



AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 10.

Respiration and multiplication of microorganisms.

Cultivation of aerobic and anaerobic bacteria.

**Bacteriological method. Isolation of the pure culture of
aerobic and anaerobic bacteria (I day)**

FACULTY: General Medicine

SUBJECT: Medical microbiology - 1

Discussed questions:

- Bacterial respiration: brodil (fermentation) and oxidative metabolism.
- The respiration types: obligate aerobes, microaerophiles, facultative anaerobes, obligate anaerobes, aerotolerants, capnophiles.
- Reproductive characteristics of prokaryotes: growth and multiplication.
- Phases of bacterial multiplication in nutrient medium.
- The inoculation methods (cultivation) of aerobes and anaerobes in nutrient media.
- Cultural method: essence and importance, cultivation conditions (temperature, aeration and duration).
- The getting of pure cultures methods of aerobic microorganisms (Drigalsky's technique, streak plate method, Shukevich methods, application of elective nutrient media).
- Cultivation of anaerobic microorganisms and isolation methods of pure culture .
- Multiplication of eukaryotes (fungi and protozoas)

Purpose of the lesson:

- To inform students about the main types of respiration in microorganisms, reproduction and development of various microorganisms. To acquaint students with the essence and importance of the bacteriological examination method. To teach them the methods of obtaining pure culture of aerobes and anaerobes.

Energy metabolism (biological oxidation)

- There are two types of biological oxidation (energy metabolism), depending on whether they are oxygen-free and oxygen-free:
- fermentation metabolism
- oxidative metabolism

Respiration of microorganisms

- ◎ The term respiration is sometimes equated with biological oxidation. This is due to the nature of the respiratory process. The process of respiration consists of complex biochemical reactions that take place in the body, as a result of which the necessary energy for life is released
- ◎ It consists of many oxidation-reduction reactions, in which the oxidizing substances give off electrons, and the reduced ones receive electrons
- ◎ However, oxidation-reduction processes in microorganisms can take place both in the presence of oxygen and without oxygen

Respiratory types of microorganisms

Microorganisms are divided into 3 main groups according to the type of respiration:

obligate aerobes

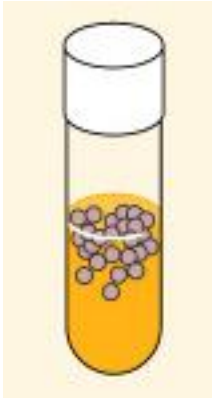
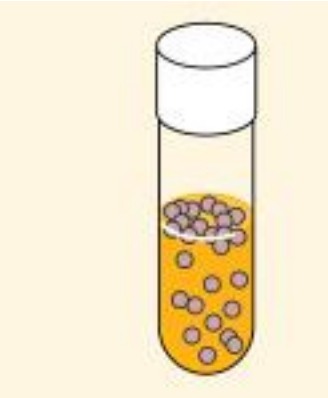
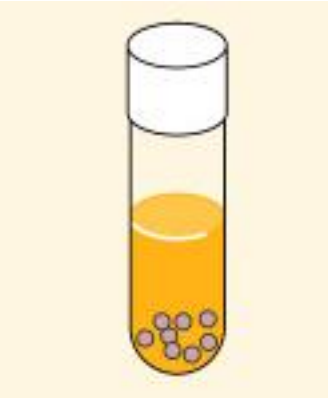
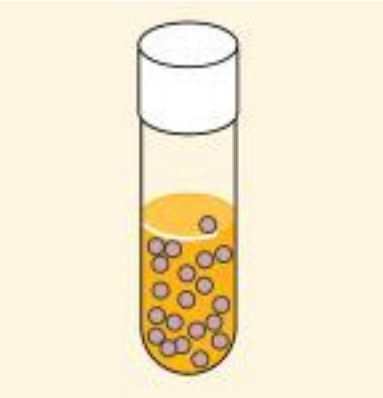
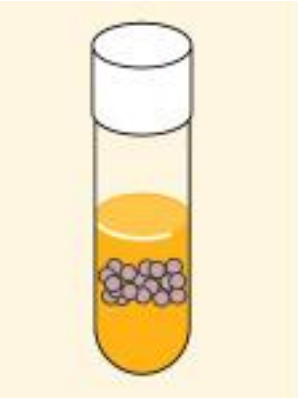
- *microaerophiles* - require 5-10% oxygen
- *Capnophiles* - need excess carbon dioxide

obligate anaerobes

- *severe anaerobes* - molecular oxygen has a destructive effect
- *aerotolerant anaerobes* - can live in an oxygenated atmosphere

facultative anaerobes (can live in both oxygen and non-oxygen environments)

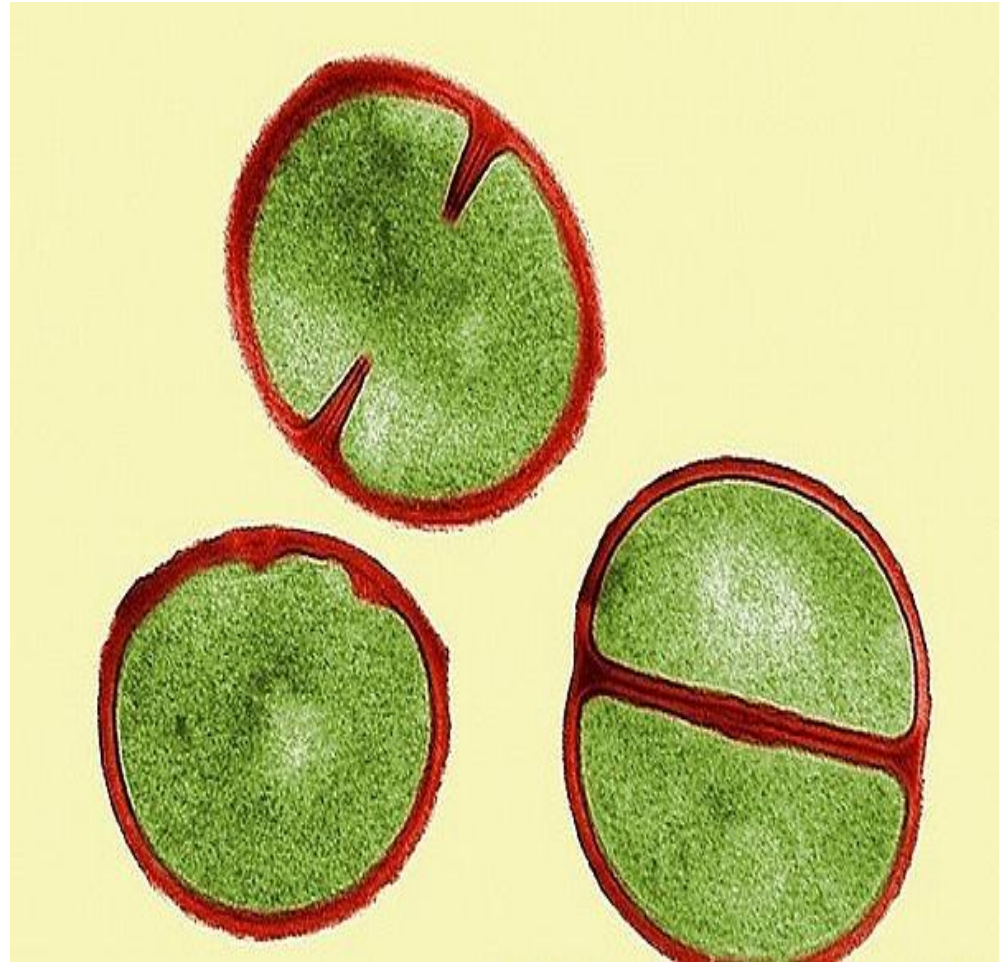
The nature of the development of microorganisms of different respiratory types in broth medium

obligate aerobe	facultative anaerobe	obligate anaerobe	aerotolerant anaerobe	microaerophile
				

Growth and reproduction of microorganisms

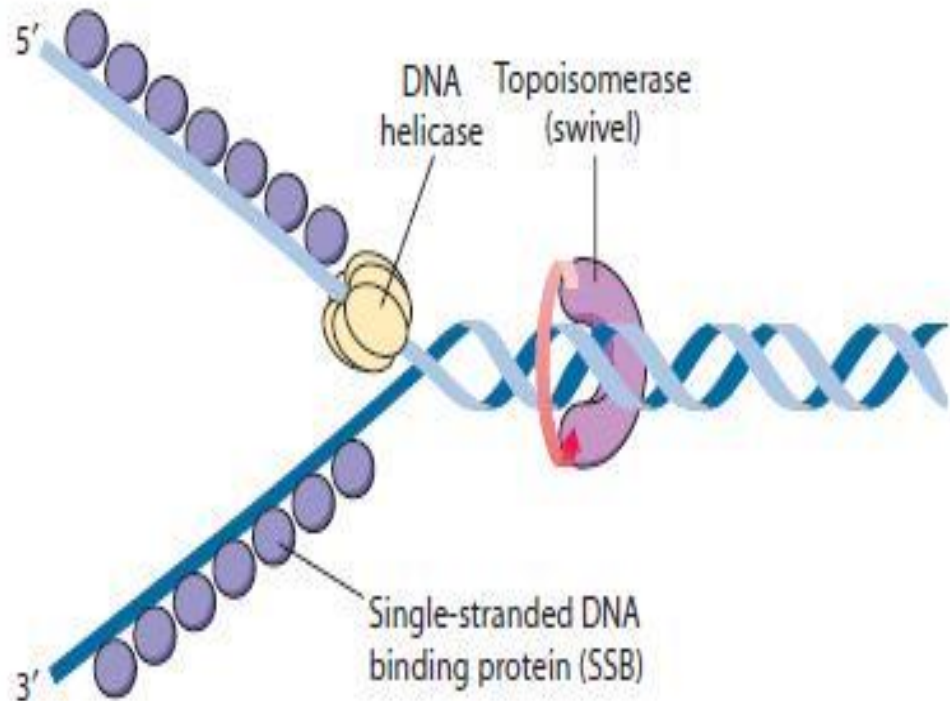
Growth is the growth of the size of each cell.

Reproduction - binary fission in bacteria - takes place through division. Through the mesosomes, a transverse partition is formed. Transversely, in coccidial bacteria, cocci are divided into different planes. Daughter cells are called isomorphic if they are the same size, and heteromorphic if they are different.



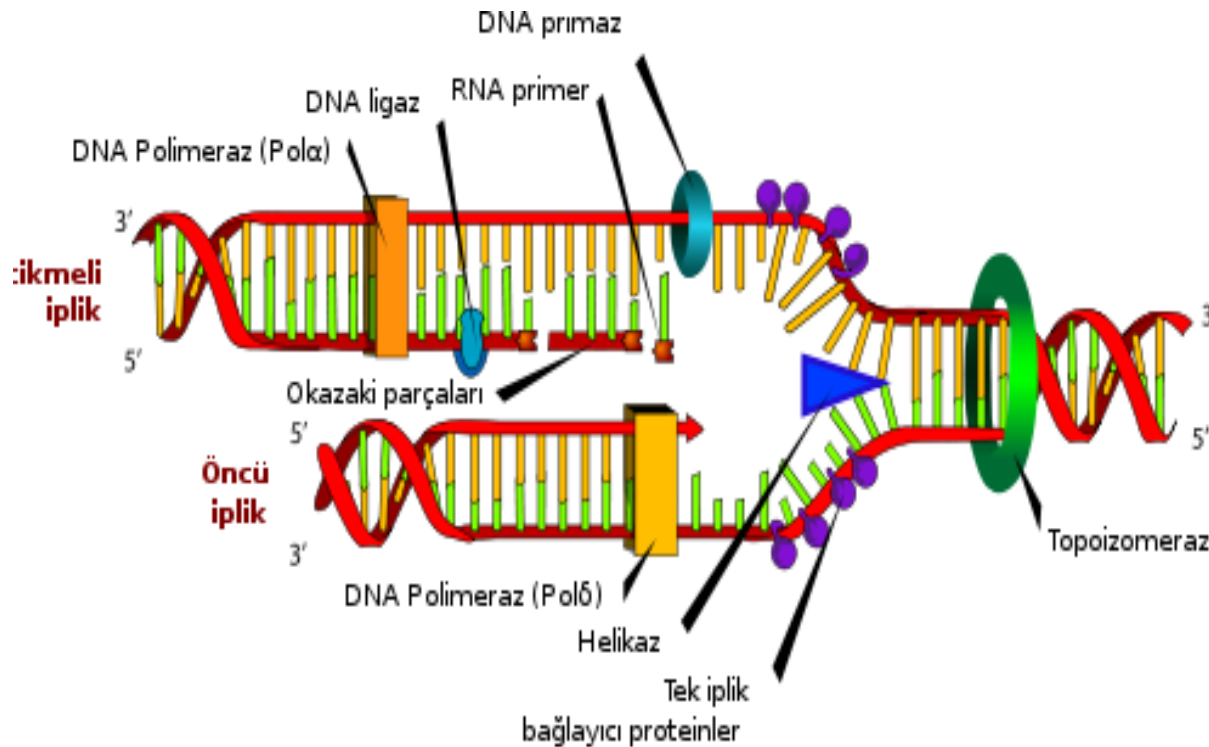
Bacterial cell division

Reproduction of Bacteria



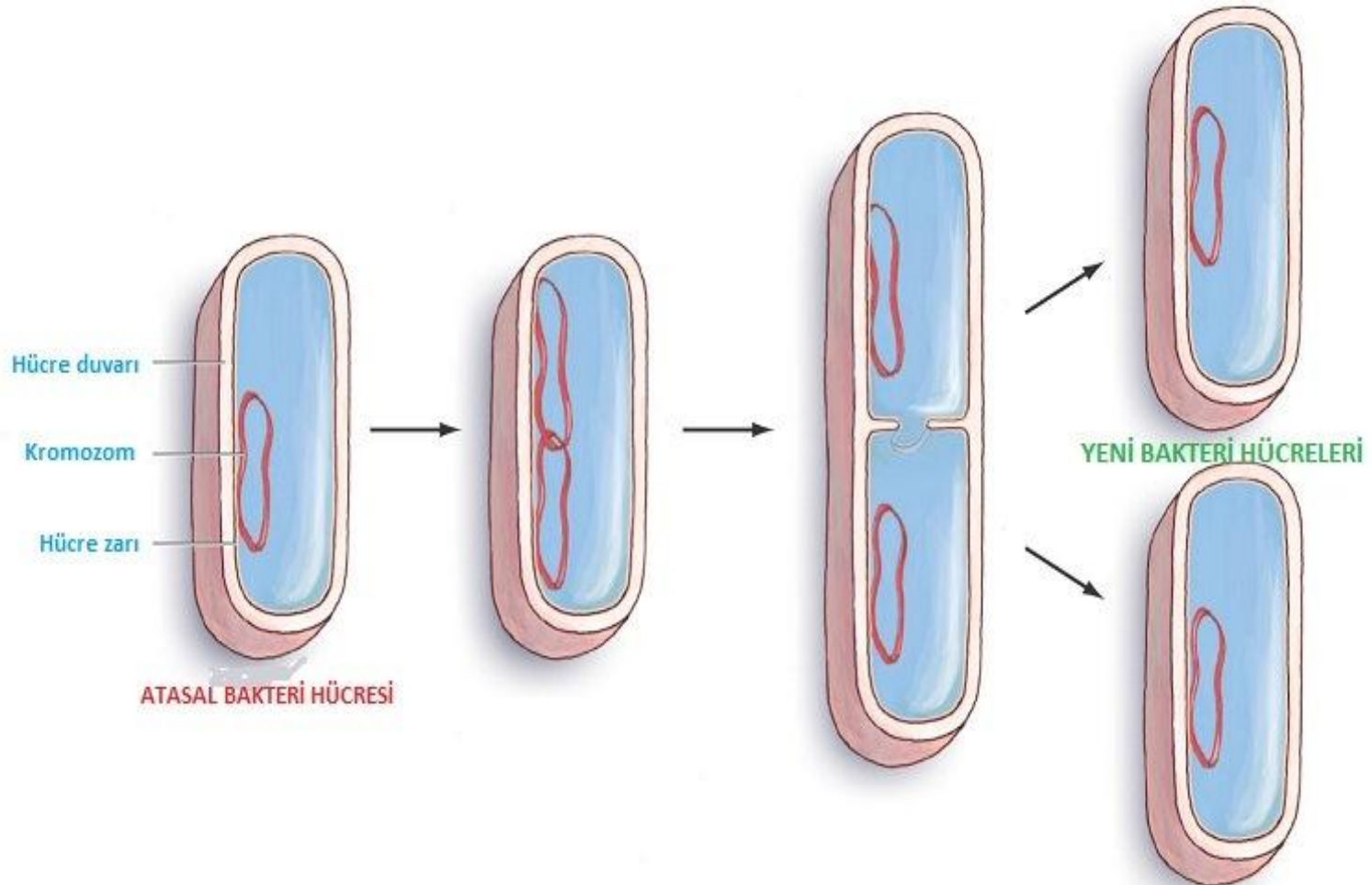
The process of DNA duplication begins with the separation of its double strand by the enzyme helicase.

Reproduction of Bacteria



A new DNA strand is synthesized on each strand formed by the enzyme DNA polymerase according to the principle of complementarity.

Reproduction of Bacteria



Generation period

- Bacteria multiply very rapidly. The concept of generation time is used to estimate the rate of reproduction. This period represents the time required for the bacterial cell to double. The generation time is different for each type of bacteria.
- Bacteria, and in general all microorganisms, multiply more rapidly under ***optimal conditions***.
- Most bacteria divide every ***15-30 minutes***. Some bacteria, such as *Mycobacterium tuberculosis*, divide relatively late (every 20-24 hours).

When a bacterial cell divides and multiplies, their number increases geometrically

Generation Number	Number of Cells	Log₁₀ of Number of Cells
0	1	0
5 (2^5) =	32	1.51
10 (2^{10}) =	1,024	3.01
15 (2^{15}) =	32,768	4.52
16 (2^{16}) =	65,536	4.82
17 (2^{17}) =	131,072	5.12
18 (2^{18}) =	262,144	5.42
19 (2^{19}) =	524,288	5.72
20 (2^{20}) =	1,048,576	6.02

(b)

Reproduction of Bacteria

- As the bacterial cell multiplies by dividing in two, their number increases geometrically in the culture: $2^0 - 2^1 - 2^2 - 2^3 \dots 2^n$, so after dividing by n , the number of bacteria in a bacterial family will be 2^n .

As they grow under such conditions, the bacteria multiply until the components needed for their development reach a minimum, after which their proliferation stops.

If no nutrients are added during this period and the metabolic products are not removed, a cycle or static culture is obtained.

Phases of bacterial reproduction in period culture

- Periodic culture behaves as if it were a multicellular organism.
- Bacterial growth in a periodic culture is subject to a certain pattern and consists of several phases — the initial phase, the exponential phase (or logarithmic phase), the stationary phase, and the death phase. The graphical representation of these phases is called the development curve.
- The graphical representation of these phases is called the ***development curve***

Phases of reproduction

- ◎ **Lag phase.** This phase follows the inoculation of bacteria into the nutrient medium. During this period, their exchange processes intensify, increase in size, and finally begin to divide.
- ◎ In the **exponential or logarithmic phase**, bacterial cells multiply rapidly. During this phase, bacterial cells have the highest biochemical and biological activity.
- ◎ In the **stationary phase**, the rate of bacterial growth begins to decrease due to the depletion of nutrients and the accumulation of toxic metabolic products.
- ◎ During the **death phase**, the number of viable cells begins to decrease progressively, they are destroyed. This is mainly due to the depletion of nutrients in the environment and the accumulation of toxic metabolic products.

Bacterial Growth Cycle

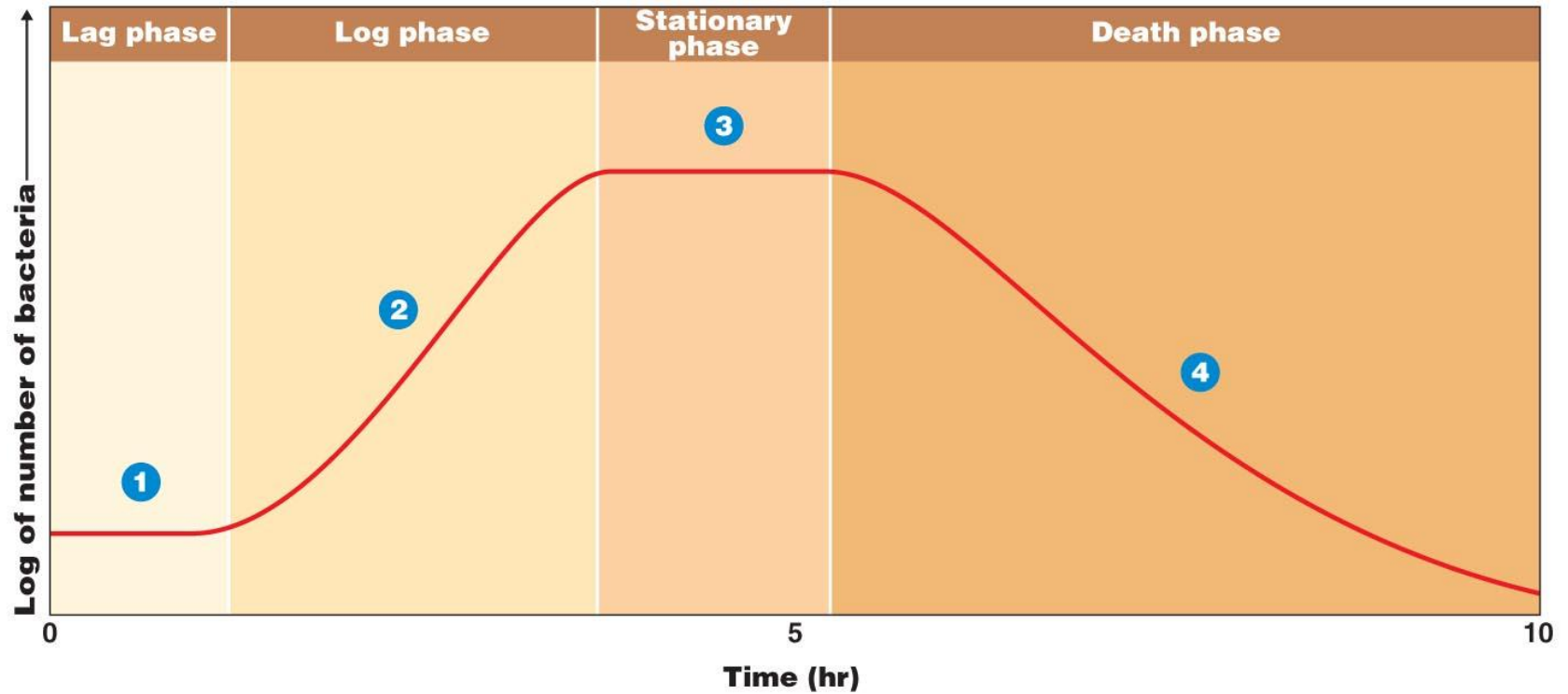


Figure 6.15

Reproduction of other prokaryotes

- ***Reproduction of Spirochetes and Rickettsiae***, like other bacteria, goes through a simple division. Rickettsiae multiply only within the host cell (nucleus or cytoplasm).
- ***Reproduction of Chlamydia*** occurs with a complex developmental cycle within the host cells.
- ***Reproduction of Mycoplasma***. The main reproductive forms of ***Mycoplasma*** are spherical or ovoid-shaped elementary bodies. In the process of development, spherical bodies are formed from the derivatives formed from them. Thus, chains of spherical bodies are formed. Then, as a result of fragmentation of derivatives, elementary bodies are formed.
- ***Reproduction of Actinomycetes*** occurs through the fragmentation of mycelium, or spores formed in aerial mycelium.

Principles of cultivation of microorganisms

- With the exception of obligate parasites (rickettsiae, chlamydia and viruses), it is possible to artificially cultivate all microorganisms, ie to ***obtain their culture in the laboratory.***
- By cultivation, it is possible to obtain a culture of microorganisms and thus study their chemical composition, morphological and biological properties, as well as to develop a number of biological preparations and vaccines of microbial origin.

Cultivation of microorganisms

- ◎ In order to obtain cultures of pathogens and study their characteristics in microbiological laboratories, it is necessary to cultivate them.
- ◎ In order to cultivate microorganisms, the material being first examined is inoculated (cultured) into appropriate nutrient media.
- ◎ Inoculation is performed in strict accordance with the rules of antisepsis. In some cases, laminar boxes are used.
- ◎ ***Laminar flow box (biosafety cabinet)*** is a laboratory cabinet used for working in sterile conditions, with lighting, ultraviolet lamp and sterile air supply system.

Laminar flow box



Cultural method, essence and significance

- The essence of the culture (bacteriological) method is to obtain a pure culture of the pathogenic bacteria from the examined material and to identify them according to their morphological, tinctorial, cultural, biochemical, toxigenic and antigenic properties.

Culture and pure culture

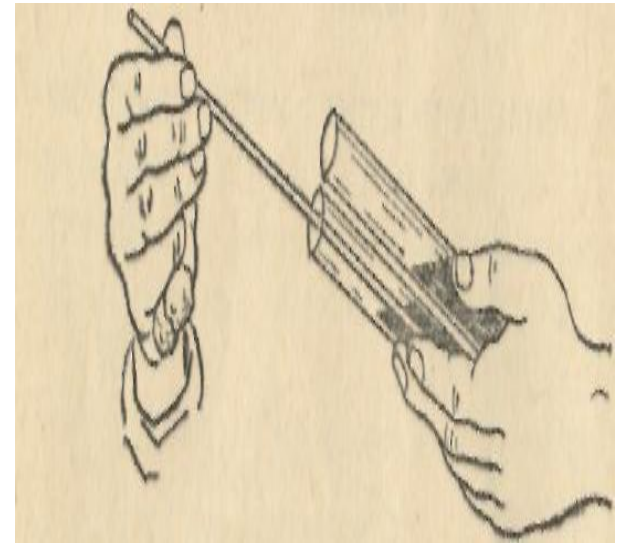
- ◎ The population of microorganisms cultivated in nutrient media is called ***culture***.
- ◎ In order to study the characteristics of microorganisms, to determine their systematic position, it is necessary to isolate different types of microbes, obtain a pure culture and identify it. However, a culture consisting of one type of microorganism is called a ***pure culture***

Inoculation on nutrient media (cultivation)

- The first step in cultivating bacteria is to inoculate them into the culture medium.
- Depending on their purpose, they inoculate bacteria in different nutrient media in different ways
- The material or microbial culture is often inoculated with a *bacterial loop*.

Inoculation to nutrient broth

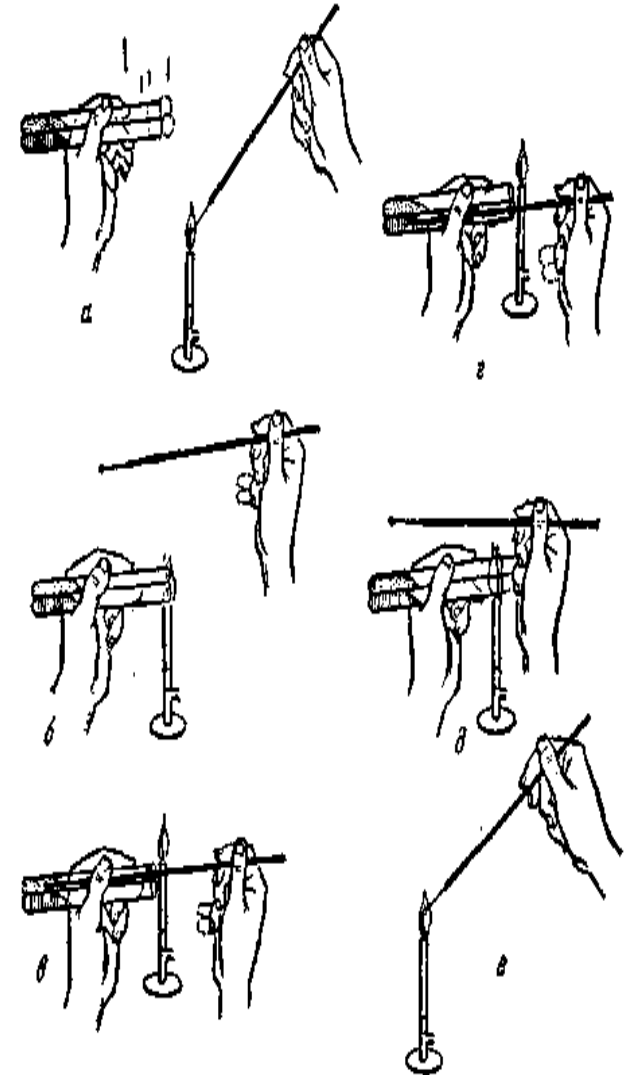
To inoculate the collected material or bacterial culture into the nutrient broth from another tube, open the mouth of the tube containing the sterile nutrient broth as described above, insert the loop into the tube without touching the walls of the tube, and apply the material to the tube wall and solve. The tube is placed vertically on the thermostat.



Inoculation on the surface of oblique agar in a tube

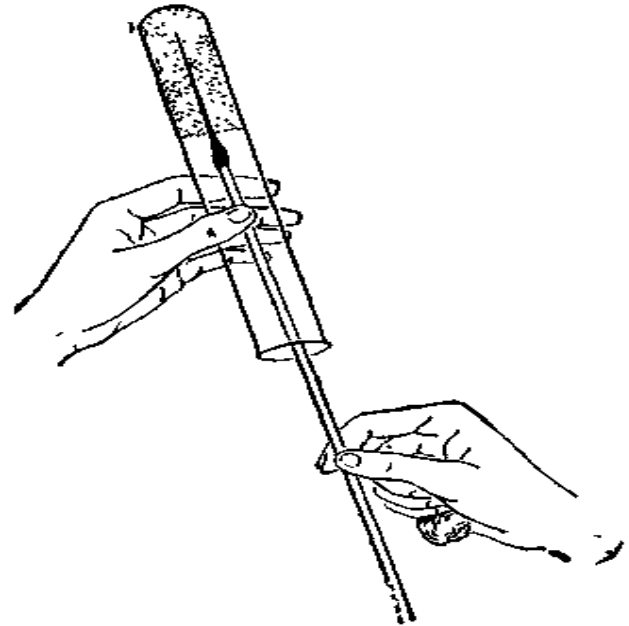
To transfer the bacterial culture from the tube to the sterile oblique agar in the other tube, both tubes are held in the left hand like a pen, with the oblique surface of each one facing upwards.

The right-handed bacteriological loop is passed through the flame. Then, with the thumb and forefinger of that hand, open the lid of both tubes. It is taken from the microbial culture with an incandescent loop and spread in a zigzag pattern on the surface of the nutrient medium in a clean tube. Incubate for 18-20 hours at 37 °C



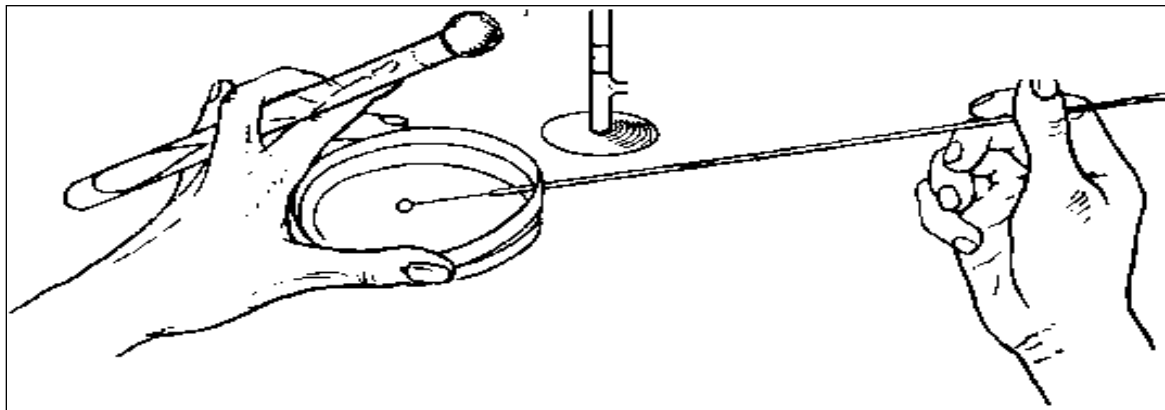
Inoculation with a bacteriological needle

The tubes, which contain an agar column, are held with the left hand, provided that the bottom is up. By opening the lid, the mouth of the tube is passed through the flame. The bacteriological needle is passed through a flame, placed in a container containing the material to be examined, cooled there, then immersed in the material and inserted into the agar column. The lid is closed by passing the flame and placed in the thermostat.



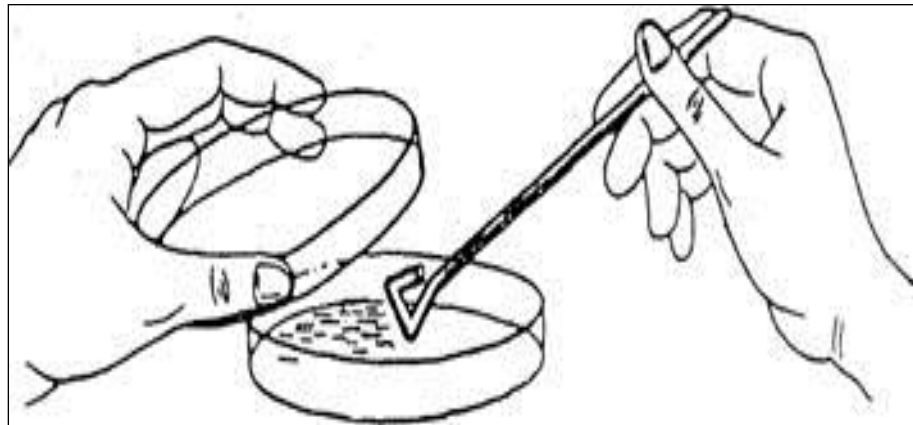
Inoculation with a loop on the surface of the solid nutrient medium in the Petri plate

- To inoculate the collected material or bacterial culture into a petri containing a solid nutrient medium, the container petri plate only be opened after the material has been taken with the bacteriological loop! The petri plate with the nutrient medium should be in the upward position on the table. Hold the lid of the petri with the fingertips of the left hand and lift it with a light motion, so that the lid of the petri plate is half open. The loop with material is carefully placed on the agar near the edge of the petri plate and spread in parallel lines.

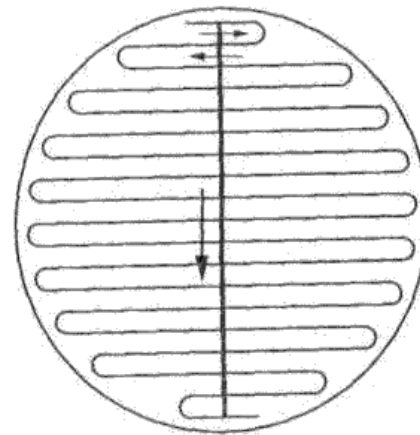
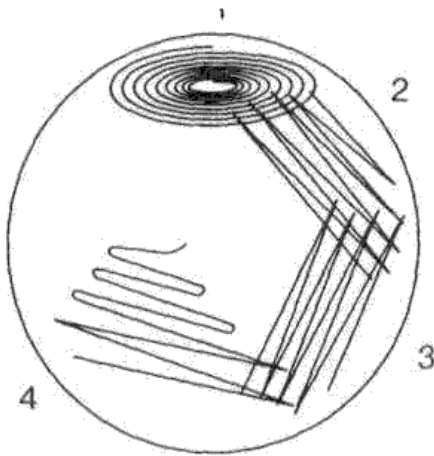


Inoculation with a spatula on the surface of the solid nutrient medium in the petri dish (plate)

- Inoculation into the petri plate with a solid nutrient medium can also be done with a spatula. It is often used to obtain growth that covers the entire surface of the nutrient medium. Carefully place the material to be inoculated on the agar surface of the plate with a loop or pipette and spread it over the entire surface of the nutrient medium with a sterile spatula.



- Depending on the purpose of cultivation, different inoculations can be made on the surface of the solid nutrient medium in the petri.
- For example, the 4-sector inoculation method is used to obtain pure cultures (isolated colonies).
- Bacteriological examination of urine to assess bacteriuria (to determine the number of bacteria) inoculation is carried out by a special method



Incubation

◎ Samples are incubated in a thermostat at a certain temperature (usually 37 ° C) for the required time (usually 1-2 days) for the growth of microorganisms after inoculation.



Cultivation conditions

- **Optimal conditions** must be created for the cultivation of microorganisms in nutrient media.

These conditions are primarily provided by the optimal temperature cultivation period and cultivation atmosphere.

Cultivation temperature

- ◎ Depending on the cultivation temperature, all microorganisms are divided into three groups: psychrophiles, mesophiles and thermophiles
- ◎ The optimum temperature for psychrophilic bacteria is 6-20 °C, for mesophylls 34-37 °C
Thermophiles require higher temperatures. Some members of this group can even grow at 70-75 °C
- ◎ Most bacteria that are pathogenic to humans are mesophilic microorganisms

Cultivation period

- ◎ The ***cultivation period*** depends on the type of microorganism. During this time, the microorganisms usually form visible cultures.
- ◎ While 18-24 hours of cultivation is sufficient under optimal conditions for most bacteria, this time varies in some microorganisms.
- ◎ For example, the causative agents of whooping cough are cultivated for 2-5 days, and the causative agents of tuberculosis for 3-4 weeks.
- ◎ In the absence of optimal conditions, the cultivation period may be extended

Cultivation atmosphere

- ◎ It is known that oxygen is required for the development of aerobes. Therefore, aerobes grow on the surface of solid nutrient media or in the top layer of liquid media.
- ◎ Microaerophiles are cultured in an atmosphere with a low oxygen concentration (1-5%). For this purpose, CO₂ incubators or "candle chambers" are used.
- ◎ Both aerobic and anaerobic conditions can be used to cultivate facultative anaerobes.
- ◎ Obligate anaerobes are cultivated without oxygen.

Cultivation of anaerobes

- ◎ Special nutrient media are used for this purpose. For anaerobes, the oxidation-reduction potential in the medium is reduced at the expense of various substances - reducing agents. For example, glucose is added as a reductant to the Kitt-Tarotsi medium used to cultivate anaerobes.



Kitt-Tarotsi medium

Cultivation of anaerobes

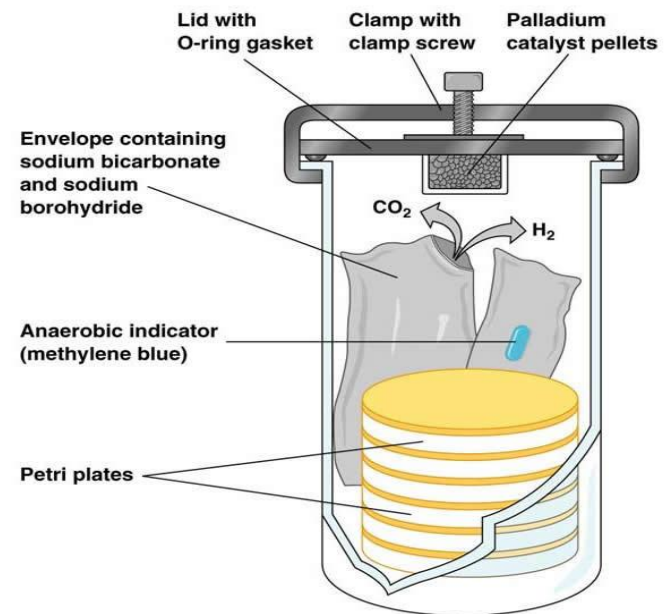
Currently anaerostats are increasingly used to cultivate anaerobes.



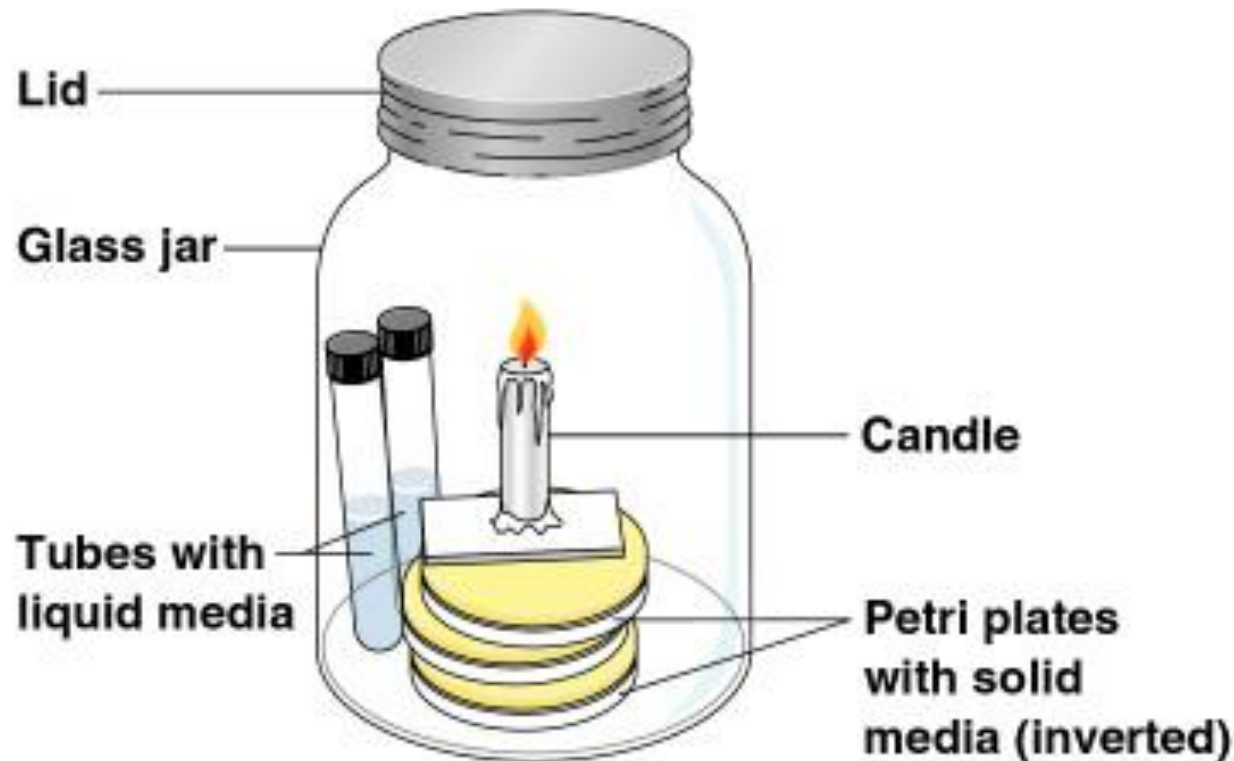
anaerostat

Cultivation of anaerobes

The Gaspak system is a new way to create anaerobic conditions. Gaspak consists of a package containing various oxygen-absorbing substances - citrate turmeric, sodium carbonate, sodium borohydride and a hermetically sealed glass chamber. When water is added, the substances in the package form hydrogen, which reacts with oxygen to form water. Thus, anaerobic conditions are created in the chamber. This method is most commonly used in the cultivation of aerotolerants.



Cultivation of microaerophiles and capnophiles in the "candle chamber"

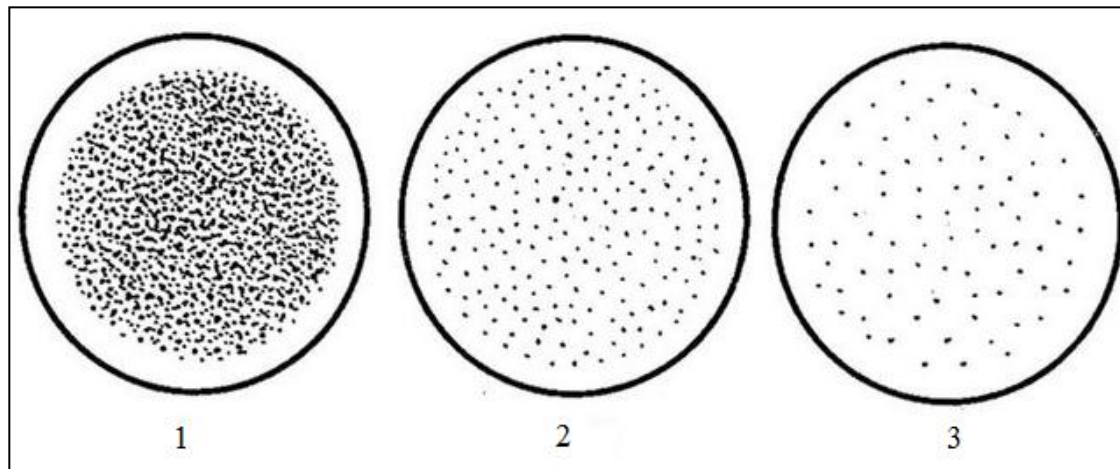


Methods of obtaining pure cultures of aerobic bacteria

- Various methods can be used to obtain pure bacterial cultures.
- Methods based on the principle of mechanical separation of microorganisms inside or on the surface of the nutrient medium are increasingly used. The general principle of these methods is to mechanically separate the materials to be examined at the depth or surface of the nutrient medium to ensure their development in isolated colonies.
- It is believed that a colony develops from a microbial cell. Thus, since the colony of any microbe usually consists of the same type of microbial cells, it can be considered a pure culture.

Method of separation of microbial cells on the surface of solid media (Drigalski method)

- The essence of the method is the sequential spreading of the studied material (inoculum) on agar with a glass spatula or loop in several Petri dishes with nutrient medium. The studied material is inoculated into 3 numbered Petri dishes containing MPA. To do this, add a drop of the test material to the MPA with a loop or a glass paste pipette and spread with a glass spatula. The spatula is removed from the first plate, the mouth is closed and transferred to the second plate without touching anywhere, carefully rubbed on the surface of the nutrient medium, and then successively transferred to the third plate. After incubation, a gradual thinning of microorganisms is observed on the surface of the nutrient medium in the inoculated plates.

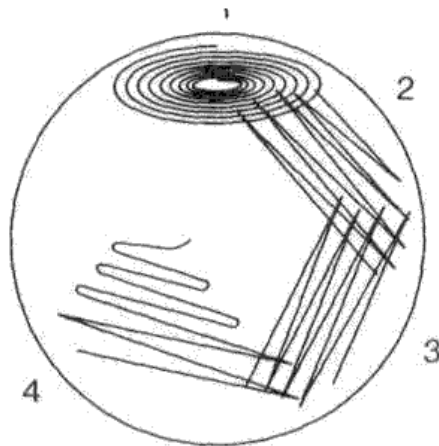


Sector inoculation method

- Currently, the 4-sector inoculation method is widely used to obtain pure culture (isolated colonies). To do this, the material taken through the bacteriological loop is planted with lines parallel to the surface of the solid nutrient medium in the Petri dish. In this case, the nutrient medium in the bowl is theoretically divided into 4 sectors. The loop is initially inoculated into the first sector, after the loop is lit, it is inoculated into the second sector by making several parallel strokes starting from the initial inoculation site, and then to the third and fourth sectors in the same manner.
- After incubation, depending on the number of microorganisms in the primary material, a gradual thinning of microorganisms is observed on the surface of the nutrient medium, and usually in the latter sectors the microorganisms develop in isolated colonies.

Sector inoculation method

- Using the 4-sector inoculation method, it is also possible to obtain approximate information on the number of microorganisms in the source material. Thus, if a certain microorganism develops only in the first sector (++) , if it develops in the first and second sectors (++) , if it develops in the first, second and third sectors (+++) , if it develops in all sectors (+++ is evaluated as +).
- When recording the results of bacteriological examinations, the number of microorganisms is positive (+) for non-measurable materials (e.g, swabs), and for quantifiable materials (e.g, urine, sputum, etc.) as a colony-forming unit (CFU) / ml. is displayed.



Making clones

- ◎ Under a microscope, a microbial cell is taken through the needle of a micromanipulator and incubated in a sterile nutrient medium.
- ◎ A culture that develops from a microbial cell is called a clone.
- ◎ This method is mostly used in genetic research.
- ◎ A clone of a particular microorganism is considered to be its ideal pure culture.

Methods based on the principle of using the biological properties of microorganisms

- Obtaining a pure culture of motile bacteria
- Obtaining a pure culture of spore bacteria
- Obtaining a pure culture using selective nutrient media
- Obtaining a pure culture by infecting susceptible laboratory animals

Methods of obtaining pure cultures of anaerobic bacteria

- ◎ The studied material is inoculated with sectors on the surface of the solid nutrient medium with a bacteriological loop, incubated under anaerobic conditions at 37 ° C for 24-72 hours.
- ◎ After incubation, one of the isolated colonies formed on the surface of the nutrient medium is transferred to the Kitt-Tarotsi medium, or another nutrient medium for anaerobes, and re-incubated to obtain a pure culture of anaerobic bacteria.

Methods of obtaining pure cultures of anaerobic bacteria

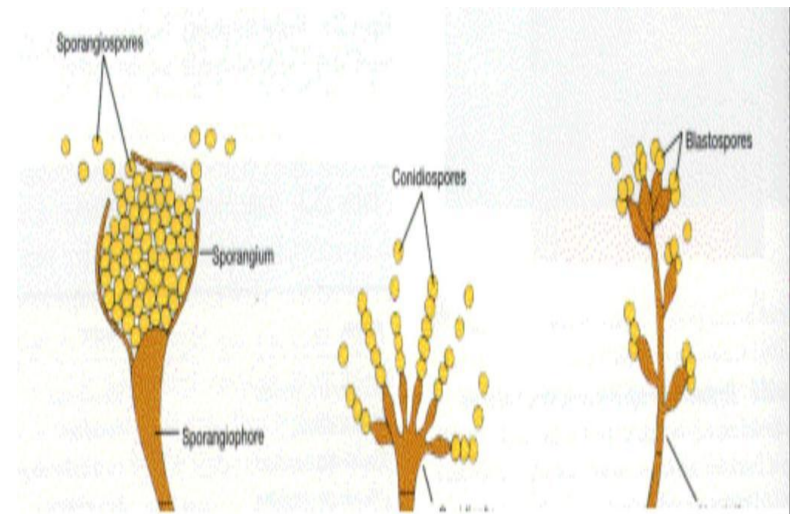
- ◎ **Weinberg method.** A few drops of the test material are injected into a test tube containing 0.9% sodium chloride solution, mixed, and transferred to a test tube containing melted and cooled sugar agar. After mixing, the sugar agar is inoculated sequentially into two more test tubes and rapidly cooled under cold water.
- ◎ After 24-72 hours of incubation, the isolated colonies formed in the depths of the agar are transferred to the Kitt-Tarotsi medium, or another nutrient medium for anaerobes, and re-incubated to obtain a pure culture of anaerobic bacteria..

Thermostat for anaerobic growth



The main reproductive organ of fungi is spores

- If the spore is located inside the mycelium, it is called an endospore
- Endospores are formed within special structures - sporangia. This type of spore formation is characteristic of fungi of the genus *Mucor*
- Spores that form outside the mycelium are called exospores or conidia



endospores **exospores**

Sexual spores	Types
Basidiospores (<i>basidiomycetes</i>)	Cryptococcus
Ascospores (<i>askomycetes</i>) <i>septate hyphae</i>	Histoplasma, Blastomyces, Piedraia hortae, Coccidioides, Candida, Saccharomyces cerevisiae
Zygosporos (<i>zygomycetes</i>) <i>aseptate hyphae</i>	Mucor Rhizopus, Apsidia Pilobolus
Oospores	Don't cause infection in human

Asexual spores	Types
Blastospores	Candida albicans
Arthrospores	Dermatofitlar, Trichosporon beigeli, Coccidioides immitis, Geotrichum candidum
Chlamydospores	Candida albicans
Sporangiospores	Mucor, Rhizopus, Prototheca
Conidiospores	

In addition to endo and exospores, thallospores are also present

Blastospores	Arthrospores	Chlamydospores
Specific to yeast-like fungi. These are formed as a result of the budding of the mother cell	They are barrel-shaped or rectangular. They are formed as a result of breaking and fragmentation of the mycelium at the ends. The formed mycelial segment is covered with a membrane and turns into spores (Geotrichium, Coccidioides species)	It is formed as a result of the transformation of mycelial or pseudomycelial cells into thick-walled spores (Candida species).

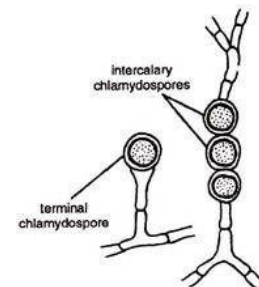
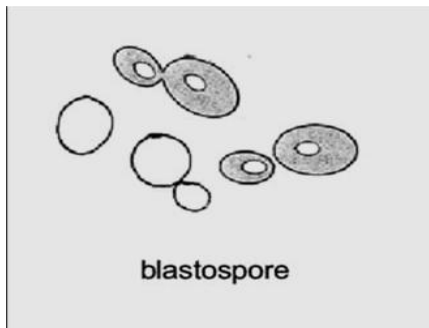


Fig. 4. *Fusarium* : Chlamydospores

Morphology of Protozoa

- Some protozoa move with pseudo legs - pseudopods, some with flagella and others with lashes. In some protozoa without motion organelles, motion is provided by microtubules. Cytoplasm of protozoa contain folding and unfolding vacuoles that perform a secretory function. Protozoa reproduce asexually and sexually. Some pathogenic protozoa undergo a complex developmental cycle by changing primary and intermediate hosts. Under unfavorable conditions, some protozoa form cysts that are resistant to the environment.