# Practical lesson 6
# Cultivation of viruses Rickettsia and Chlamydia. Bacteriophages and their applications. Ecology of microorganisms. Microflora of environment and human organism. Microflora of pharmaceutical raw materials and medicinal preparations. Genetics of microorganisms

**Viruses, Rickettsia and Chlamydiae – obligate intracellular parasites**

Viruses, Rickettsia and Chlamydiae are obligate intracellular parasites and not cultivated in artificial media

Multiplication of Rickettsia occurs inside the host cell (nucleus and cytoplasma) by binary fission

Multiplication of Chlamydiae occurs inside the host cell via complex development cycle

Virus reproduction occurs by special way - replication.

Viruses – reproduction

Viruses after entering the organism can not multiply in all cells – they infect only cells sensitive for particular virus.

Mutual interaction between virus and sensitive cell occurs in several stages

Virion attachment

Penetration of virion inside the host cell (*endocytosis– viropexis*, *fusion of cell membrane and viral envelope*)

Virion “uncoating”, disintegration or deproteinsation

Replication of viral nucleic acids and synthesis of viral proteins

Virion formation

Release of viruses from the cell (*lysis of host cell, «budding»*)

Types of virus-host interaction

Productive infection - reproduction

Abortive infection– noncomplete reproduction

Integrative infection– integration (virogeny)

---

Main principles of viral cultivation

Organism of laboratory animals

Embryonated eggs

Cell (tissue) culture

Cultivation of viruses in laboratory animals organism

Virological investigations commonly involves newborn laboratory animals (white mice, rats, monkeys, mountain mice etc.)

Infection routes of laboratory animals (subcutaneous, intramuscular, intravenous, intranasal, intraperitoneal etc.) are selected in accordance with virus tropism.

Currently application of this method is limited due to inability of human viruses to cause infection in animals, their contamination by microorganisms, ethical and economic issues.

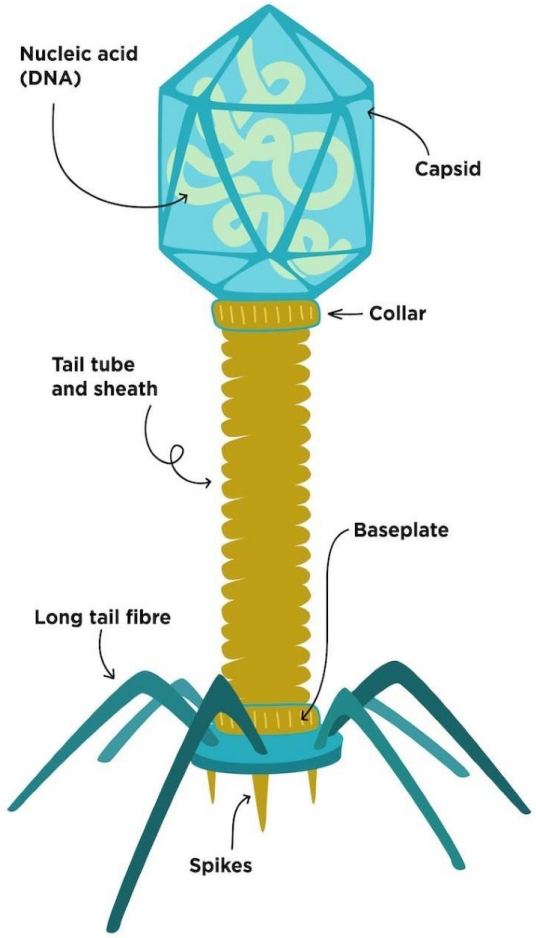
***Bacteriophages***

Reproduce in bacteria and other microorganisms and in special conditions cause their lysis. First was observed in 1917 F.D'Erell when he detected lysis of pathogen obtained from patient with dysentery by filtrate obtained from stool specimen of the same patient. D'Erell concluded that factor causing the lysis is a virus which can pass through bacterial filterHe called this virus as bacteriophage(«eating bacteria»), and phenomenon - as baceriophagy . Phage sizes are similar to other viruses and vary between 20- 800 nm. They have thread, cube and spermatozoid like morphology. E.coli phages have been (T phages) studyed well. T (type) group phages are represented by 7 members, 4 of which single (T1, T3, T5, T7) and paired 3 (T2, T4, T6). Paired T phages, especially T2 have complex structure. Due to character of interaction with bacterial cell phages divided to virulent and temperate one.

Virulent phages enter and reproduce in bacterial cell causing its death – lysis. It is represented with loss of turbidity of microorganism broth culture -phage lusate. In solid nutrition media they visible by eyes zones of lysis – phage negative colonies.

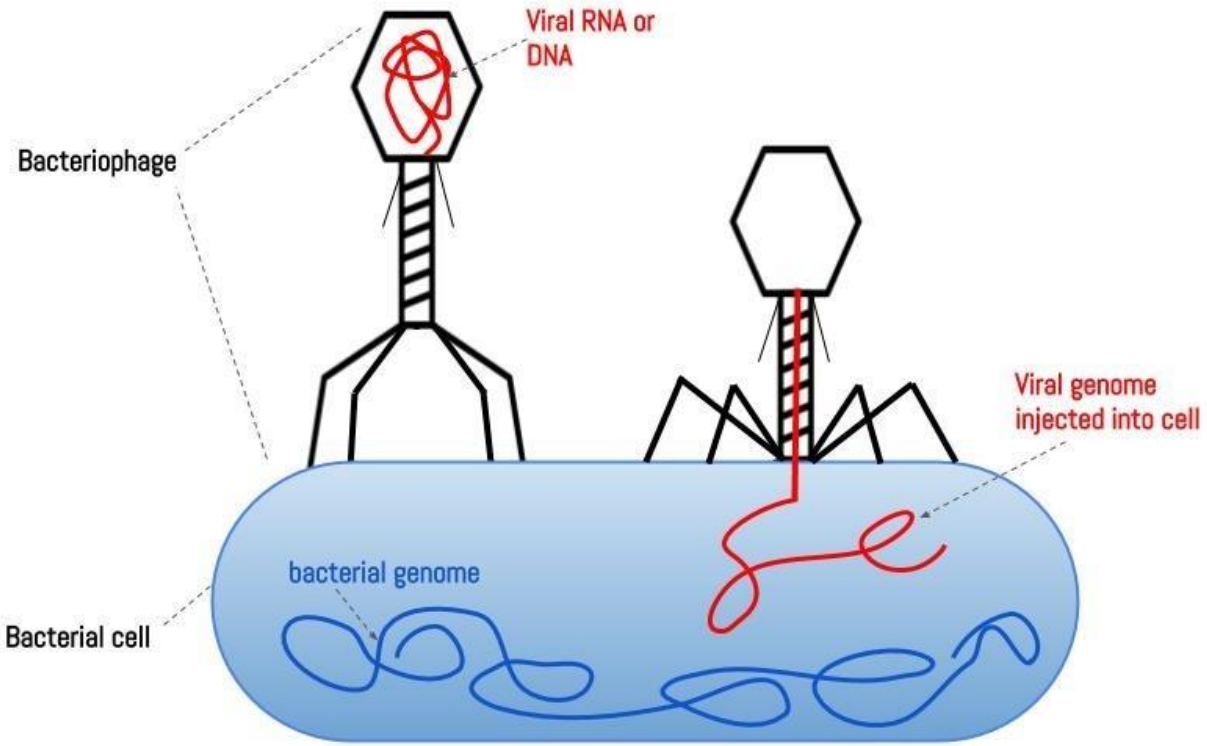
Interaction of virulent phage with bacterial cell: 1. Adsorption of phage to bacterial cell 2. Entrance of phage nucleic acid inside the bacteria 3. Reproduction of phage nucleic acid and protein synthesis 4. Assembly of phage 5. Release of phage from the cell

# BACTERIOPHAGE



After entering the bacterial cell nucleic acid of temperate phage integrate with bacterial cell chromosome. It does not cause lysis of bacterial cell. Nucleic acid of phage connected to chromosome is called prophage. Symbiosis of bacterial cell with phage is called lysogeny while bacteria is called lysogenic bacteria. Prophage of lysogenic bacteria is able to disintegrate from chromosome and become virulent phage. At this circumstance phage causes lysis of bacteria. The process of conversion prophage to virulent is triggered by various factors, especially by radioactive rays.

During lysogeny with defective phage possessing genes responsible for some features lysogenic bacteria obtain new features. Defective phages temperate phage wich are unable to carry out complete infectious cycle. Using this way bacteria can obtain ability to produce toxins, new antigens, morphological features, etc. It is called phage conversion or lysogenic conversion. They are used in genetic engineering as transductive phages.


Viral RNA or
DNA
Bacteriophage
Viral genome
injected into cell
bacterial genome
Bacterial cell

***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.

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. 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.

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

**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 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.

***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. 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.


*Microbial flora of soil:* Various pathogenic and opportunistic pathogenic microorganism are excreted in the environment by human and animals. The sanitary indicator microorganisms of soil are *Escherichia coli* and *Clostridium perfringens*.

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.

***Microflora of water***

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

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.

***Microflora of air***

• 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 m3 air;

- The number of hemolytic streptococci and Staphylococcus aureus in 1 m3 air;

- The number of pathogenic and opportunistic bacteria 1 m3 in 1 m3 air.

***Microflora of pharmaceutical raw materials and medicinal preparations.***

While there are no publications about the use of medicines for the treatment of microorganisms and the possibility of infection.Orally taken orally administered contaminant products after 1963 caused some infections. It was understood that the drugs could also be a source of infection.Oral medications include food type infections-Salmonella, eye ointments containing P. aeruginosa, eye drops are common eye infections. In the past - when the pharmacist prepared the medicine according to the patient's prescription and consumed it in a short time. Today - the drug is being prepared in factories and used by a large patient population after a long time in the factory.

---

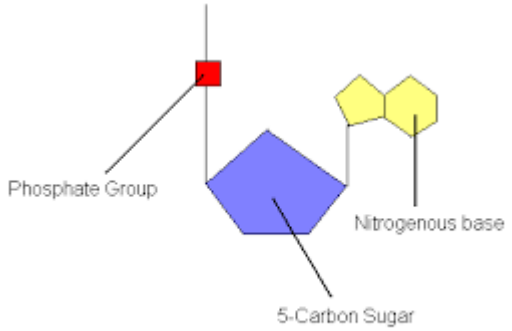
Standard, set of rules for quality production = GMP (Good Manufacturing Practice): reduce the risk of error in production to a minimum,concept that provides quality production suitable for its intended use. First introduced in 1963 by the Food and Drug Administration (FDA) in the United States. It was accepted and published by the World Health Organization (WHO) in 1968. In 1984, practiced in our country as a compulsory drug producer. The rules governing the minimum requirements of the methods, installations and controls applied to the production, packaging and presentation of a product (medicine). The aim is; it is safe to use the drug, and it ensures that it carries the desired purity and quality.

**A pharmaceutical preparation;**

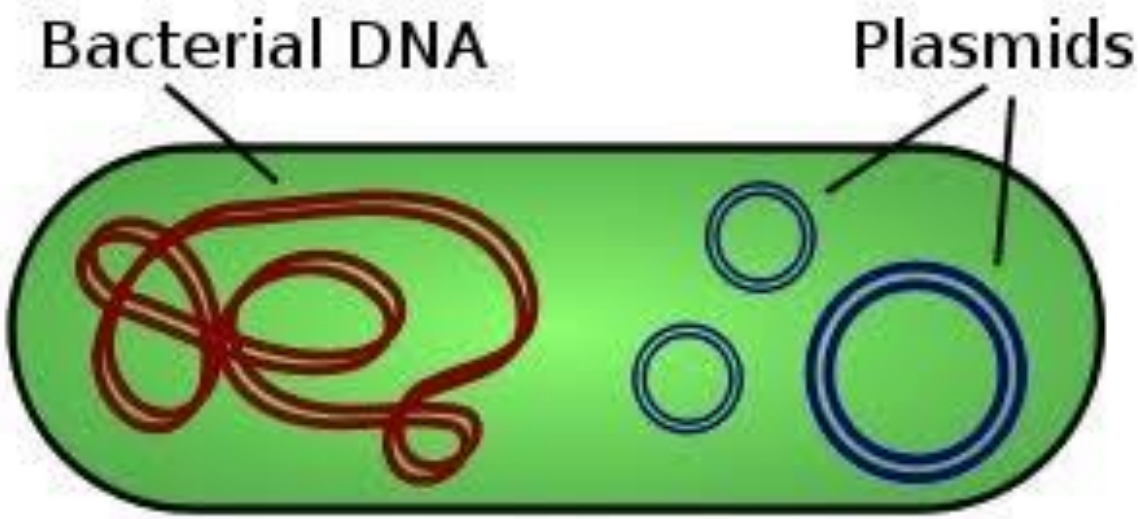
Contains pathogenic or potentially pathogenic microorganisms.Possession of toxic metabolic residues of microorganism. In the case of obvious and obvious physical and chemical changes, the preparation is regarded as completely degraded in terms of microbiology.Contamination is the activation of the active substances in the drug and may lead to some. Types of microorganisms contained in a drug that is contamine; Air, water, human, animal and vegetal fluoride.Aeropers are the dominant microorganisms. The majority, except Bacillus anthracis, are saprophytic bacteria. Sports forms are particularly resistant to heat and antimicrobial agents. Gram (-) basil is another group of bacteria that can be found in contaminating prep. E. coli, Klebsiella, Enterobacter, Hafnia, Serratia, Citrobacter, Salmonella, Proteus and Pseudomonas group microorganisms. Most of these microorganisms are opaque (opportunistic, potential pathogen). These bacteria, which are found in human and animal normal microflora, gain pathogenicity . Yeast and Mold (Aspergillus, Penicillium, Saccharomyces) Are among the microorganisms encountered in medicines and most of them are heat resistant.

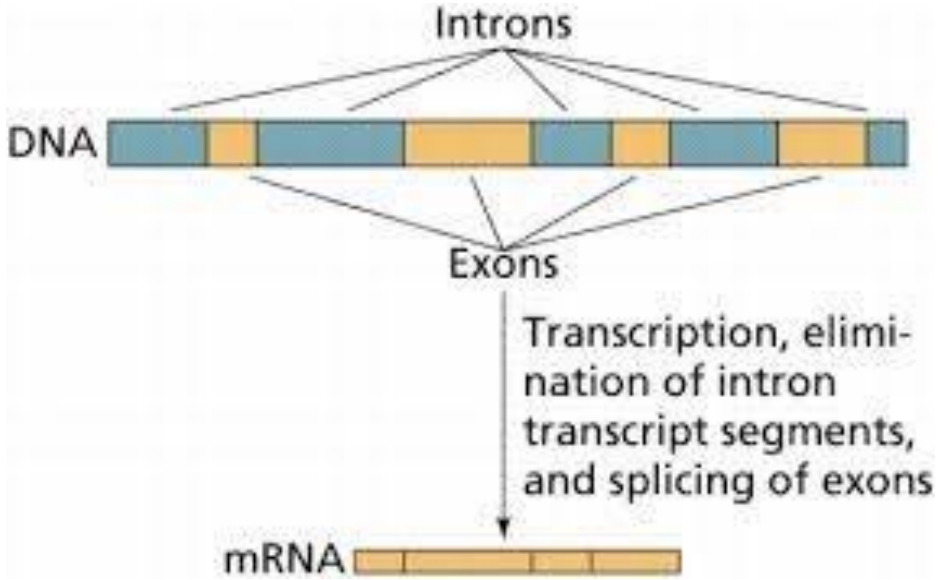
**Genetics of microorganisms.**

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.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 5x106 nucleotide pairs (if compare human genome consists of 2,9x109 nucleotide pairs). The length of the chromosome of a bacterial cell (Escherichia coli) is about 1 mm. 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 typical nucleotide


Bacterial DNA
Plasmids


Introns
DNA
Exons
Transcription, elimi-
nation of intron
transcript segments,
and splicing of exons
mRNA

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

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 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 Sfactor provides a specific connection with the promoter.  Operator is area for repressor protein binding and located between promoter and structural genes.

***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.***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 mutantstrains.

---

*Mutations* :

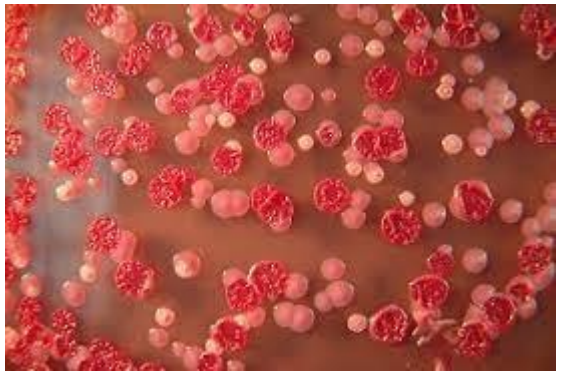
**Spontaneous mutations** - reversible

**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)

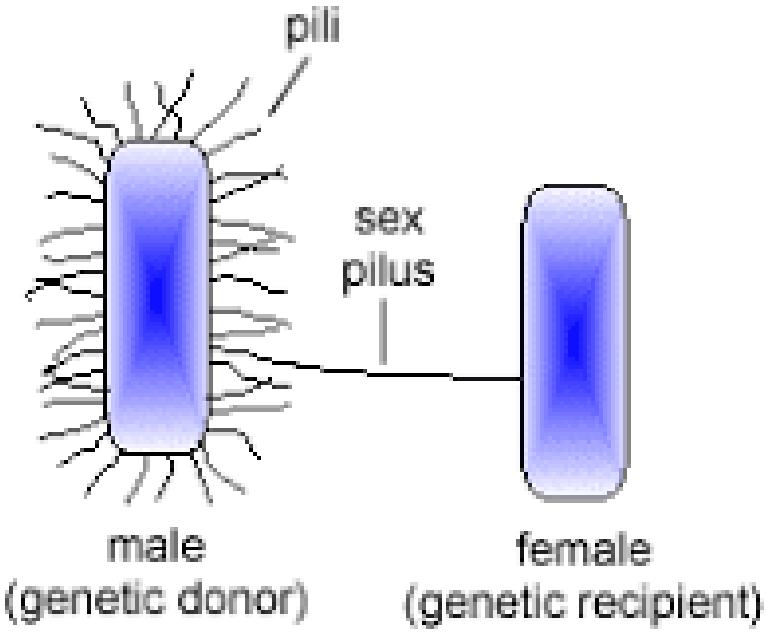
**According to phenotypic results**- neutral mutations, conditional lethal, lethal mutations



| 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 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.*




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

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

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

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.

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.

***Genetics of viruses.***

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

-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.