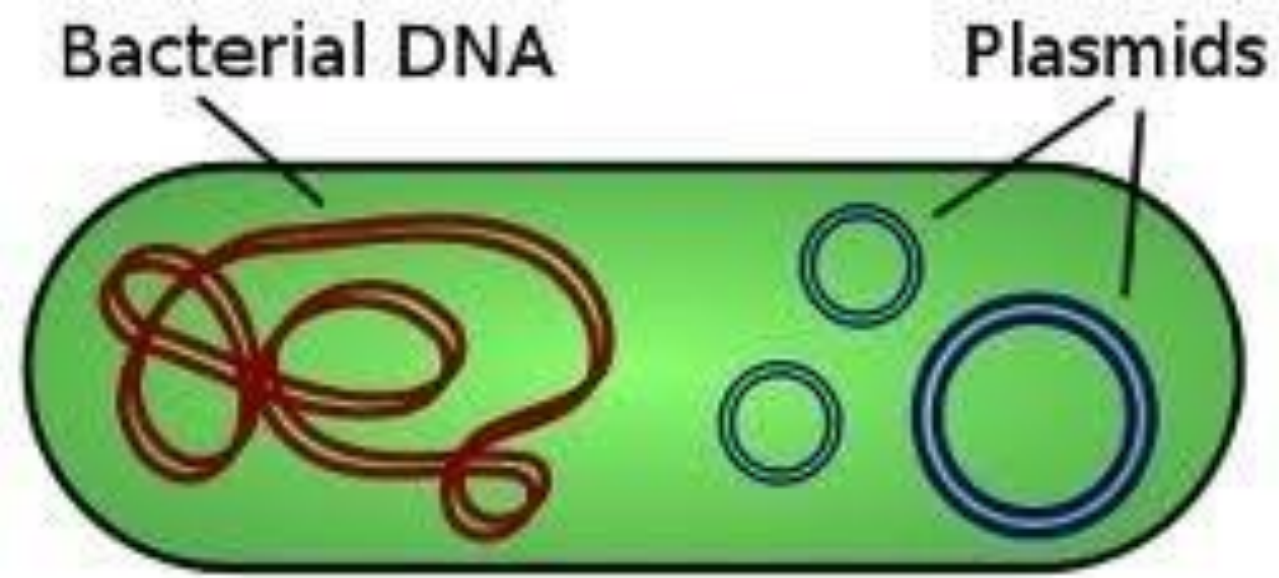
Treatment of dysbiosis and dysbacteriosis

- 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.

Genetic apparatus 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



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

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

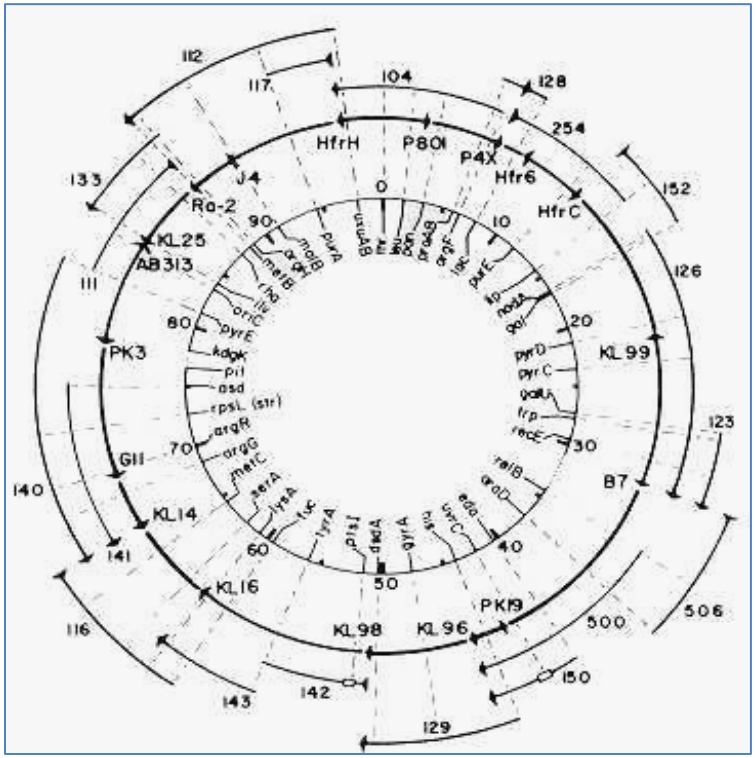
- The whole set of cell genes comprises its genotype
- The genes responsible for synthesis of substance is named by initial letters of corresponding substance. For example, aminoacide arginine gene *arg*$^{+}$, lactase gene - *lac*$^{+}$
- Susceptibility to antibiotics and phages is denoted by **s**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

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.

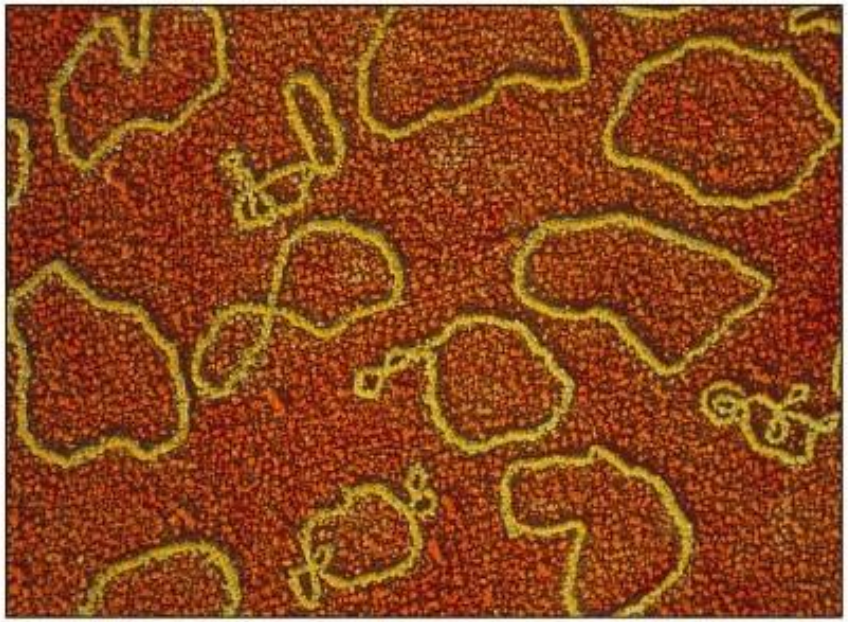
Plasmids

- **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;

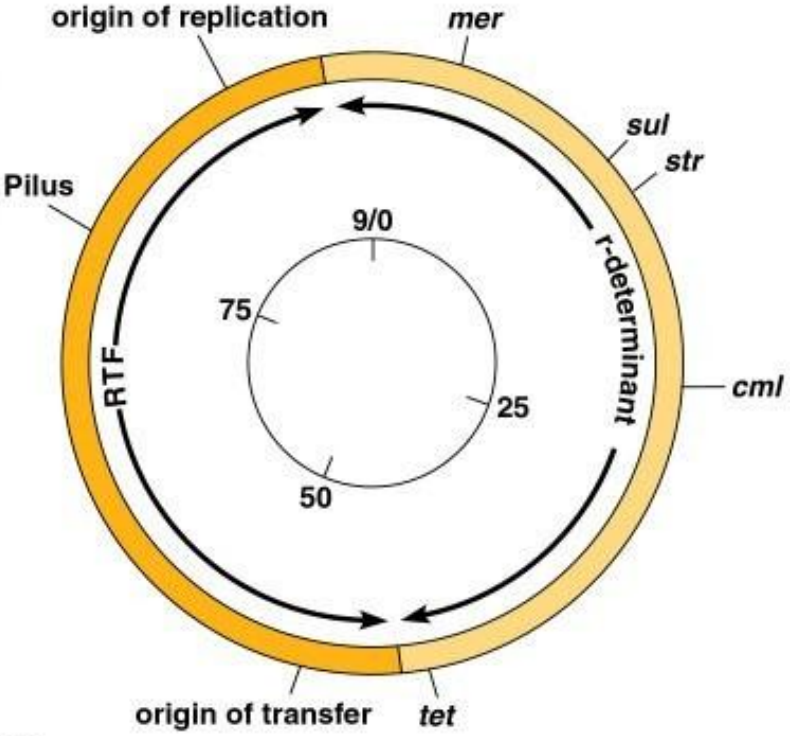
Plasmids

- 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

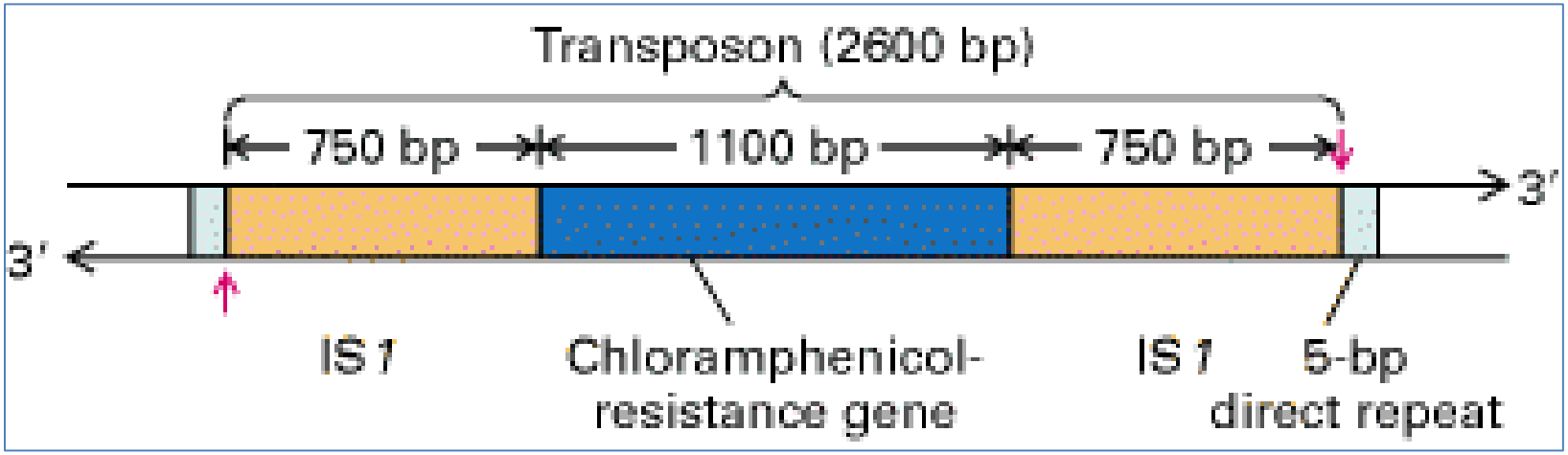
  - insertion sequences (IS-elements),
  - transposons(Tn-elements),
  - defective phages.

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

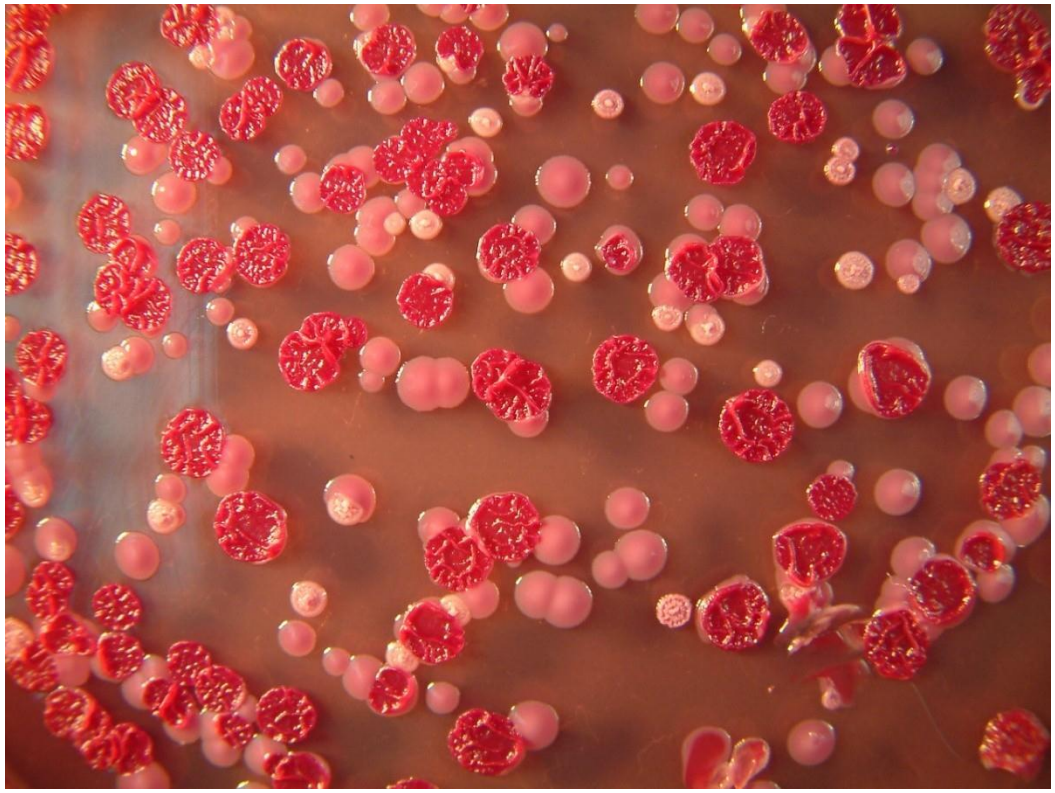
- Through modification microorganisms attain morphological, cultural, biochemical changes.
- Modification in ***morphological features*** is accompanied by changes in form and size of microorganisms***.***
- Modification can be represented by changes in***:***
- ***cultural features,***
- ***Biochemical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

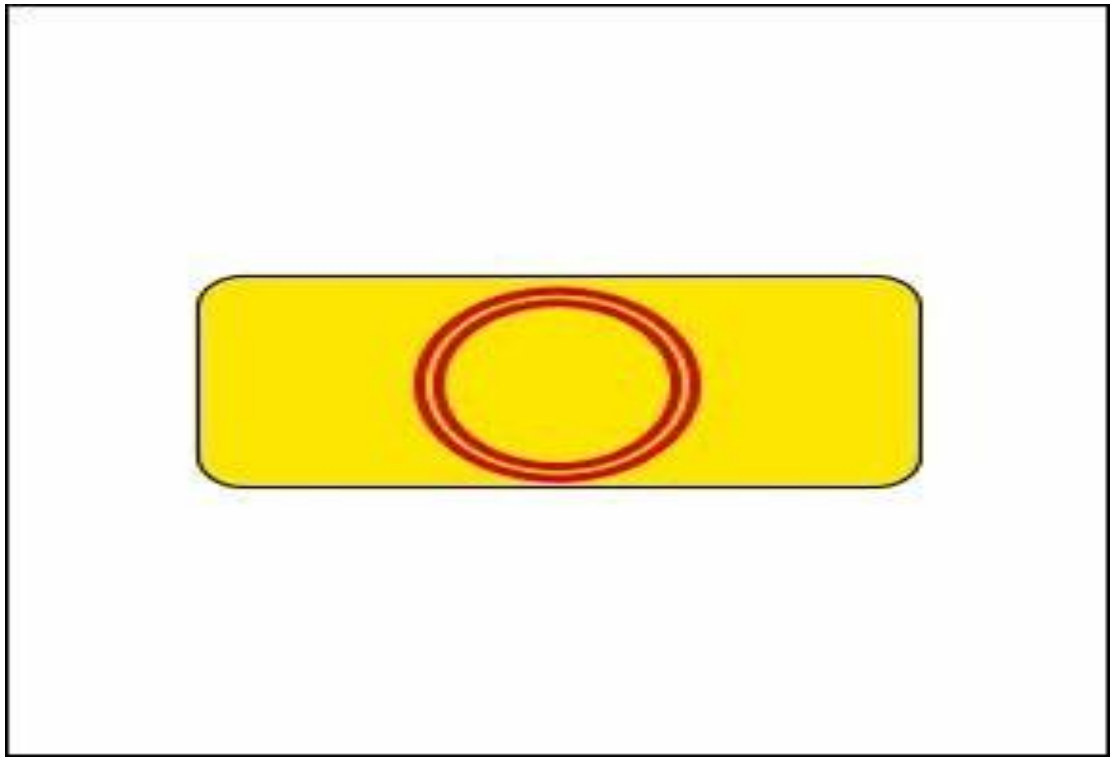
- ***According to phenotypic results- neutral mutations, conditional lethal, lethal mutations***

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**.

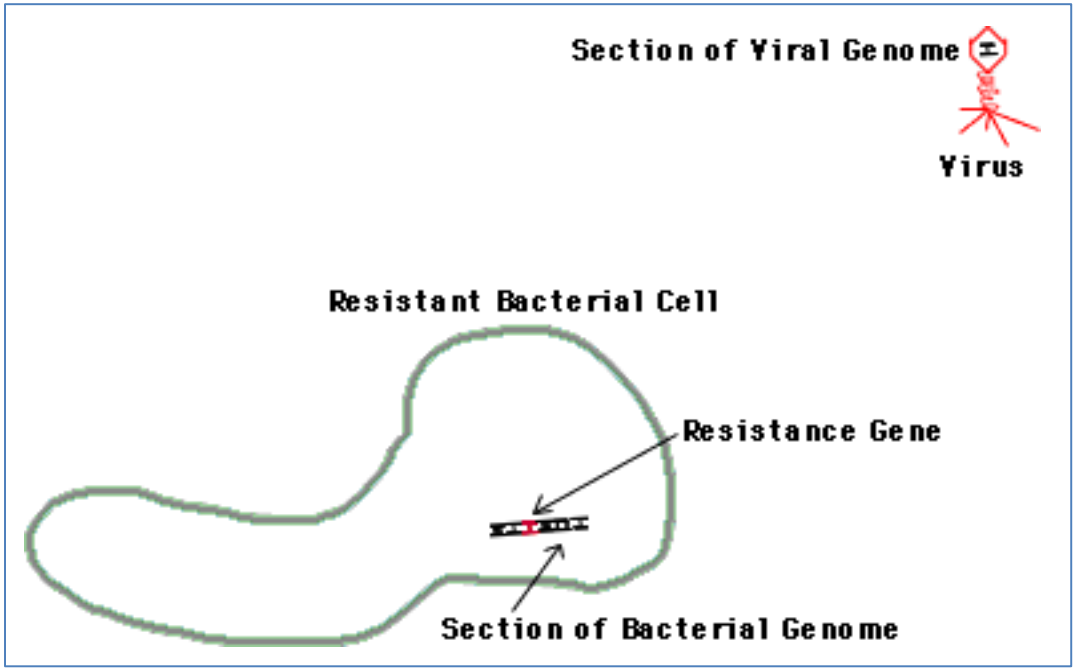
Transformation

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



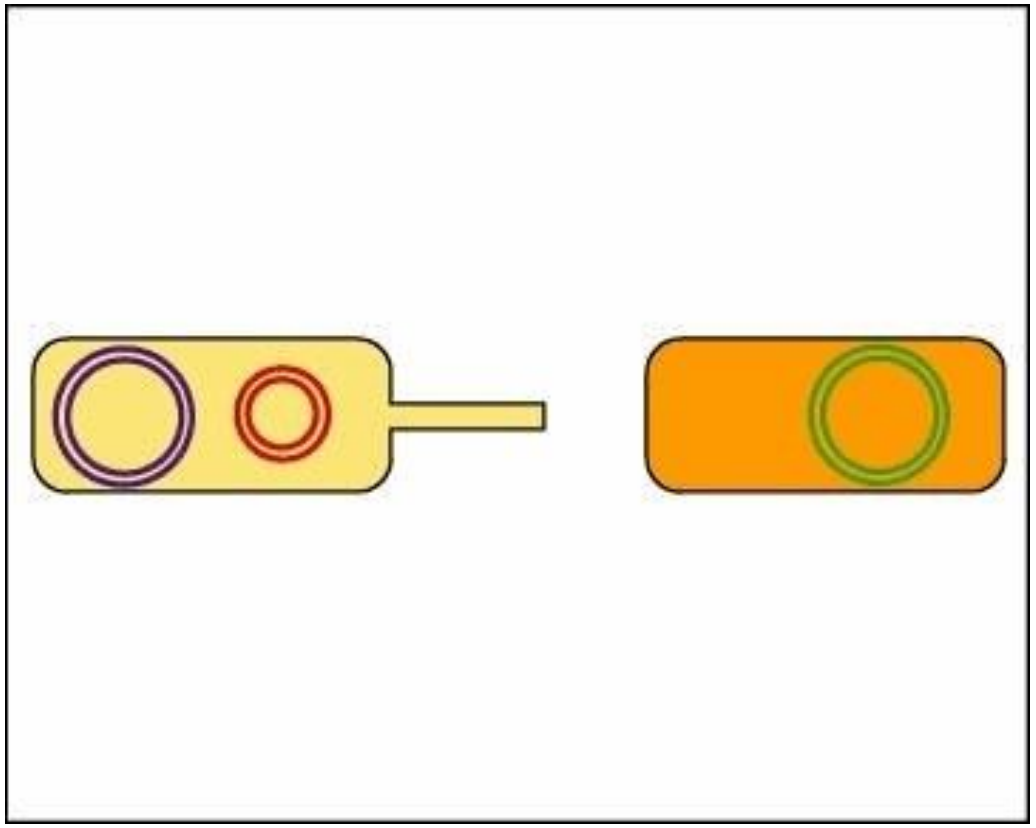
Transduction

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

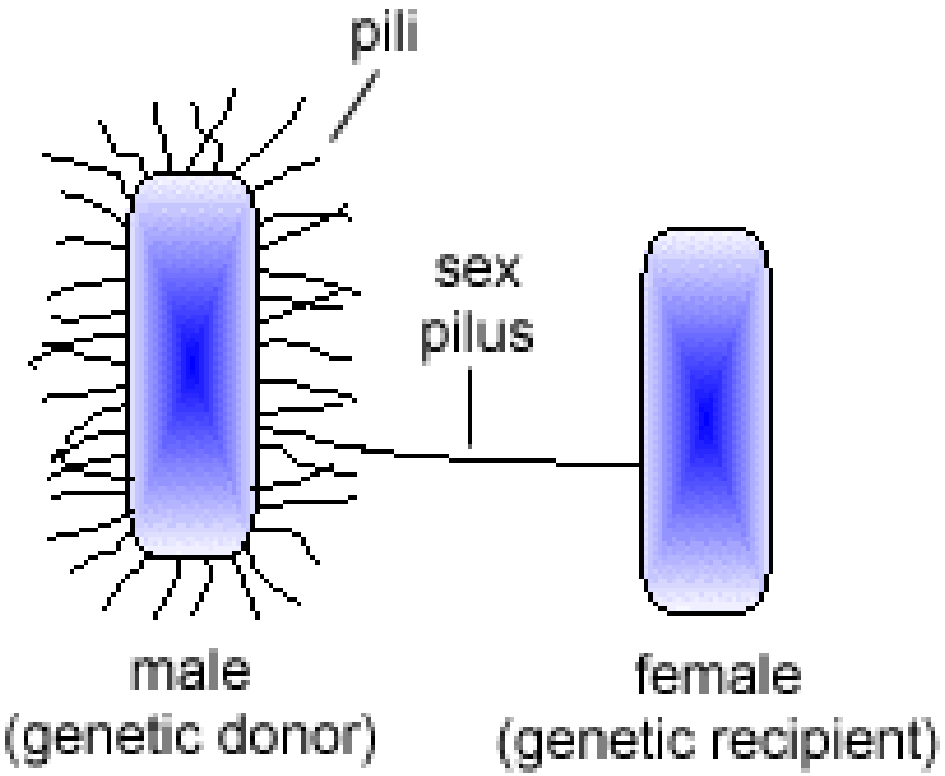


Conjugation

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.



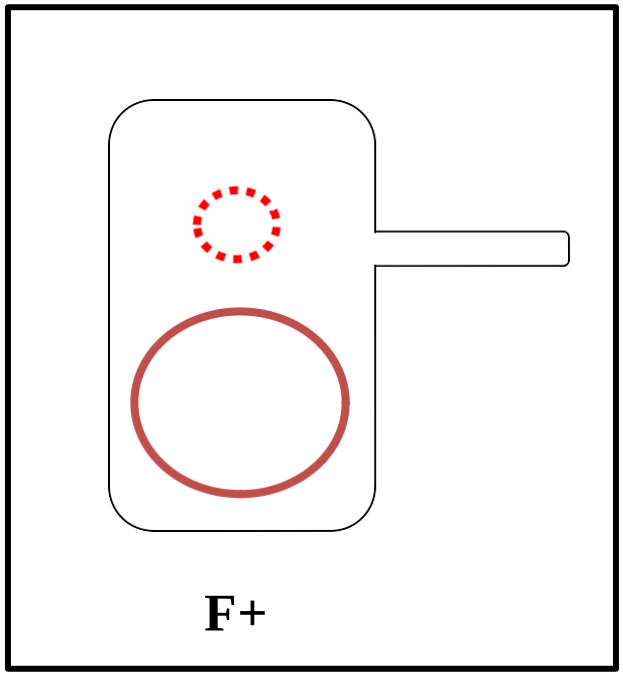
Conjugation



Conjugation

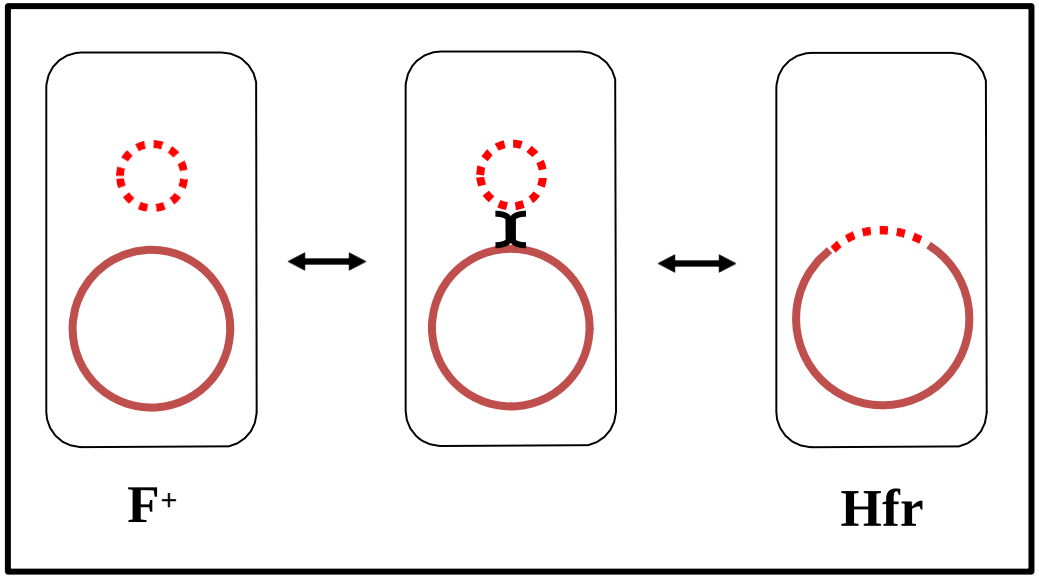
- As other recombination mechanism 2 cells participate in conjugation. The donor must have F-plasmid or F-factor (fertility), and called F + cell. Since this factor is not present in the recipient cell, it is referred to as F- cell.
- During conjugation the F-factor is transferred to the recipient cell 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

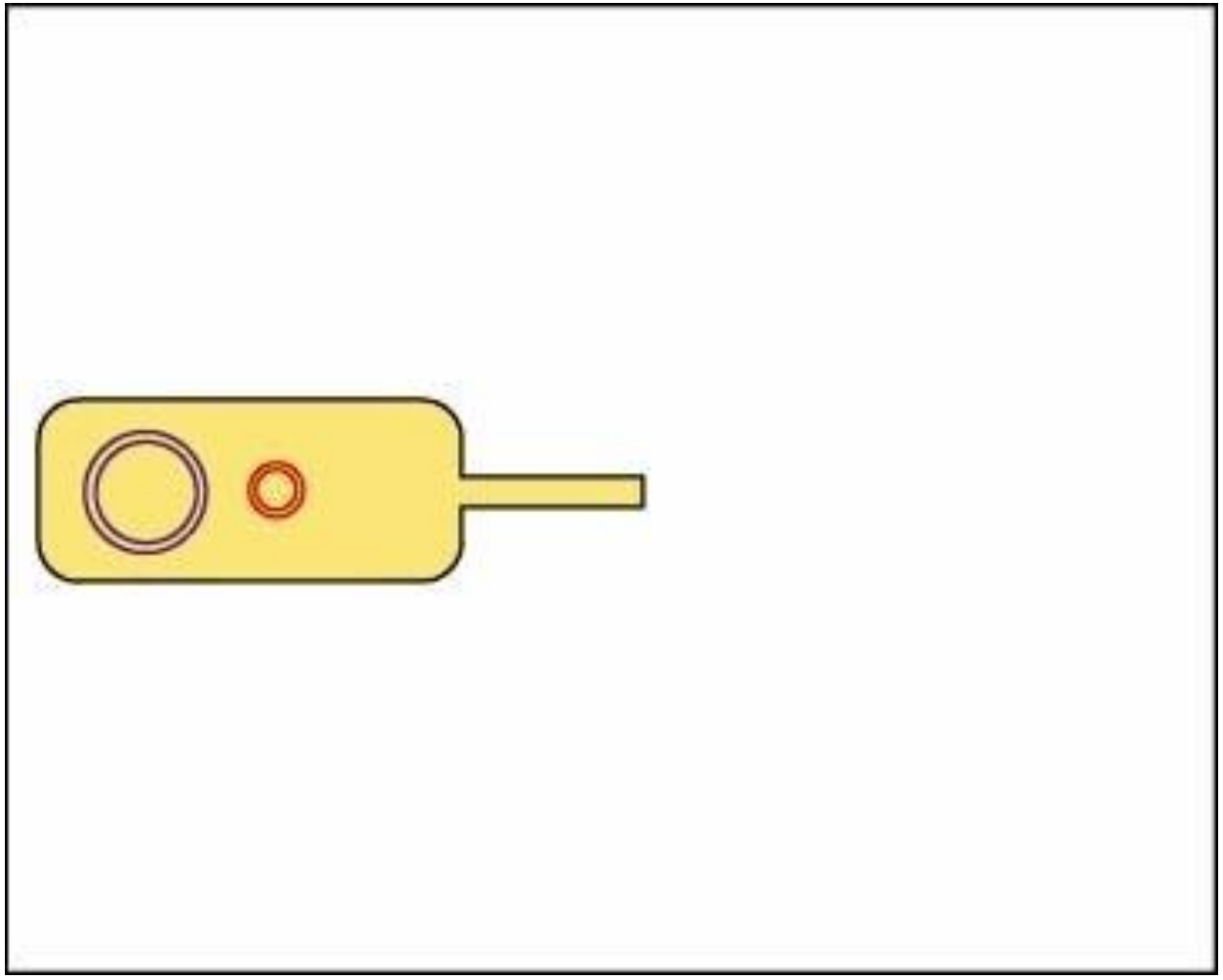
If F-plasmid integrates to cell chromosome it forms Hfr-cell (*high frequency of*). They are able to transfer chromosomal genes to recipient cells with high frequency



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



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***.