Ministry of Health, Republic of Belarus

Institution of Education

“Grodno State Medical University”

Department of Microbiology, Virology and Immunology named after S.I.Gelberg

GENERAL MICROBIOLOGY

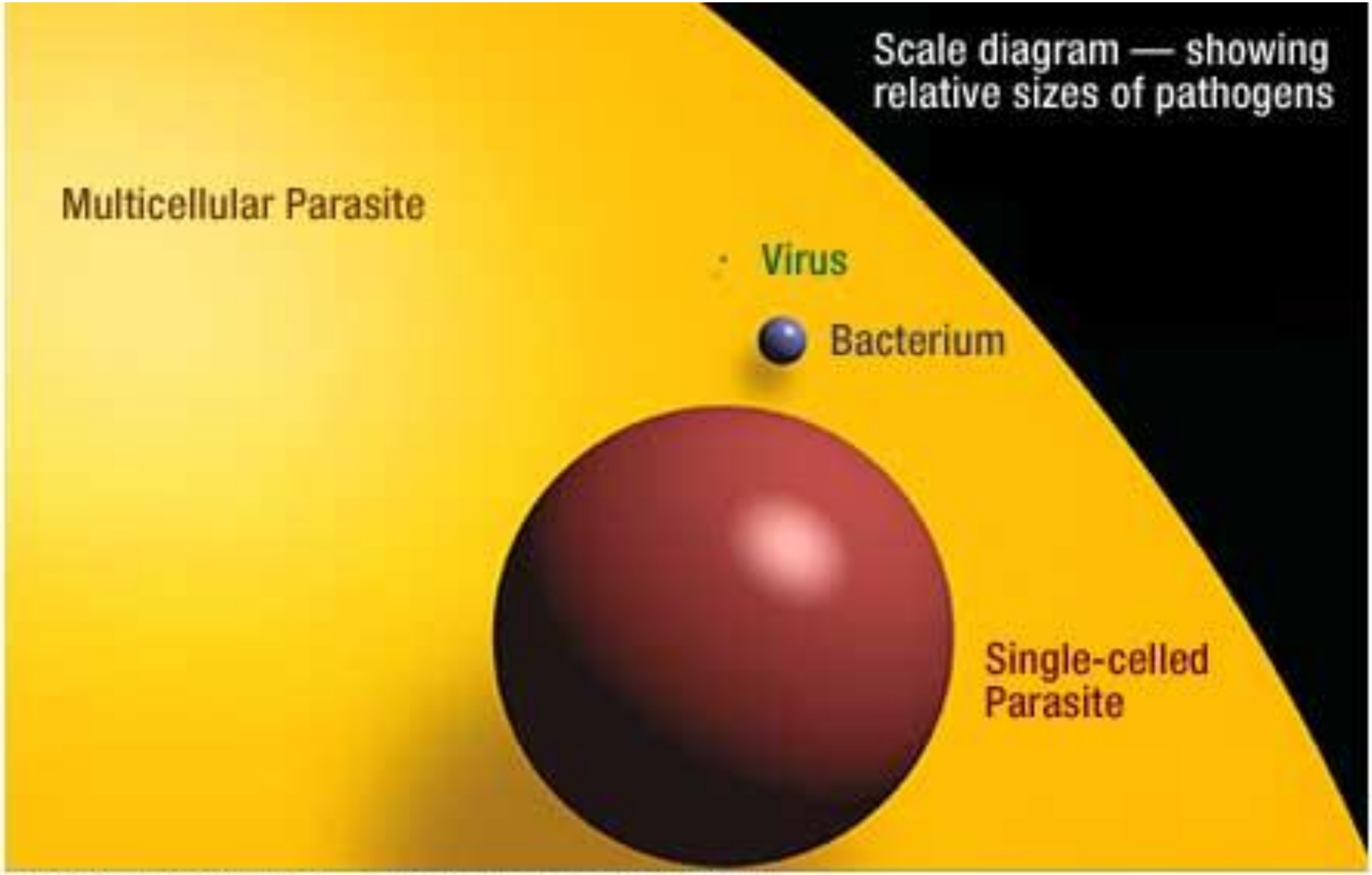
Training appliance for students of the Department for International Students

The Subject of Microbiology Microscopic Method of Investigation and Staining Techniques

Theme No1

DEFINITION OF THE TERMS "MICROBIOLOGY" AND "MICROORGANISM"

- Science studying microorganisms.
- Organisms, invisible by the unaided eye (microscopic object = microbe)



Eade Creative Services, Inc. / George Eade, Illustrator

CLASSIFICATION OF MICROBIOLOGICAL SCIENCES

- According to the topic (object) of research

- **General microbiology**

- **Individual microbiological sciences**

- bacteriology (prokaryotes)
- mycology (eukaryotes-fungi)
- protozoology (eukaryotes - multicellular parasites)
- virology (viruses)
- According to their application
- medical
- sanitary
- veterinary
- industrial
- soil
- sea
- space

CLASSIFICATION OF MICROBIOLOGICAL SCIENCES

According to the topic (object) of research

General microbiology

Individual microbiological sciences:

- bacteriology (prokaryotes)
- mycology (eukaryotes-fungi)
- protozoology (eukaryotes - multicellular parasites)
- virology (viruses)

According to their application

- medical
- sanitary
- veterinary
- industrial
- soil
- sea
- space

TASKS OF MEDICAL MICROBIOLOGY

- Study of structure and biological properties of microorganisms
- Study of cointeraction of microorganism with human organism (i.e. infection), namely:

- pathogenesis
- diagnostics
- treatment
- preventive maintenance

MICROBIOLOGICAL METHODS OF RESEARCH (DIAGNOSTICS)

Microscopy	Cultivation	Experimental (biological)
Pathological material (specimen) ↓ smear ↓ microscopy	Pathological material (specimen) ↓ pure culture of microbe ↓ identification	Pathological material (specimen) ↓ experimental animal ↓ effect (disease, death)

MICROBIOLOGICAL METHODS OF RESEARCH (DIAGNOSTICS)

<table>
<tr><td colspan="4">Immunological (immunobiological) method (methods)</td></tr>
<tr><td colspan="3">Serological tests</td><td>Skin testing</td><td rowspan="3">Methods of estimation of immune status of patient</td></tr>
<tr><td colspan="2">Revealing of antigenes of microorganisms:</td><td rowspan="2">Revealing of antibodies in blood serum of patient (serological diagnostics)</td><td rowspan="2">Revealing of specific hypersensi-tivity (allergy)</td></tr>
<tr><td>In pathological material (express-diagnostics)</td><td>In pure culture (serological tests)</td></tr>
</table>

HISTORY OF MICROBIOLOGY: DESCRIPTIVE PERIOD

The end of XVII – middle of XIX century:

- Discovery of the world of microorganisms, description of microorganisms.

Anthony van Leeuwenhoek – discoverer of first microorganisms

HISTORY OF MICROBIOLOGY: PHYSIOLOGICAL (PASTEUR'S) PERIOD

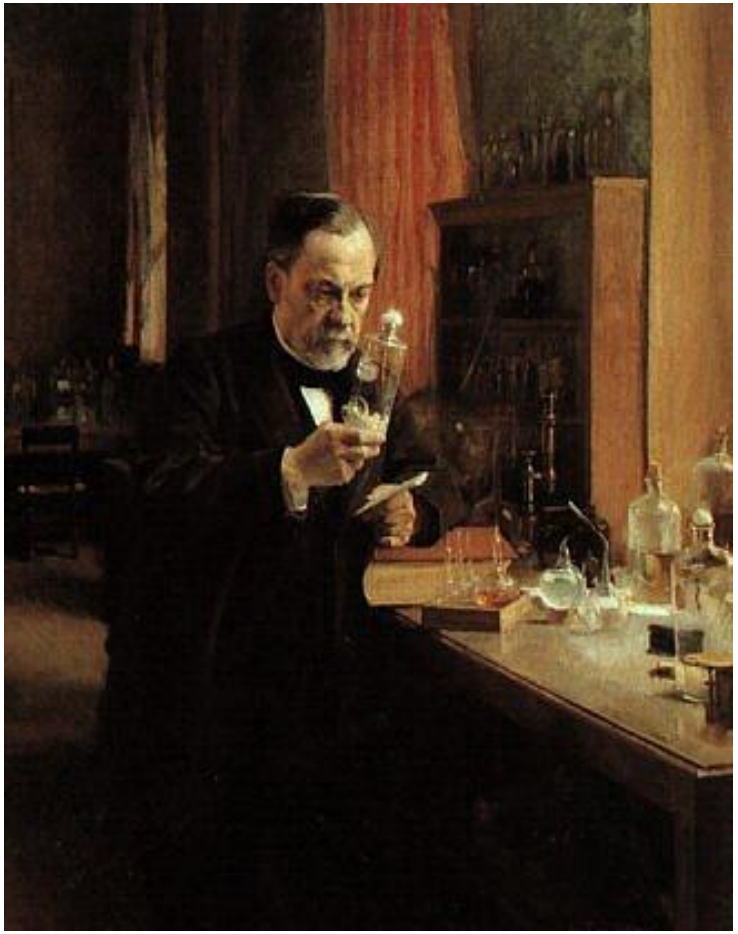
Middle of XIX – beginning of XX century:

- Study of living activity of microbial cell, discovery of infectious (causing disease) bacteria, beginning of scientific microbiology.

Louis Pasteur

Robert Koch

Louis Pasteur **Robert Koch**



SCIENTIFIC CONTRIBUTION OF PASTEUR

- Discovery of pathogenic microorganisms
  - Staphylococcus
  - Pneumococcus
  - Clostridium
- Development of active (live weakened) vaccines
  - chicken cholera
  - anthrax
  - rabies
- Other discoveries
  - microbial nature of fermentation
  - microbial nature of “disease” of wine and beer
  - impossibility of spontaneous self-origin of microorganisms
  - methods of dry heat sterilization and pasteurization

SCIENTIFIC CONTRIBUTION OF KOCH

- Discovery of pathogenic microorganisms
  - anthrax rod
  - cholera vibrio (Koch's comma)
  - tuberculosis rod (Koch's rod)
- Development of basic principals of identification of pathogenic microbes causing disease
  - Henle-Koch postulates:
    1. Microbe has to be isolated from individuals suffering from the disease
    2. The etiological agent (microbe) must be cultivated in pure culture
    3. Pure culture of the pathogen when introduce into susceptible host (experimental animal) has to produce the symptoms characteristic for the disease
- Other discoveries
  - solid agar media for cultivation of microorganisms
  - aniline dyes
  - immersion objective for light microscopy
  - microphotography
  - sterilization by steam

HISTORY OF MICROBIOLOGY: IMMUNOLOGICAL PERIOD

Beginning – middle of XX century

- Discovery of immune response

  Metchnikoff

  Ehrlich

Metchnikoff

- developer of cellular theory of immunity

Ehrlich

- founder of humoral theory of immunity
- developer of chemotherapy of infectious diseases

HISTORY OF MICROBIOLOGY: MODERN PERIOD

Middle of XX century

Molecular biological methods of research

TAXONOMY OF LIVING ORGANISMS

Type of taxonomy	Principals of composition	Area of application
Phylogenetic (natural)	From whom are they descended	Basic biology
Practical (artificial)	Who do they resemble	Microbiology

PROPERTIES USED AS A BASIS IN MODERN TAXONOMY OF MICROORGANISMS

Group of properties	Includes	Application in bacteriology	Application in virology
Morphological	Shape, size, composition	+	+
Biochemical	Biochemical activity	+	–
Physiological (cultural)	Type of colonies got during growing on artificial media	+	–
Serological	Antigenic composition	+	+
Molecular biological	•DNA composition •Composition of 16S ribosomal RNA	+ +	+ (DNA and RNA) –

HIERARCHIAL SYSTEM OF TAXONOMY USED IN BACTERIOLOGY

1. **Kingdom**
   Prokaryote
2. **Division**
   Composition of cellular wall:
   – *Eubacteria*
     - **Firmicutes**
     - **Gracilicutes**
     - **Tenericutes**
   – *Archaebacteria*
     - **Mendosicutes**
3. **Order**
   The name ends with**–ales**
4. **Family**
   The name ends with**–ceae**
5. **Genus**
6. **Species**
   The basic taxonomy in classification of Prokaryote
7. **Species' subdivisions**
   – Variants (subspecies) (morhpo-, bio-, enzymological-, resistance-, phage-, serological-, ecological-, pathogenicl variants)
   – Strain (culture – population, – isolated from certain source)
   – Clone (generation having origin from one cell)

HIERARCHIAL SYSTEM OF TAXONOMY USED IN VIROLOGY

1. **Kingdom**

   Vira

2. **Subkingdom**

   – DNA- genomic viruses

   – RNA – genomic viruses

3. **Family**

   The name ends with**–viridae**

4. **Subfamily**

   The name ends with**–virinae** (could be find in some families)

5. **Virus**

   The name ends with**–virus.**

   Basic taxonomy in classification of viruses

6. **Serological variants**

   The basis is antigenic structure

METHODS OF MICROSCOPY

- Electron microscopy
- Light microscopy
  - Basic light
  - Immersion
  - Dark field
  - Phase contrast
  - Fluorescence

METHODS OF MICROSCOPY: ELECTRON MICROSCOPY

- **Using microscope**

  electron microscope
- **Effect (the principal of the method)**

Uses beams of electrons instead of light rays
- **Application in microbiology**
  - Study of viruses
  - Study of ultra structure of microbial cell

METHODS OF MICROSCOPY: USUAL LIGHT MICROSCOPY

- **Using microscope**

  Biological light microscope
- **Effect (the principal of the method)**

  Uses visible light rays (see the course of Physics)
- **Application in microbiology**

  It is not frequently used in microbiology

METHODS OF MICROSCOPY: IMMERSION MICROSCOPY

- **Using microscope**

  Biological light microscope + immersion objective

- **Effect (the principal of the method)**

  Coefficient of refraction of immersion oil (placed between glass slide and objective lens) **=** coefficient of refraction of glass⇨ eliminates losses of light rays getting in objective lens.

- **Application in microbiology**

  It is most frequently used in bacteriology as a microscopic method of research.

METHODS OF MICROSCOPY: DARK FIELD MICROSCOPY

- **Using microscope**

  Biological light microscope + dark field condenser

- **Effect (the principal of the method)**

  Only light rays scattered from the specimen (object) reach the objective lens (see light object on a dark background)

- **Application in microbiology**

  It is used for observation of very thin objects, for example, spirochetes.

METHODS OF MICROSCOPY: PHASE CONTRAST MICROSCOPY

- **Using microscope**

  Biological light microscope + phase contrast optical design

- **Effect (the principal of the method)**

  Amplifies small differences in refractive indices (when light is coming through translucent objects we can't see these changes) ⇨ to changes of amplitude – **we can see these changes and translucent object becomes visible.**

- **Application in microbiology**

  It is used for observation of translucent objects, for example, mycoplasmas.

METHODS OF MICROSCOPY: LUMINESCENT (FLUORESCENT) MICROSCOPY

- **Using microscope**

  Luminescent (fluorescent) microscope

- **Effect (the principal of the method)**

  Luminescence of the object in ultraviolet light is registered

- **Application in microbiology**

  - microscopy of specimen stained with fluorescent dyes (auramine, rhodamine, etc.),

  - evaluation of serological fluorescence reactions.

METHODS OF STAINING: SIMPLE STAINING TECHNIQUES

- Staining by methylene blue
- Staining by aqueous fuchsine

- Revealing of presence of microbes in a pathological material
- Study:
  - shape of bacteria
  - their arrangement in a smear

METHODS OF STAINING: DIFFERENTIAL STAINING TECHNIQUES

- Gram staining (basic method of staining in bacteriology)
  - revealing of cell wall structure
- Ziehl-Neelsen staining
  - revealing of acid-fast bacteria (mycobacteria)
  - revealing of spores
- Neisser staining
  - revealing of volutine storage granules and identification of corynebacteria according the granule presence
- Burry-Hines staining
  - revealing of capsules

METHODS OF STAINING: DIFFERENTIAL STAINING TECHNIQUES

- Morozov staining
  - revealing of flagella
  - revealing of treponemas
- Zdradovsky staining
  - revealing of viruses causing chickenpox and smallpox in vesicular lesions
  - revealing of rickettsia and chlamydia
- Romanovsky-Giemsa staining
  - revealing of rickettsia and chlamydia
  - revealing of spirochetes after their preliminary differentiation by colour of staining
  - revealing of parasites

Morphology and Structure of Bacterial Cell
Gram staining technique

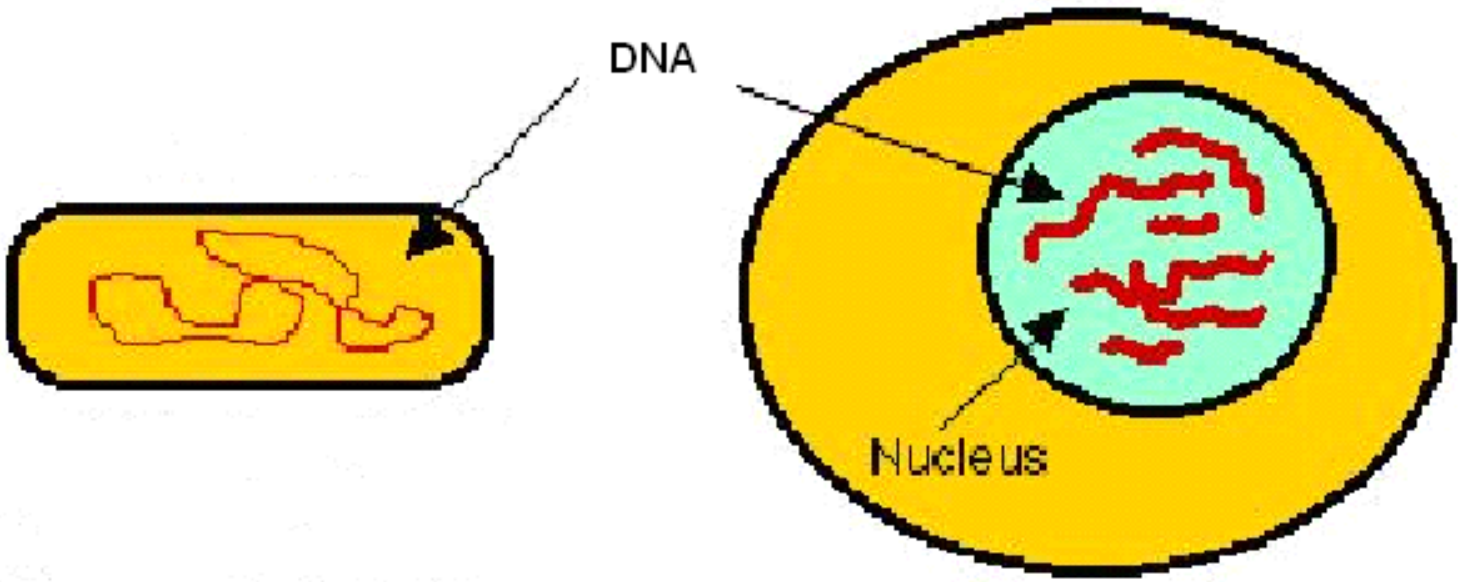
Theme No2

Main differences between pro- and eukaryotic cell

Essential differences	**Absence of membrane intracellular structures in prokaryotic cell**	
DNA: Structure Localization Number of chromosomes Histone proteins Mitosis	 Circular closed structure nucleoid + 1 – –	 Linear Nucleus+ mitochondria > 1 + +
Ribosomes	70 S	80 S
Movement of cytoplasm	–	+
Peptidoglycan (firm structure built of polymeric chains of amino sugars linked by peptide bridges)	+	–
Flagella	Protein subunits (protein flagellin), forming a spiral	Set of micro tubes assembled into bunches

Organelles of bacterial cell: basic

- **Nucleoid**
  Circular closed super spiraled double-strand DNA molecule = bacterial chromosome
- **Cytoplasm**
  Similar to cytoplasm of eukaryotic cell
- **Ribosomes**
  Similar to ribosomes of eukaryotic cell but possess lower molecular weight
- **Cytoplasmic (plasma) membrane**
  Similar to cytoplasm (cellular) membrane of eukaryotic cell but without sterols (sterols present only in the membrane of mycoplasmas)
- **Mesosomes**
  Invaginations of cytoplasmic membrane:
  – centre of energy producing metabolic reactions
  – participation in cell division
- **Cell wall**
  – creates shape of bacterial cell
  – preserves cell from osmotic lysis
  – possesses two types of a composition (Gram-positive and Gram-negative cell wall)
  – lack of cell wall found only in mycoplasmas


DNA
Nucleus

Organelles of bacterial cell : facultative

Plasmids

DNA structure is similar to DNA of nucleoid, but possesses

– lower molecular weight

– there can be several copies of plasmid in one bacterial cell

Cytoplasmic inclusions

Usually storage granules of metabolites

Protective structures

– spore (endospore)

– capsule

Flagella

Organelles responsible for motility

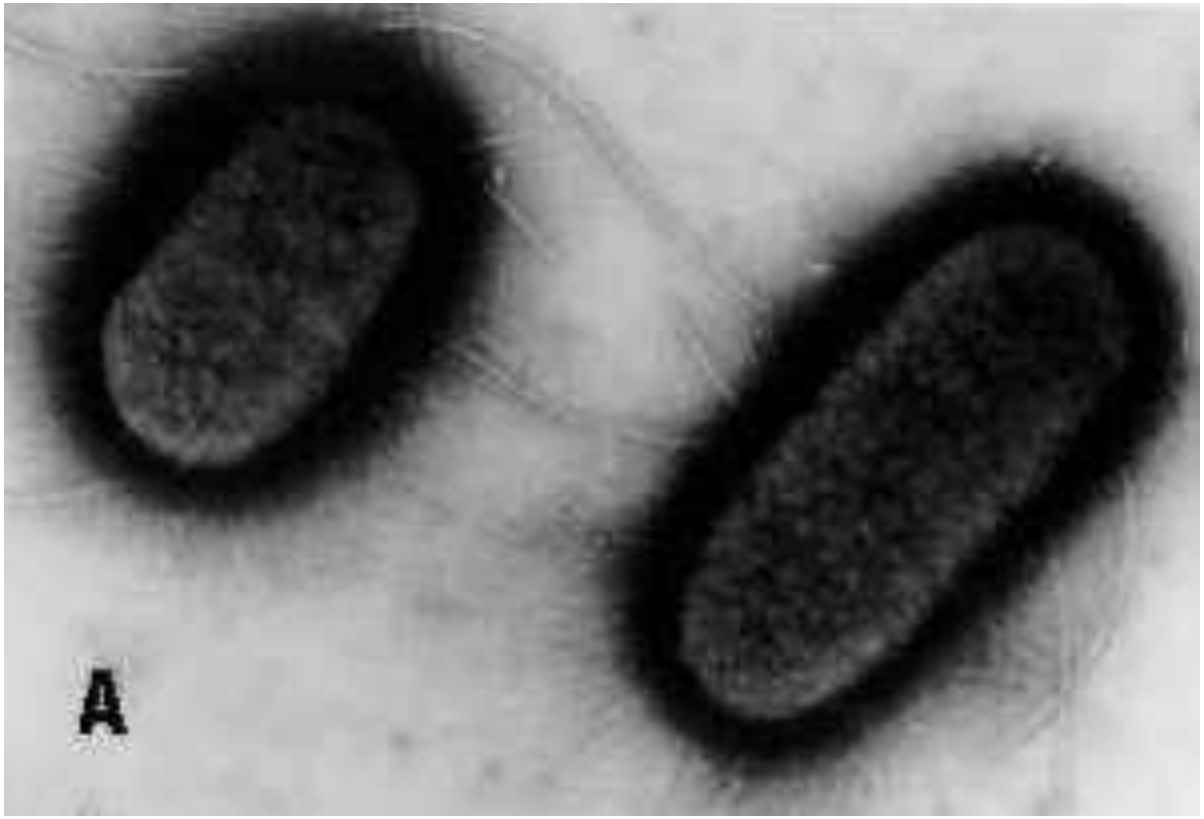
Pili (fimbriae)

Empty inside protein tubular structures (composed of protein pilin) covering the surface of bacterial cell:

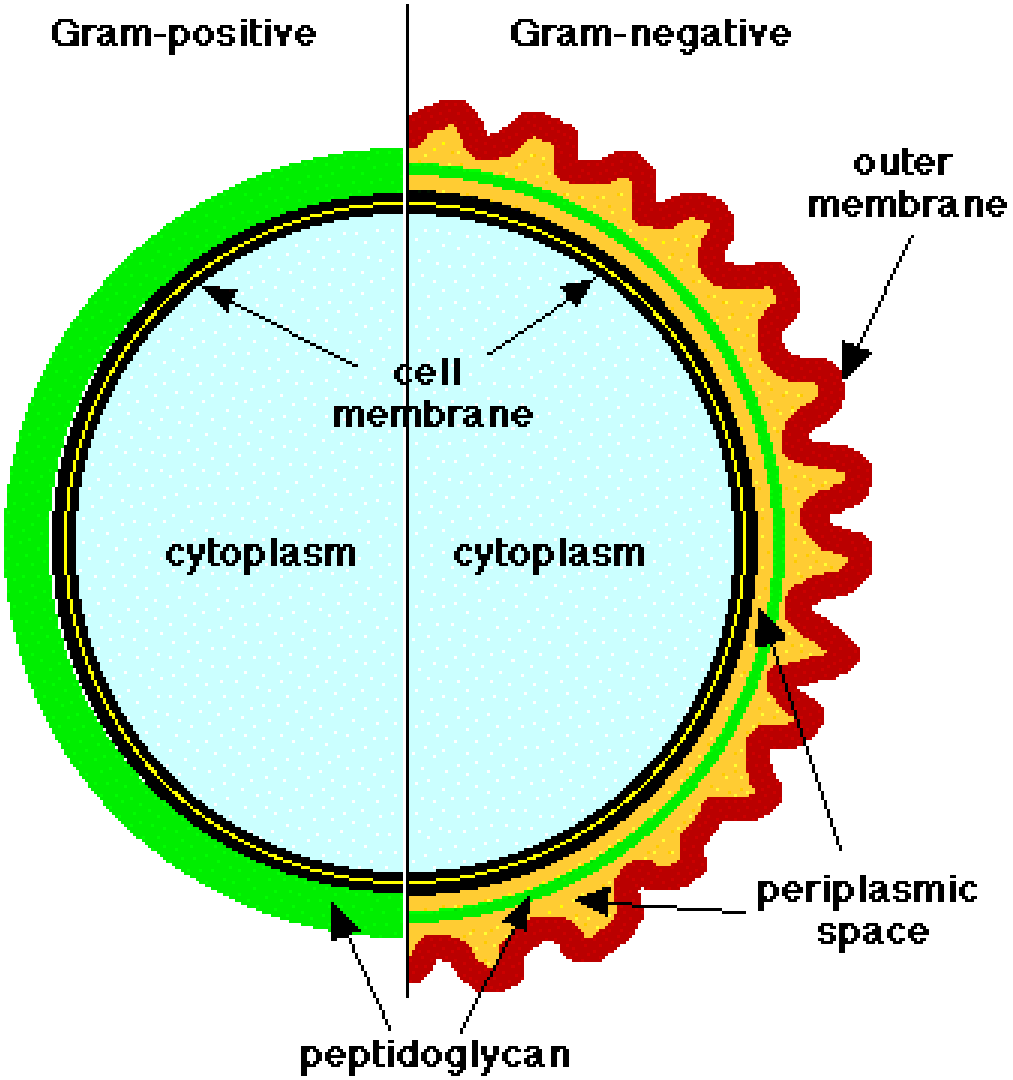
– common type - necessary for bacterial adhesion to the surface of nutrient substrates

– sex pili (conjugative pili) – participate in DNA transfer from one cell to another

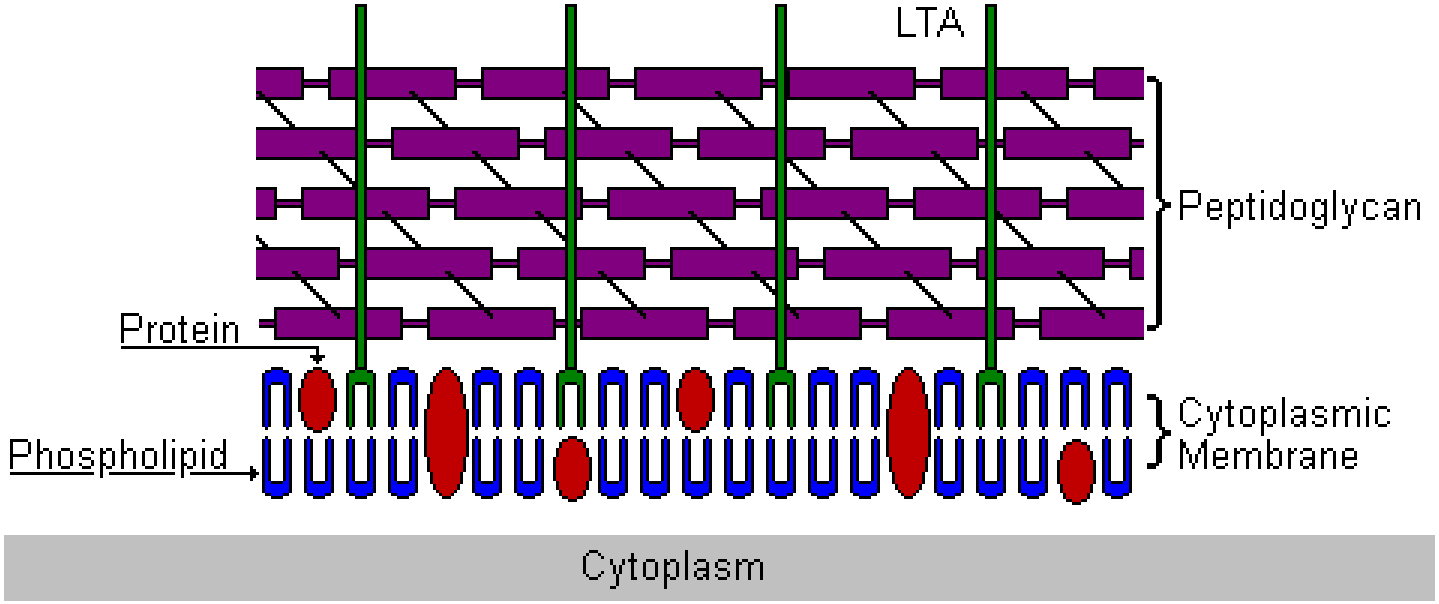
Bacterial fimbriae



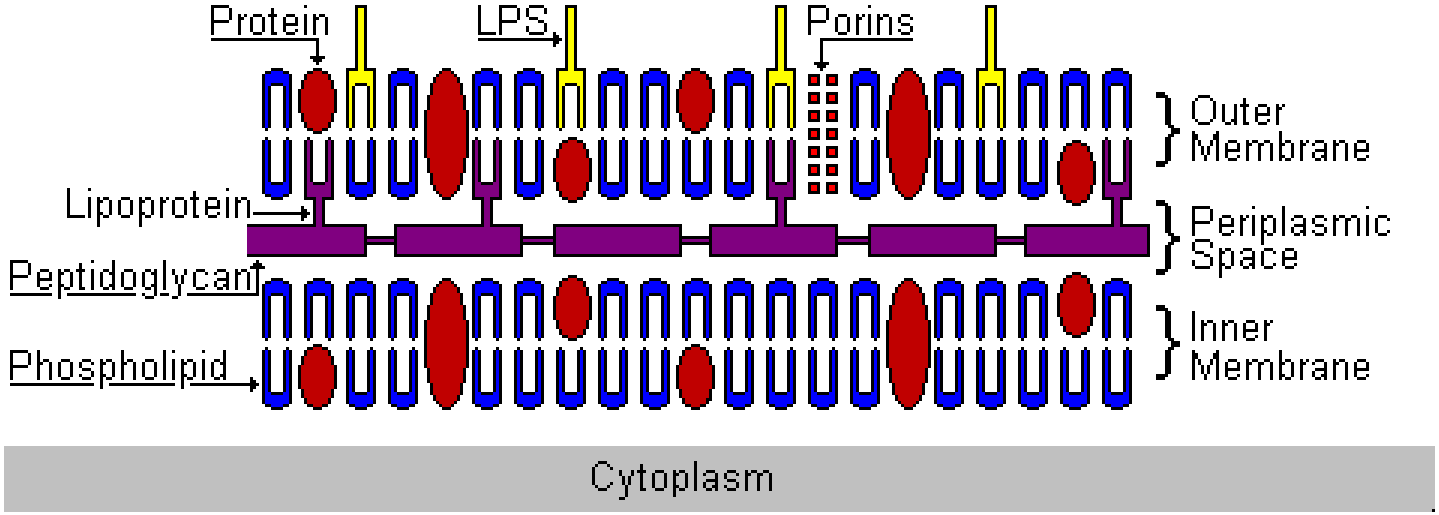
Composition of bacterial cell wall



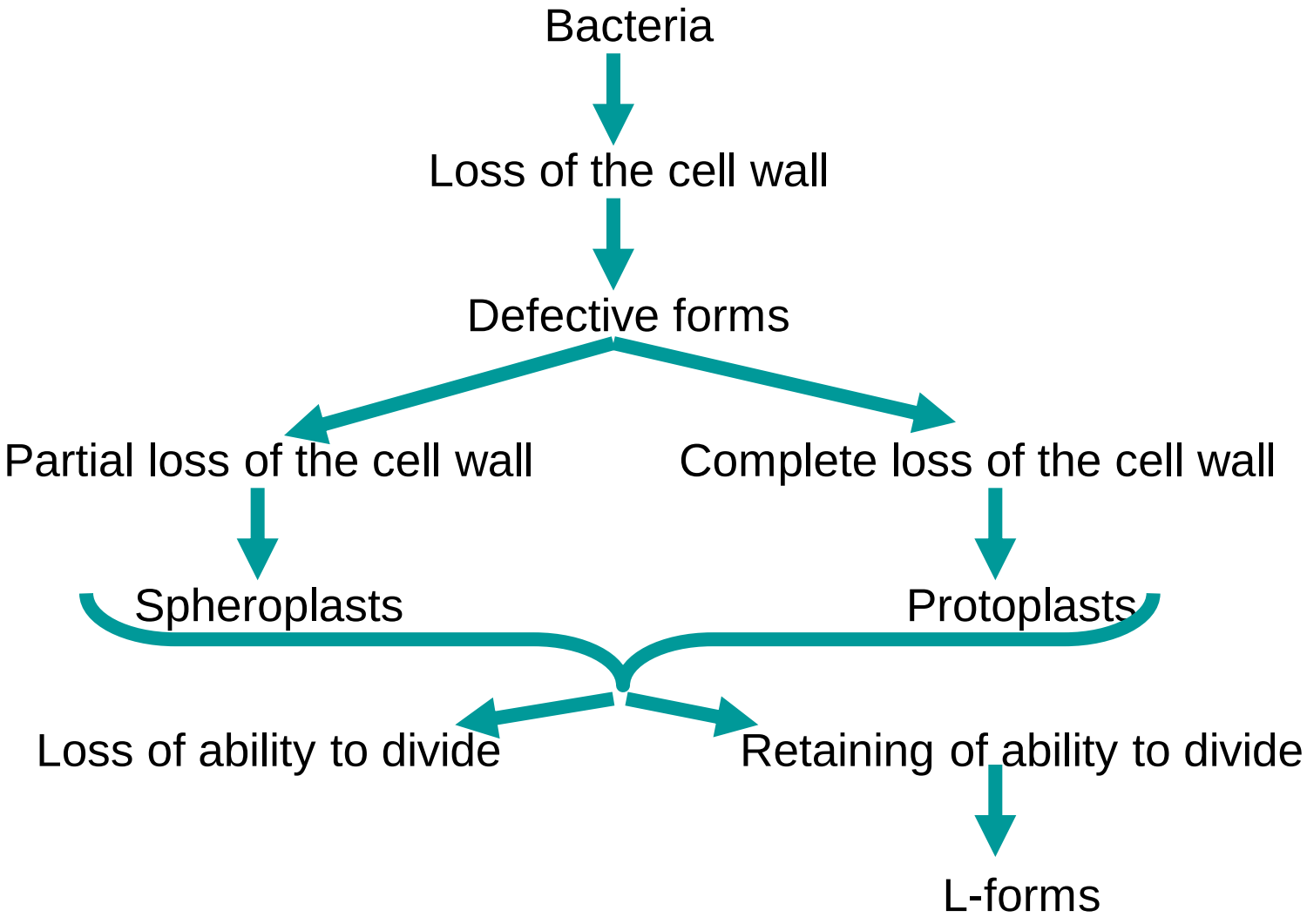
Gram-positive Cell Wall



Gram-negative Cell Wall



Defective forms of bacteria



Gram staining: techniques

Stage	Duration	Result	
		G^+ (Firmicutes)	G^- (Gracili-, Tenericutes)
Crystal violet	1 – 2 minutes	blue	blue
Iodine solution	1 – 2 minutes	blue	blue
Alcohol decolori-zation(following by washing with H_2O)	½ minutes	blue	uncoloured
Aqueous fuchsine or safranin	1 – 2 minutes	blue	red

Gram staining : Gram-positive and Gram-negative bacteria

Group of bacteria	Gram-positive (Firmicutes)	Gram-negative (Gracilicutes + Tenericutes)
Cocci	all bacteria excluding neisseria	neisseria
Rods	•spore-forming •branch-forming •listeria	all others

Morphological features of bacteria

- Gram-staining
- Shape of bacterial cell
- Size of bacterial cell
- Presence of protective structures
- Motility (presence of flagella)
- Arrangement of bacteria in a smear

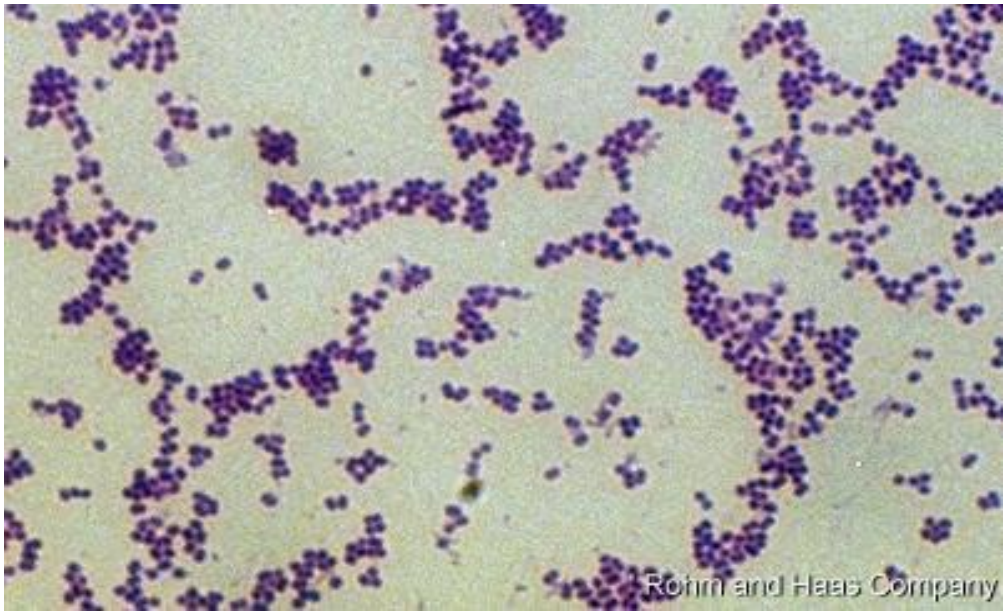
Shape of bacteria

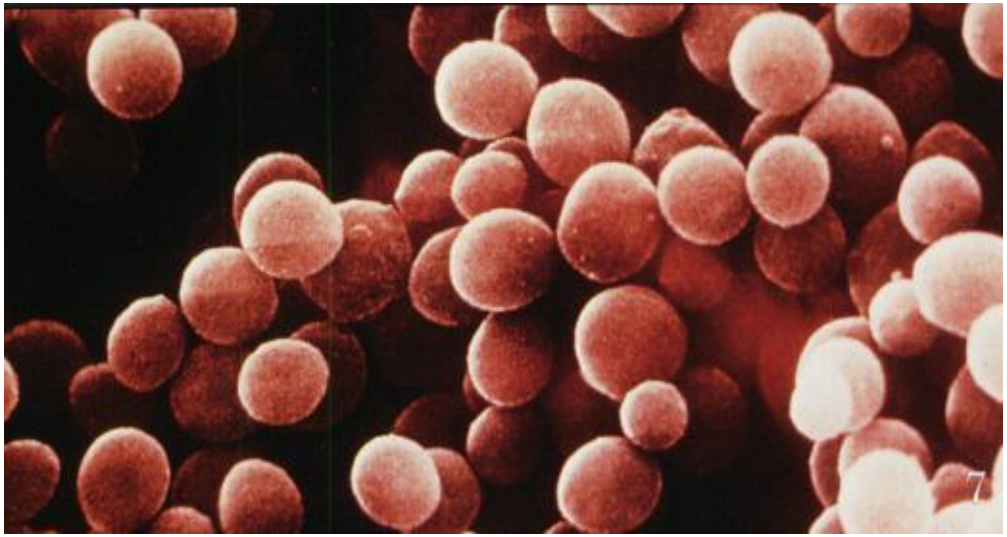
1. Having certain shape (Firmicutes и Gracilicutes)
   - round (cocci)
   - rods
   - helical (spirochetes)
2. Without certain shape (Tenericutes)
   - mycoplasmas

Shape of bacteria

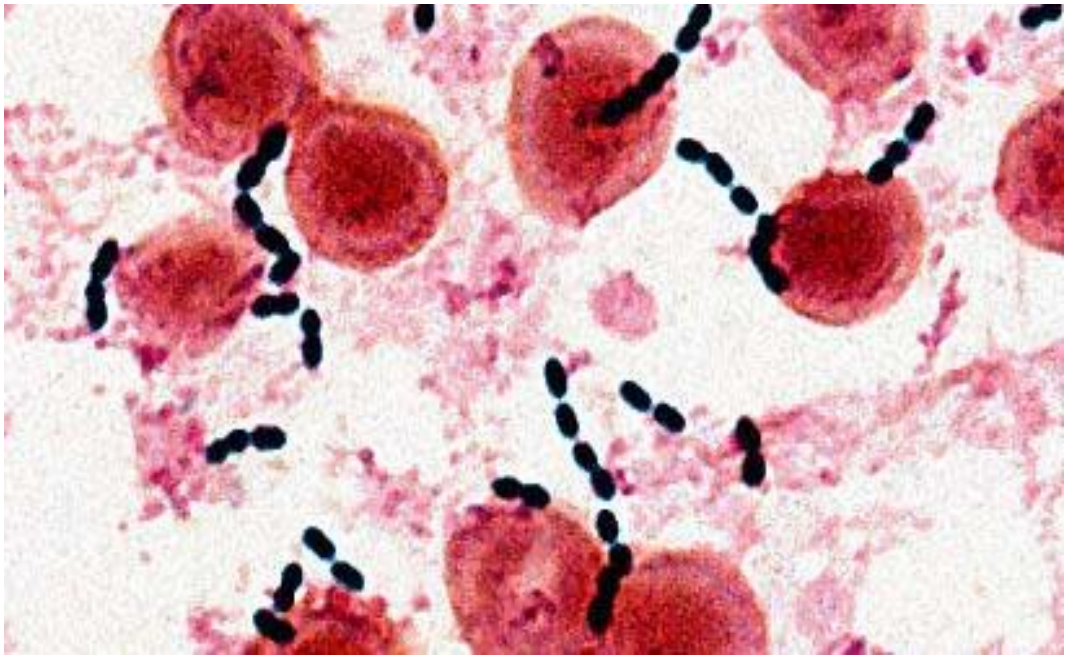
1. Having certain shape (Firmicutes и Gracilicutes)
   - Round (cocci)
     - ideal sphere - staphylococci
     - oval - streptococci
     - lanceolate – pneumococci
     - fabiform – neisseria

Staphylococci

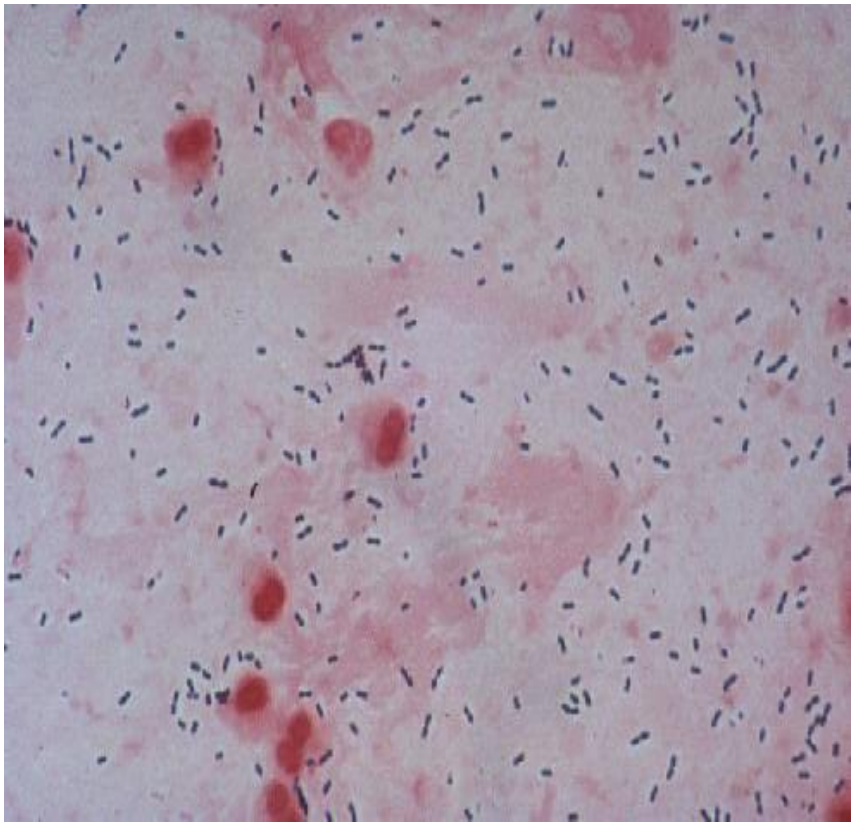




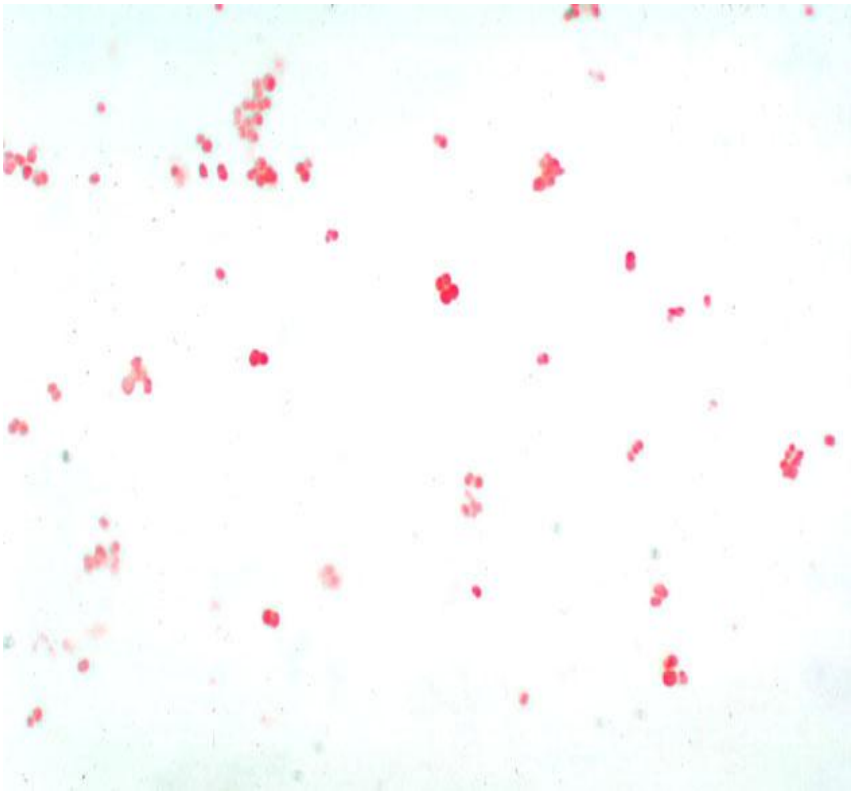
Streptococci



Pneumococci



Neisseria



Shape of bacteria

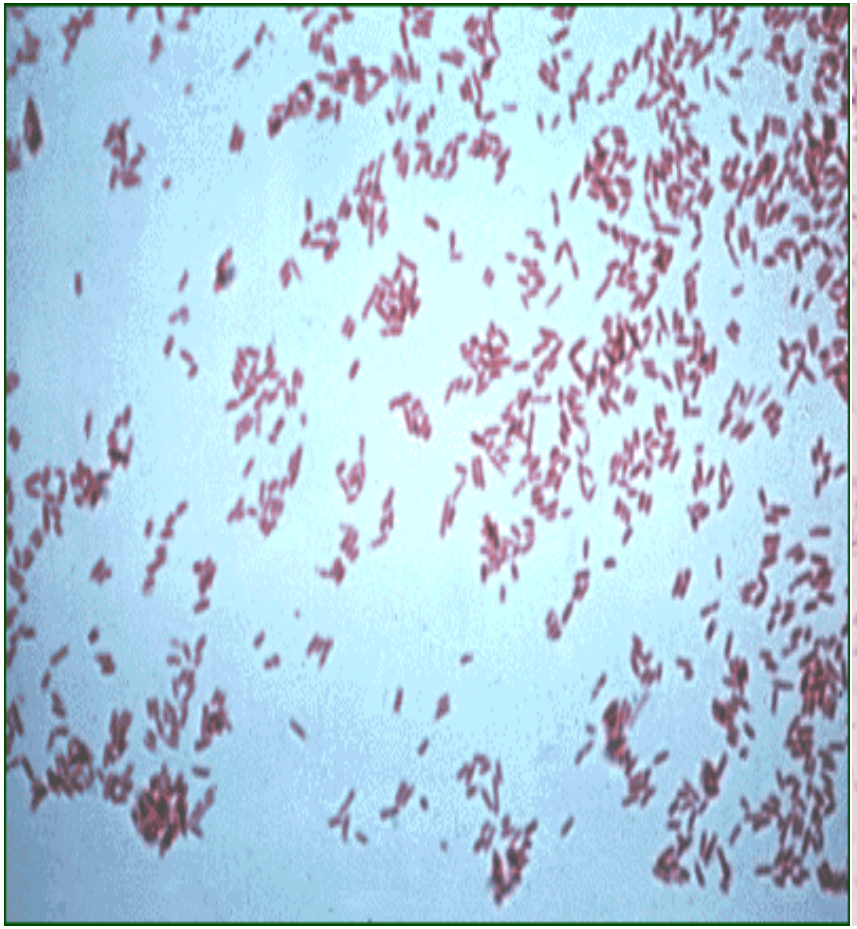
1. Having certain shape (Firmicutes и Gracilicutes)

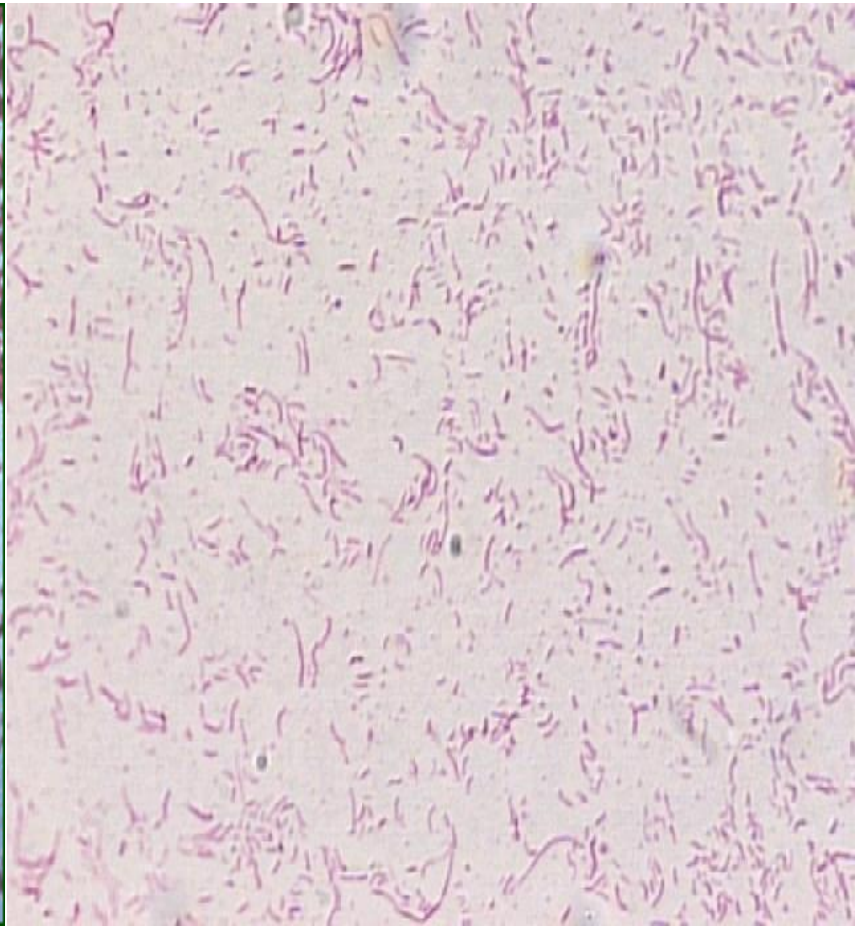
   rods

   - cylindrical – most of them
   - curved
     - one curve - vibrio
     - 2-3 curves – campylobacteria and helicobacteria
   - branch – forming

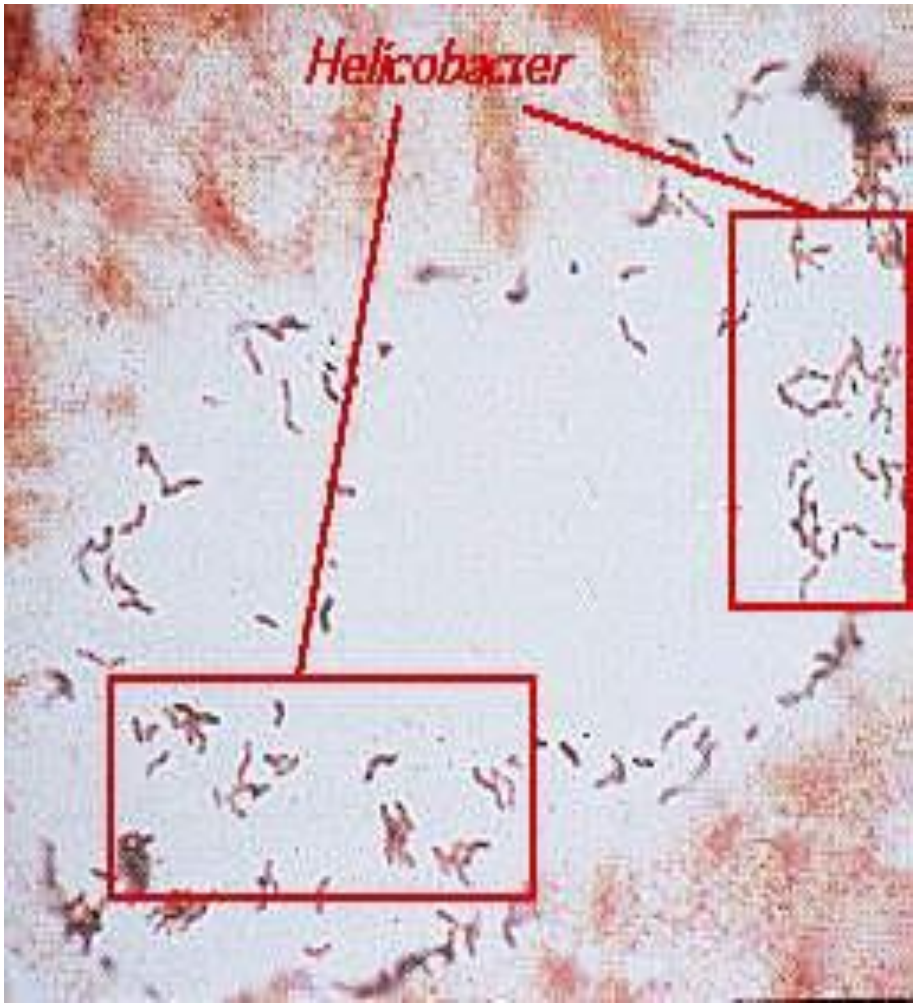
   – actyninomycetes, mycobacteria, corynebacteria

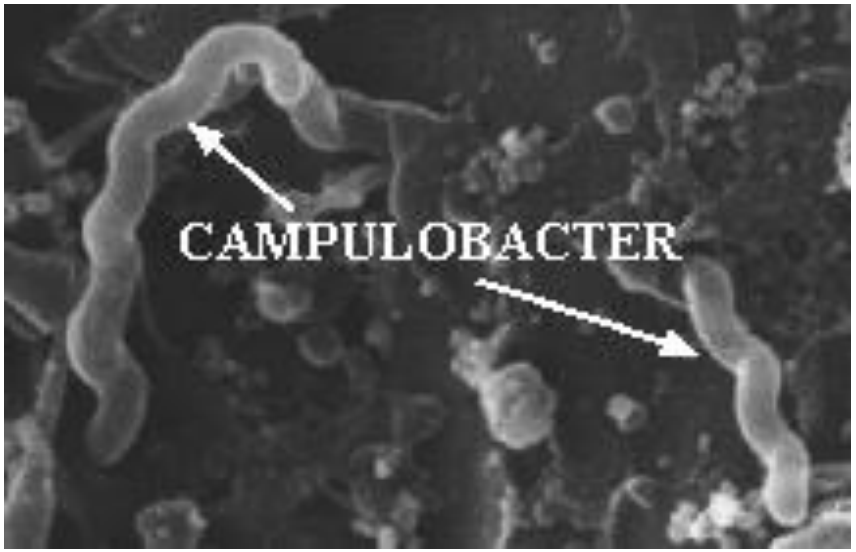
Cylindrical rods

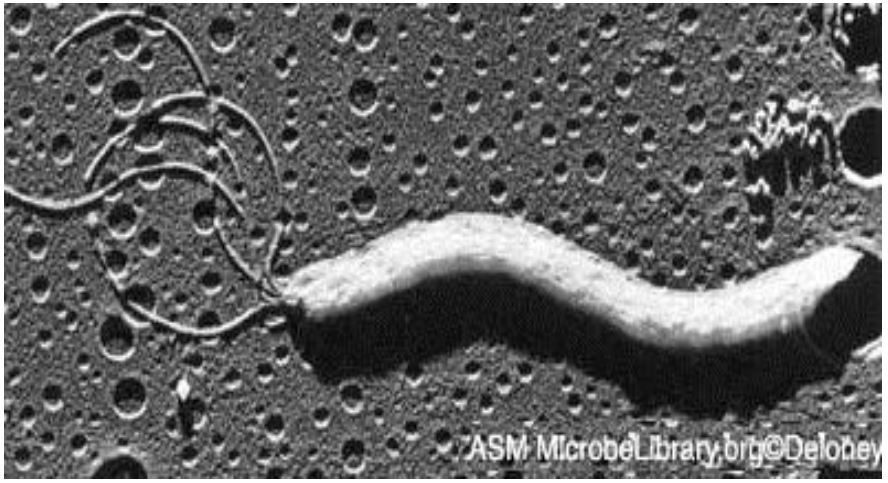




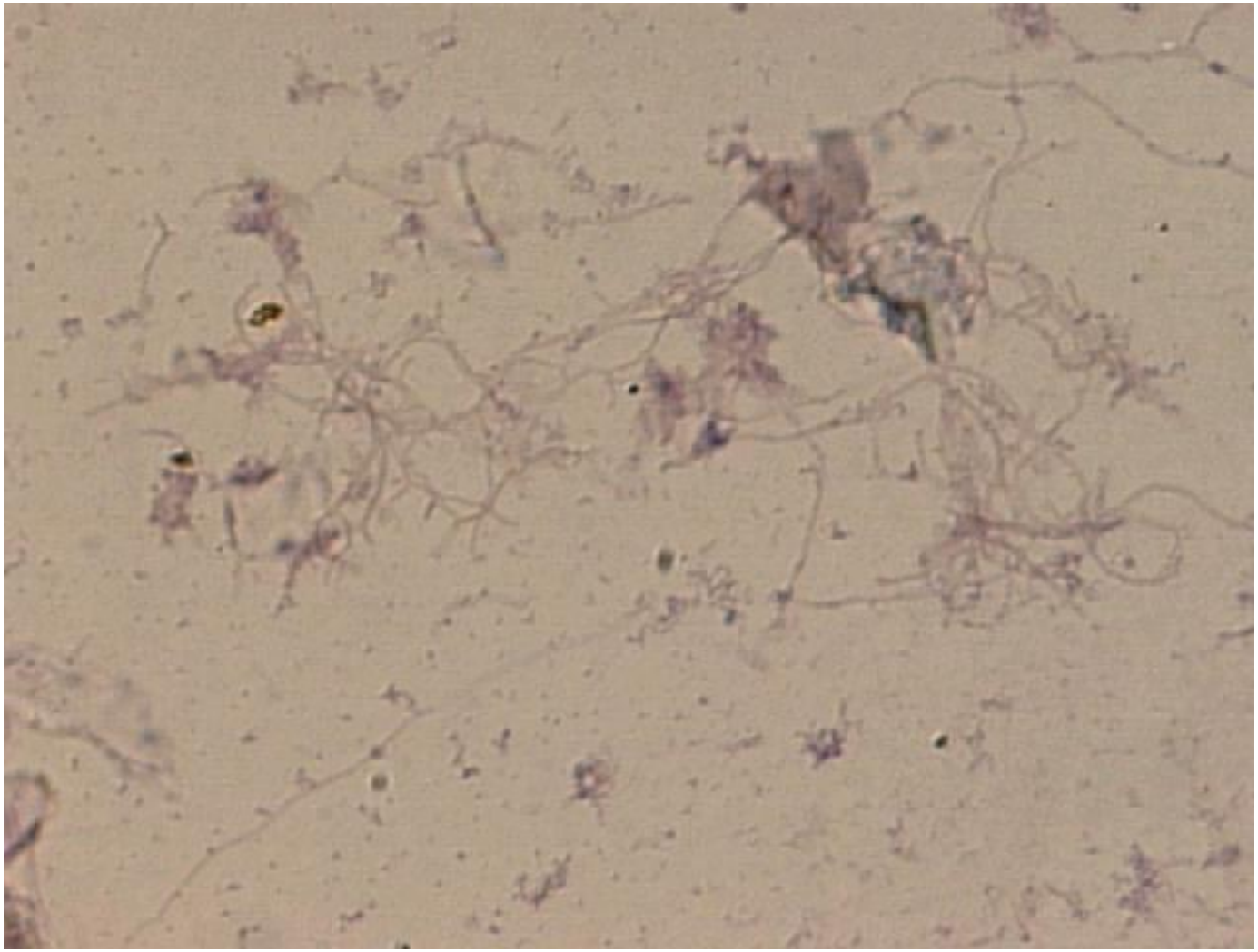
Curved rods


Helicobacter


CAMPULOBACTER


ASM MicrobeLibrary.org©Delobey

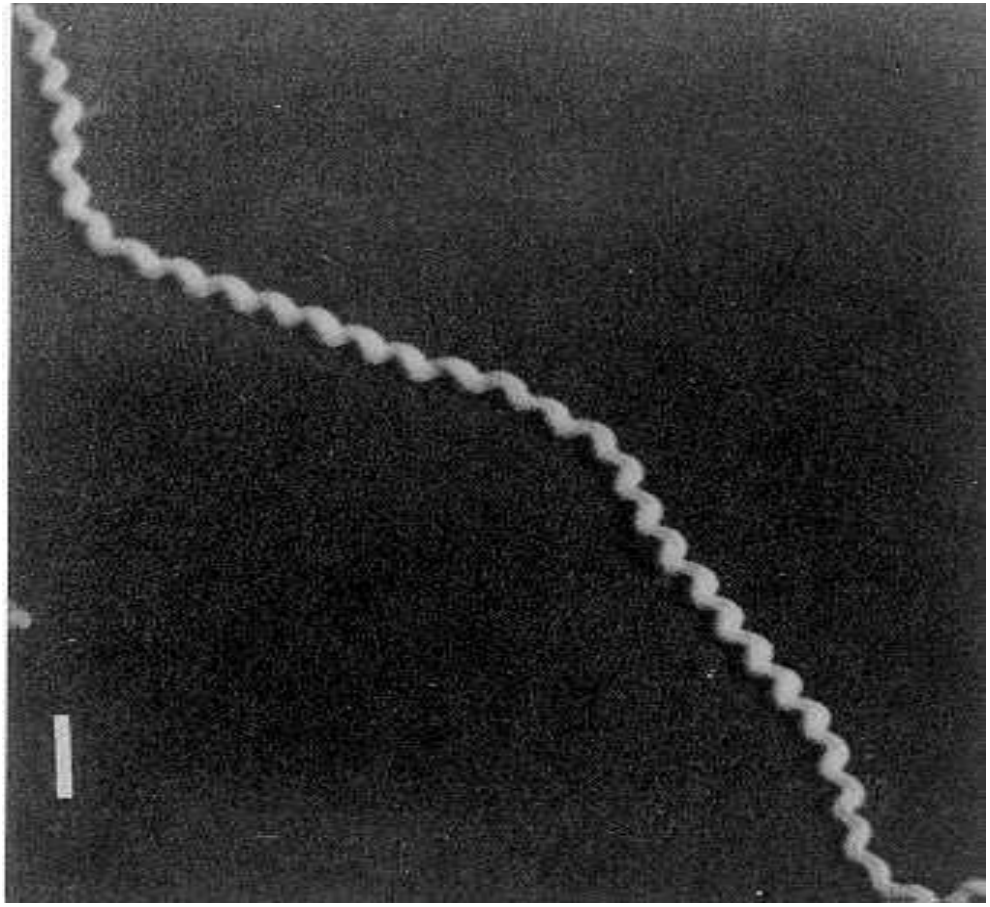
Branch – forming rods



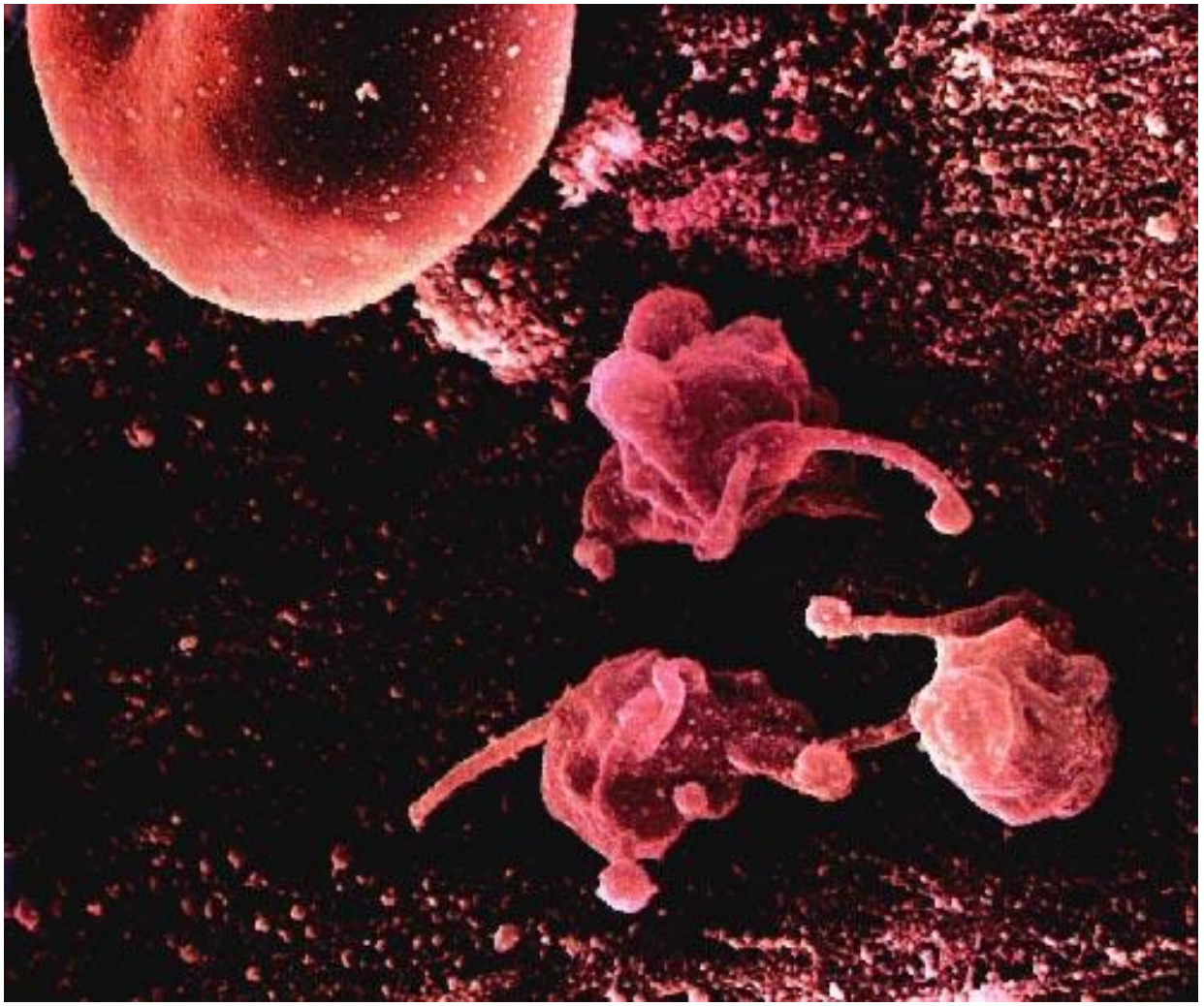
Shape of bacteria

1. Having certain shape(Firmicutes и Gracilicutes)
   - helical
     - spirochetes – treponemas, leptospiras and borrelias
2. Without certain shape (Tenericutes)
   - mycoplasmas

Helical rods



Bacteria without certain shape



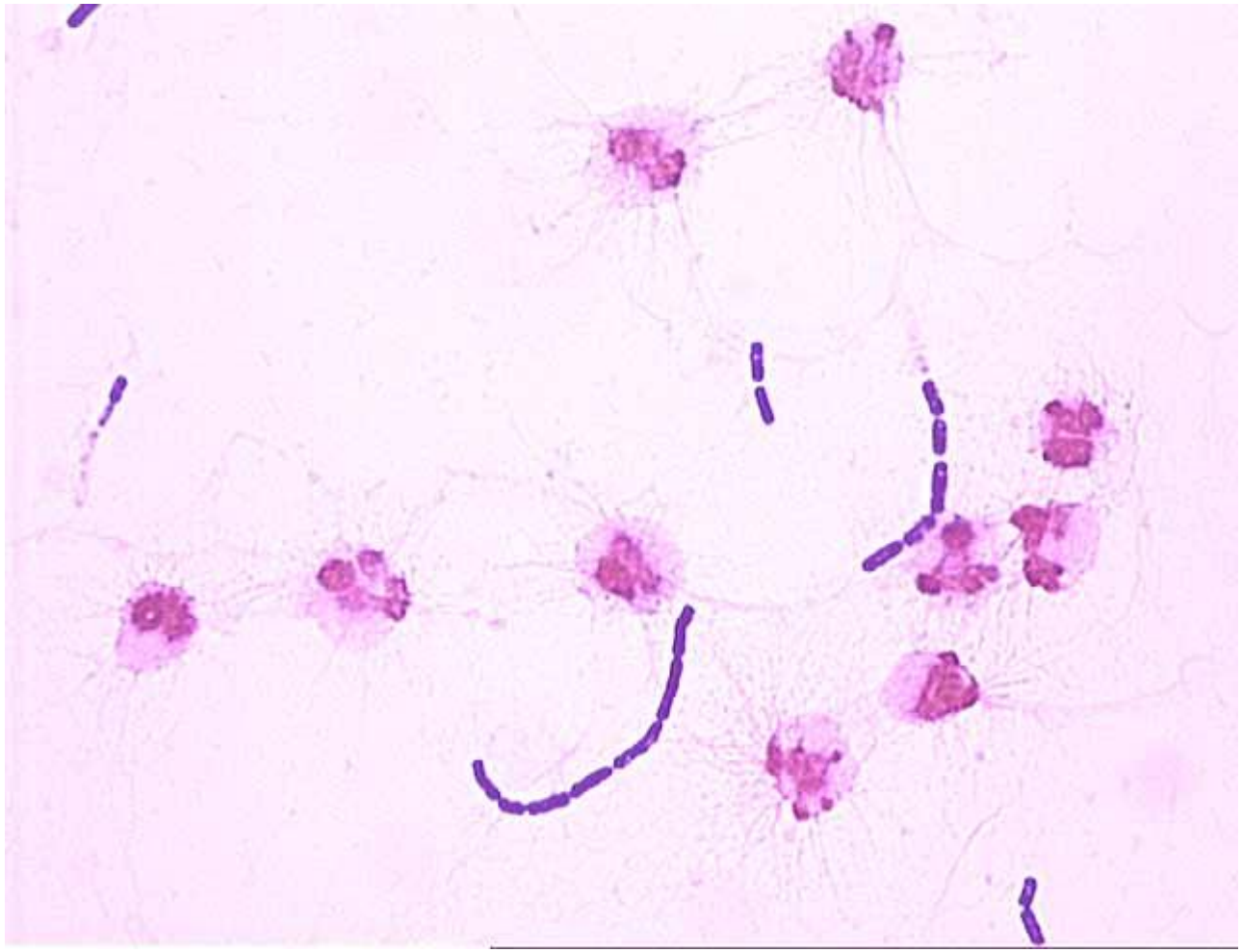
Size of bacteria

- cocci – ~1 micrometer
- rods
  - very small – coccobacteria
  - small and average – most of the rods
  - large – branch-forming and spore-forming
- spirochetes – thin and long
- mycoplasmas – have no constant size

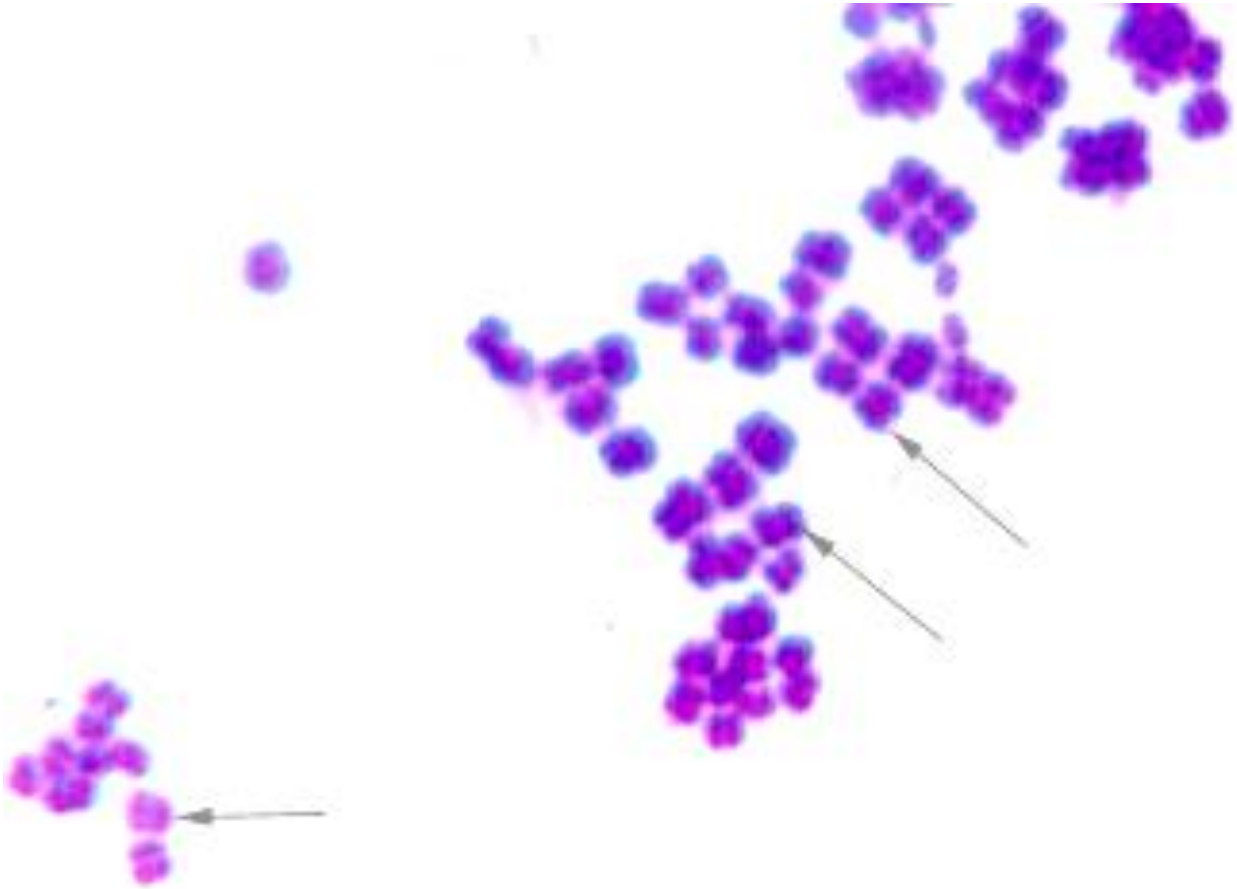
Arrangement of bacterial cells in a smear

- cocci
  - single cells without special arrangement – micrococci
  - groups of two cells (diplococci) – pneumococci, neisseria, enterococci
  - tetrad (packet) arranged from the number of cells, multiple to 4 – sarcinas
  - chains of cells – streptococci
  - arrangements reminding bunches of grapes – staphylococci

Chains of streptococci



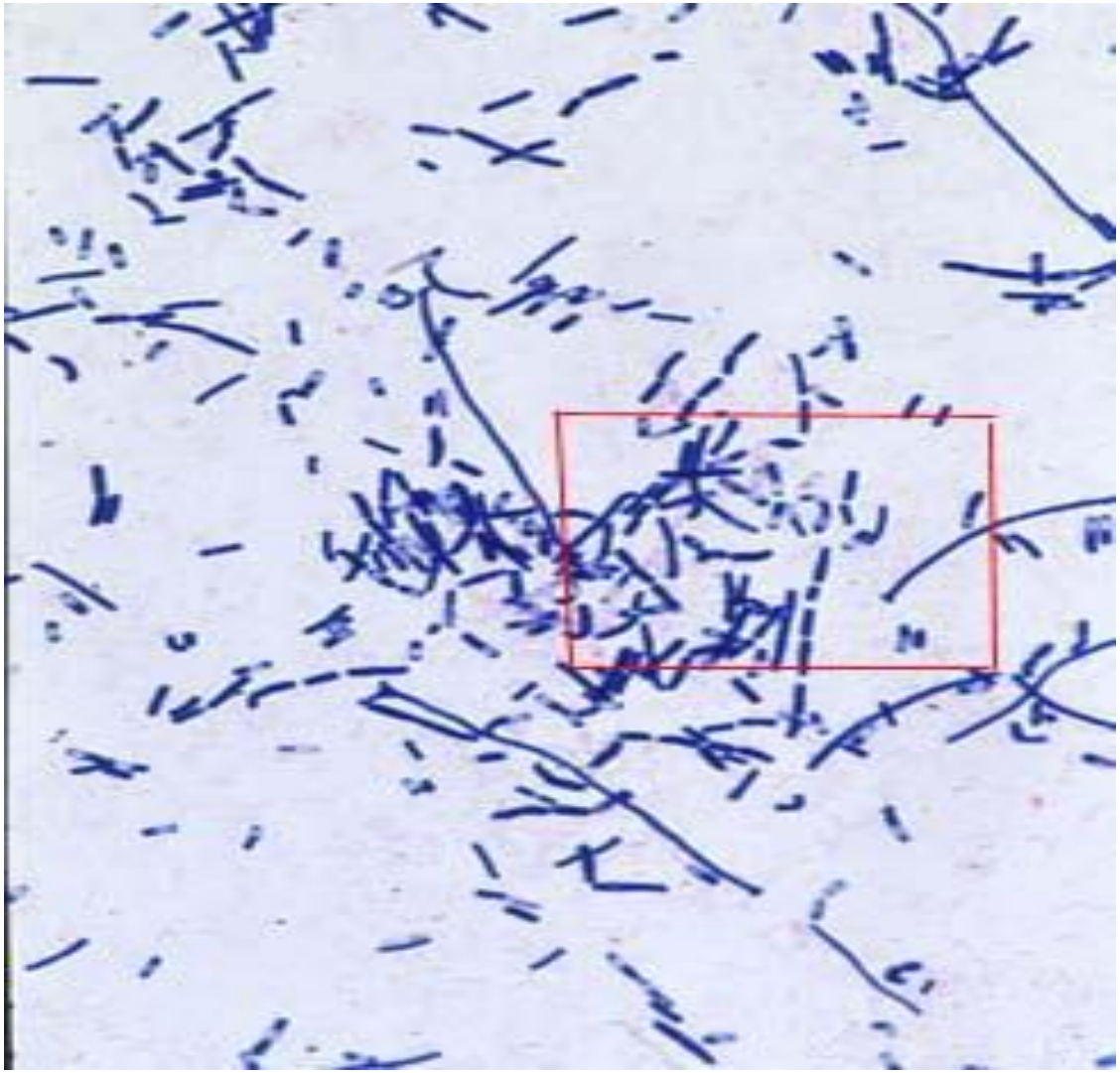
Tetrads of sarcinas



Arrangement of bacterial cells in a smear

- rods
  - without any order – most of the rods
  - forming pairs – klebsiellas, corynebacteria
  - forming chains – bacilli

Chains of bacilli



MORPHOLOGY AND STRUCTURE OF BACTERIAL CELL (CONTINUATION). MORPHOLOGICAL AND ULTRA STRUCTURAL PECULIARITIES OF ACTINOMYCETES, SPIROCHETES, RICKETTSIA, CHLAMYDIA, MYCOPLASMAS AND FUNGI.

Ziehl-Neelsen staining technique

Theme No3

Micro- and macrocapsules of bacteria

	Macrocapsules (capsule)	Microcapsules
Definition	The expressed mucous layer covering cell wall and having a fibrillar composition	Mucopolysaccharide fibrils close-fitting the cell wall
Composition	•Most frequently - polysaccharides •rare - polypeptides	Mucopolysaccharides
Function	Defense of bacterial cell from: •phagocytosis •binding by antibodies	

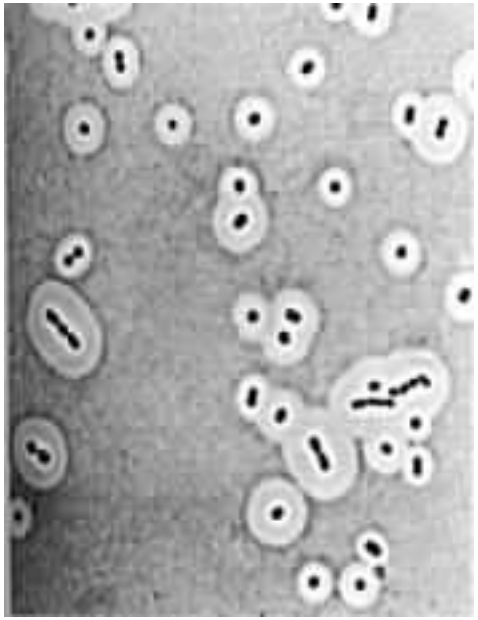
Micro- and macrocapsules of bacteria

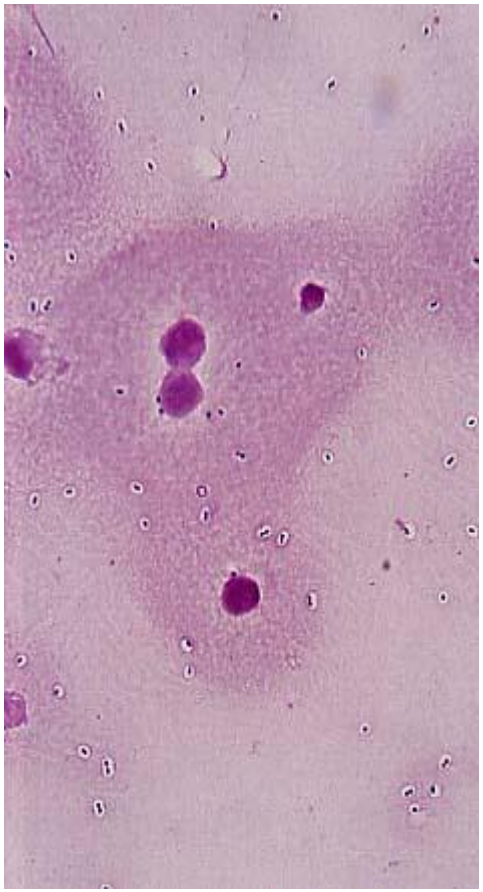
	Macrocapsules (capsule)	Microcapsules
Present in bacteria	•penetrated into the human organism •growing in artificial media containing blood serum	
Bacteria having capsule	It is most pronounced in: •klebsiella (always form it even when growing on simple artificial media) •pneumococci •bacilii causing anthrax •Clostridium perfringens •coccobacteria (excluding brucellas)	Many bacteria

Micro- and macrocapsules of bacteria

	Macrocapsules (capsule)	Microcapsules
Detection (methods of the revealing of the capsule)	Light microscope: •In smears prepared from a pathological material - any method of staining (can be seen as uncoloured aura around bacterial cell) •With use of special staining techniques	Electron microscope

Macrocapsules of bacteria

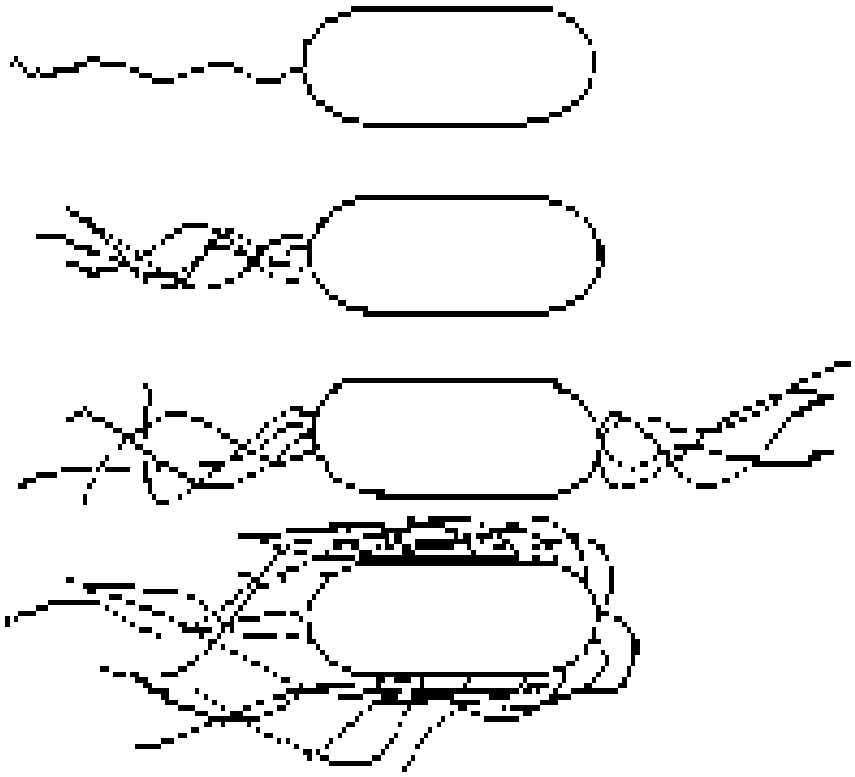


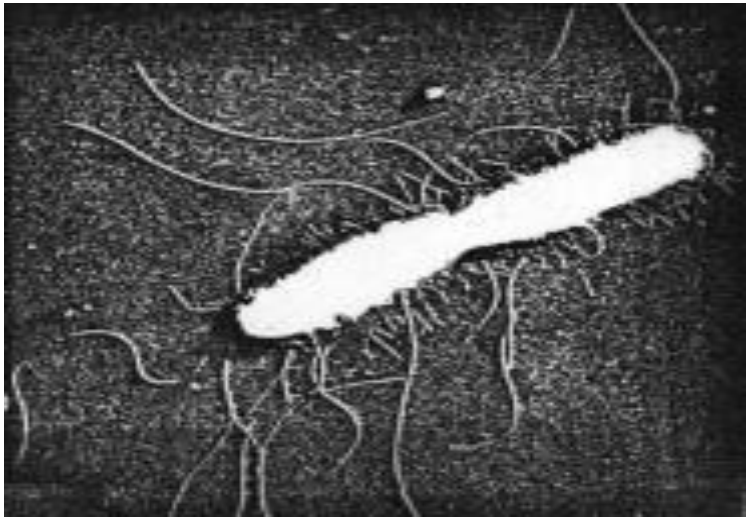


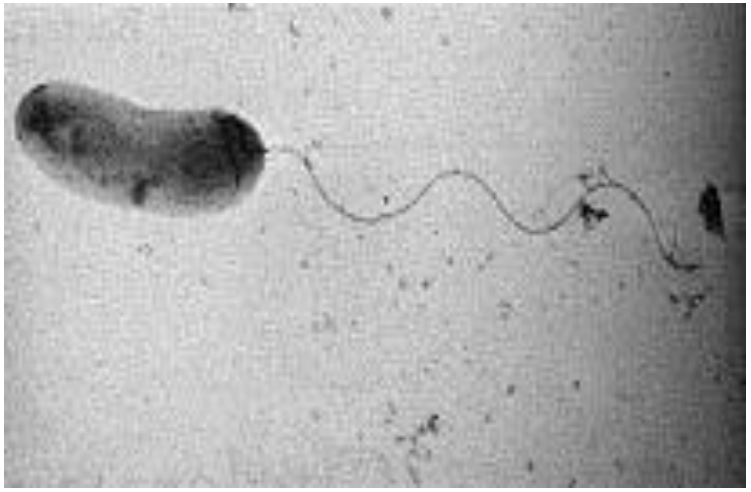
Bacterial flagella

- Organelles responsible for motility of bacteria
  - flagella
  - axial filament (spirochetes)
- Character of movement of flagella
  - rotary
- Bacterial classification according to the number and localization of flagella
  - Monotrichous – single flagellum located at one of the end of the cell
  - Polytrichous – more then one flagella:
    - amphitrichous – two separate flagella localized at the opposite ends of the cell
    - lophotrichous – bunch of flagella localized at the end of the cell
    - peritrichous – many flagella distributed over the surface of bacteria
  - Atrichous – lack of flagella
- Detection of flagella
  - indirect – according to the existence of motility of bacteria
  - direct:
    - Special staining techniques
    - Phase contrast microscopy (for lophotrichous)
    - Electron microscopy

Bacterial flagella







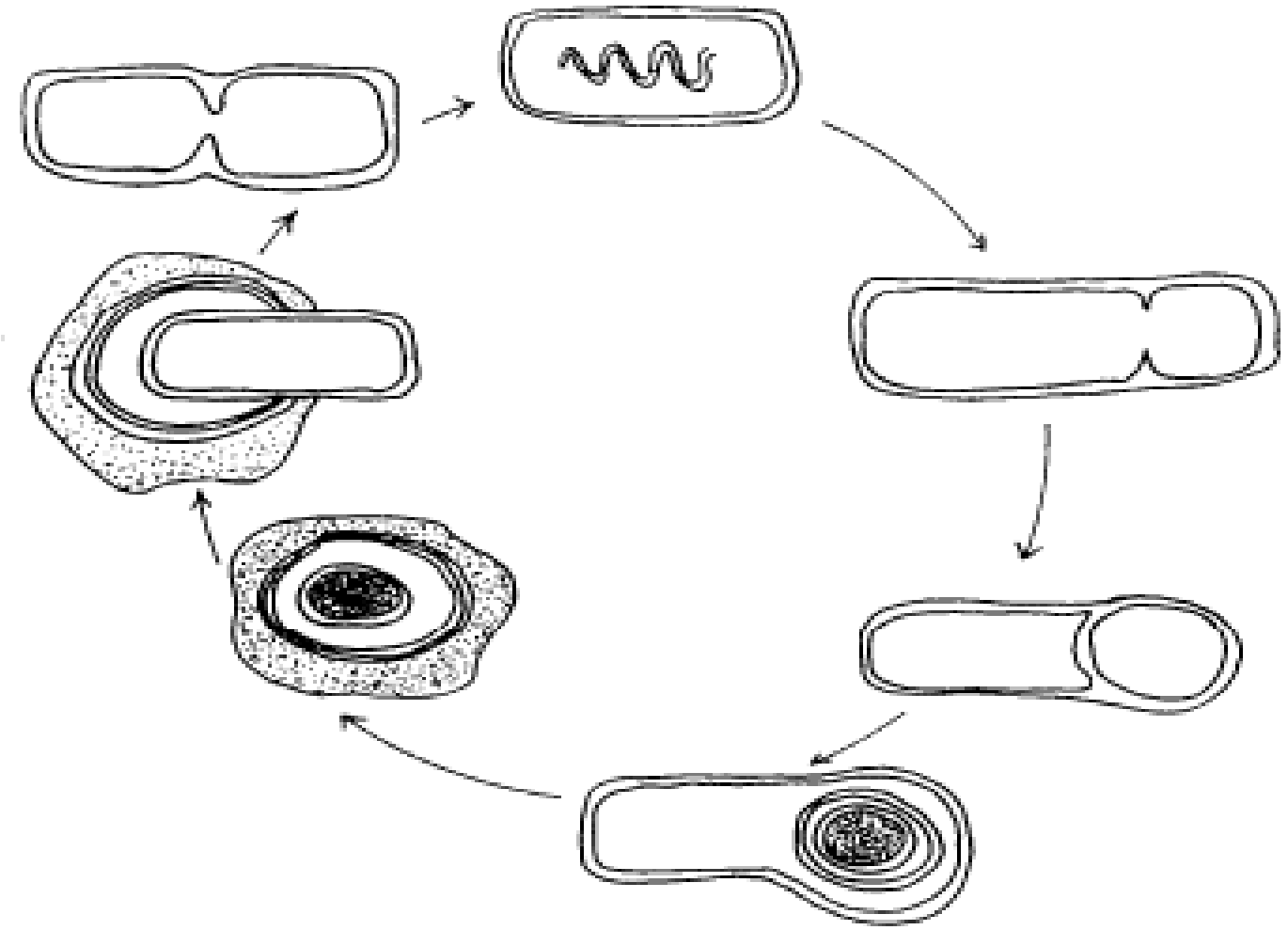
Spore and sporulation in bacteria: endospore

- Definition
  - Reposing (resting) form of the cell which allows it to keep the inheritable information of bacteria in unfavourable conditions of external environment
- Function
  - Defence from:
    - unfavorable physical-chemical factors of external environment
    - exhaustion of nutritive components in medium
- Composition
  - DNA, covered with multilayer spore coat containing peptidoglycan (cortex)
- Conditions for sporulation
  - external environment (not in human organism)
  - artificial nutritive media

Spore and sporulation in bacteria: endospore

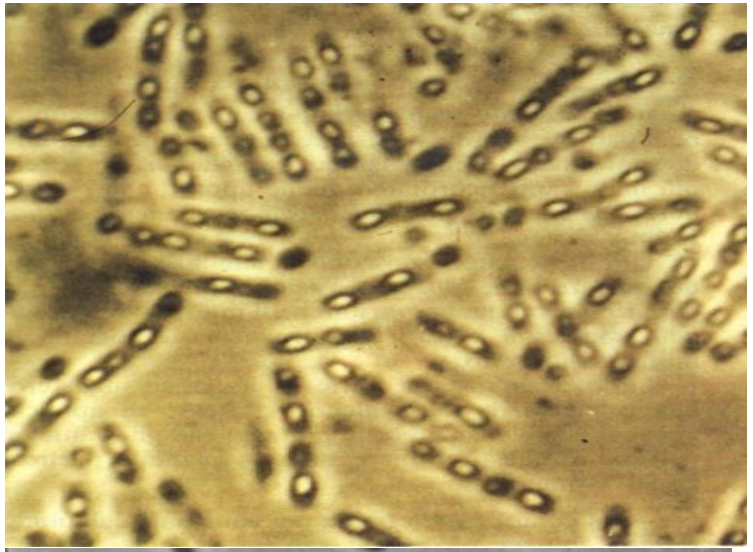
- The factors ensuring thermal resistance
  - practically complete absence of unbound water
  - increased calcium concentration
  - presence of dipicolinic acid
  - especial composition of protein
  - especial composition of peptidoglycan of the cortex

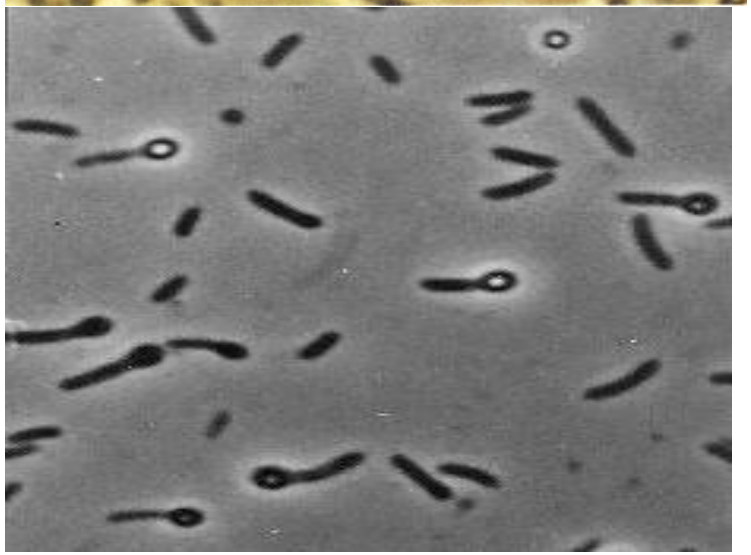
Sporulation in bacteria



Spore and sporulation in bacteria: endospore

- Spore – forming bacteria
  - bacilli (spore doesn't change the cell diameter)
  - clostridia (size of the spore is bigger then cell diameter)





Spore and sporulation in bacteria: endospore

- Detection
  - Ziehl-Neelsen staining technique

Spore and sporulation in bacteria: exospores

- Definition
  - Reproductive structures in streptomycetes
- Differences between exospore and endospore
  - not resistant in unfavorable conditions of external environment
  - forms outside of the bacterial cell
  - one bacterial cell contains many (not single) exospores

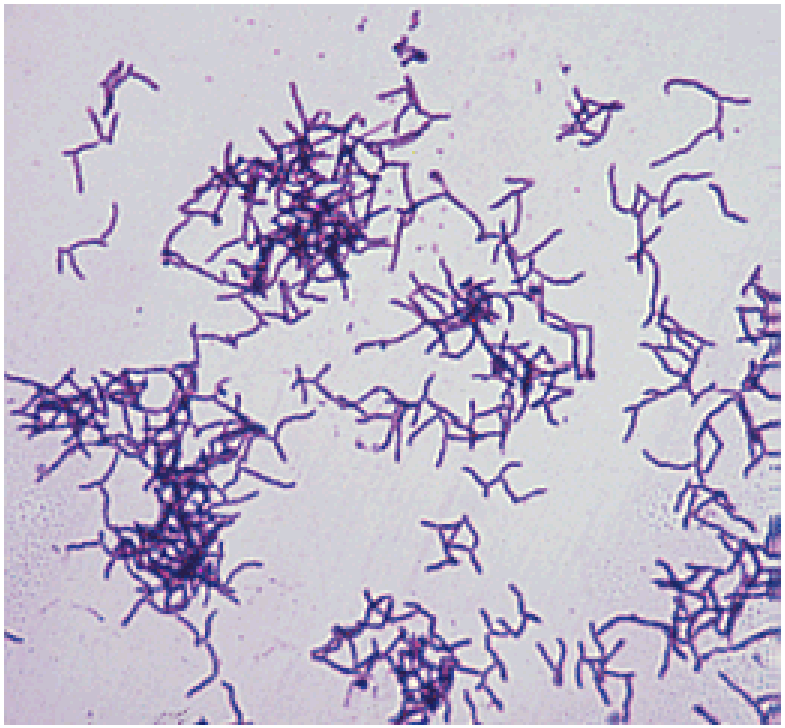
Morphological and ultra structural peculiarities of actinomycetes

Classification	Order	Actinomycetales	
	Family	Actino-mycetaceae	Strepto-mycetaceae
	Genera	Actinomyces	Strepto-myces

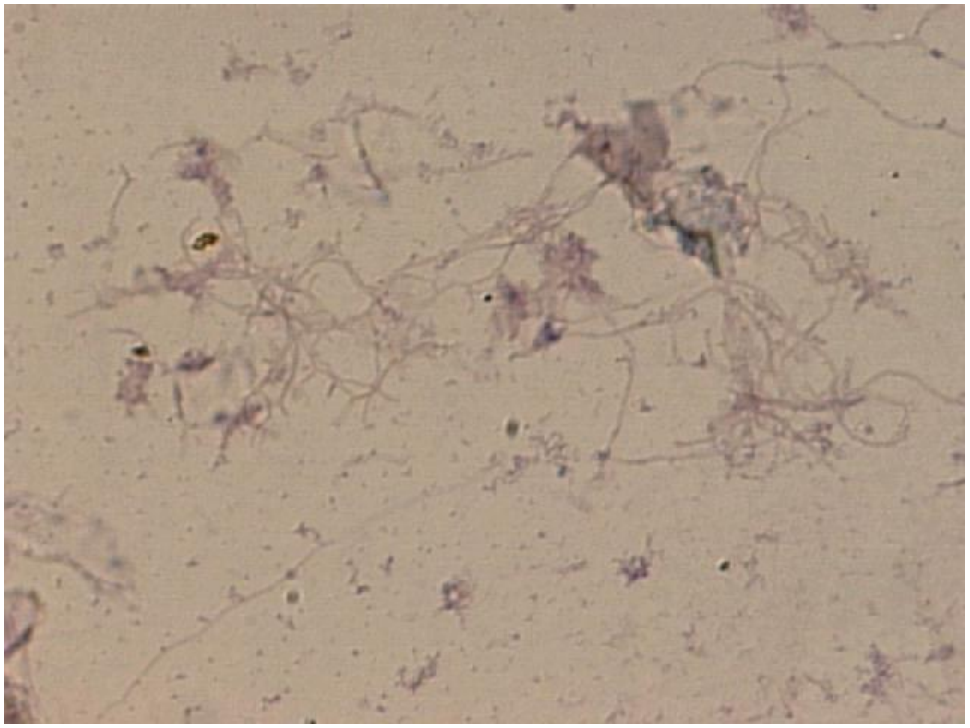
Morphological and ultra structural peculiarities of actinomycetes

	Actinomyces	Streptomyces
Importance for medicine	Cause actinomycosis: in infected tissues form interlaced hyphas – druses, which are calcified in centre	Produce antibiotics
Morphology	Slightly branched rods having flask-like thickening localized at the ends of the cells	Strongly branched threads (hyphae)

Actinomyces



Streptomyces



Morphological and ultra structural peculiarities of actinomycetes

	Actinomyces	Streptomyces
Bacteria forming exospores	–	+
Ultra structural features	Peptidoglycan of the cell wall contains unusual sugars which are not present in other Prokaryotes	

Morphological and ultra structural peculiarities of spirochetes

Classification

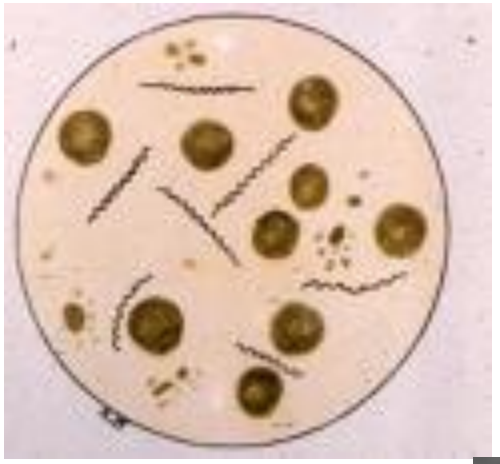
- Order
  - Spirochaetales
- Family
  - Spirochaetaceae
- Genera
  - Treponema
  - Leptospira
  - Borrelia

Morphological and ultra structural peculiarities of spirochetes

Morphological features

- *Treponema*
  - 8-12 coils having regular amplitude
- *Leptospira*
  - Primary coils – practically not visible
  - Secondary coils -(«hooks») localized on the ends and directed to the same or opposite direction
- *Borrelia*

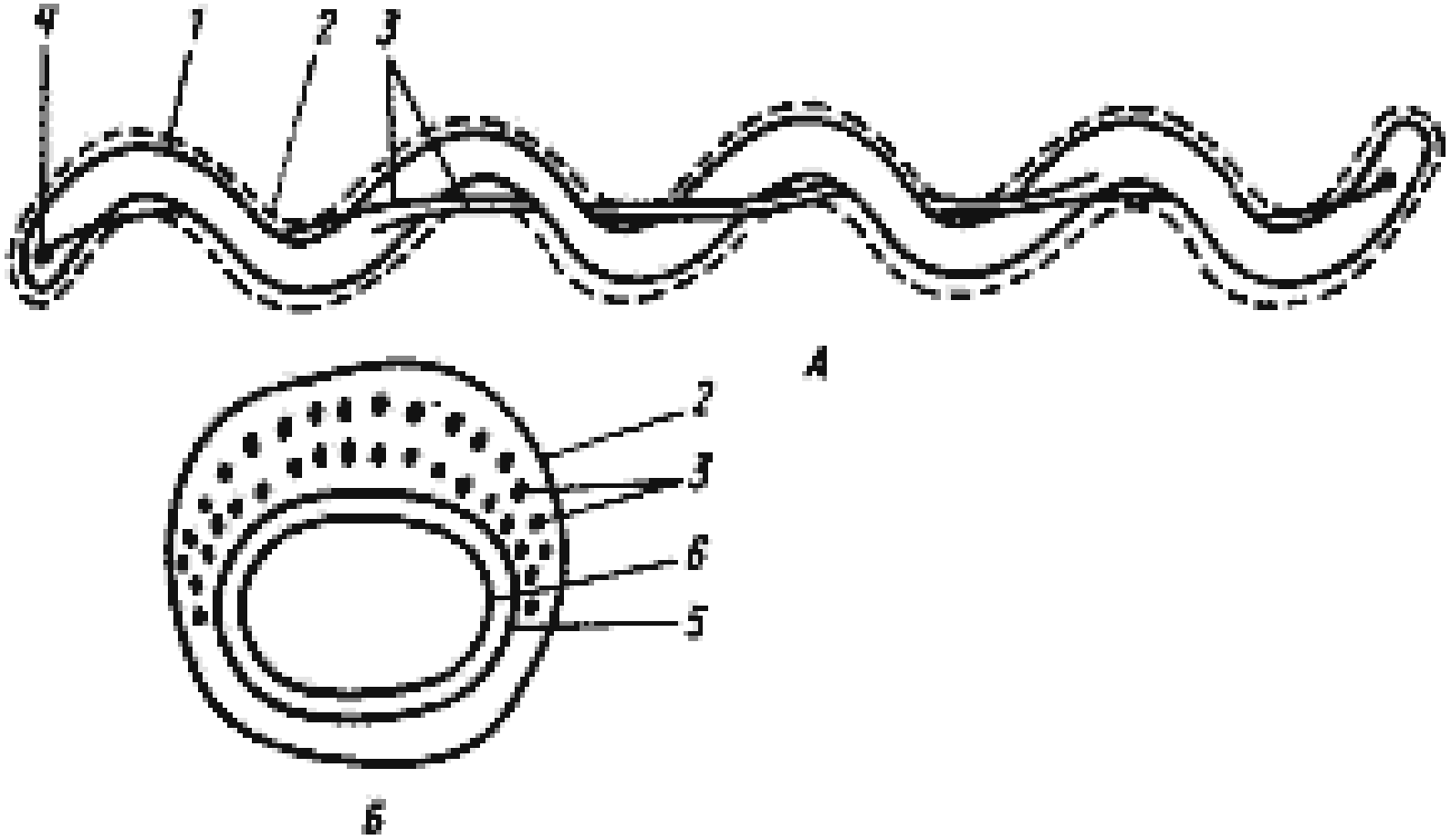
Irregular coils having different number and amplitude



Morphological and ultra structural peculiarities of spirochetes

Ultra structural peculiarities

The organ of motility is an axial filament (fibril) which is localized in periplasmic space of the cell wall and situated along the cell. The filament is built of contractive protein flagellin (the same as flagella). Therefore spirochetes move by the way of contraction of the body of bacterial cell.



1 — protoplasmic cylinder; *2* — outside cover; *3* — axial fibrils; *4* — place of anchoring of axial fibrils; *5* — peptidoglycan layer of the cell wall; *6* — cell membrane.

Morphological and ultra structural peculiarities of spirochetes

Romanovsky- Giemsa staining technique

- Treponema

- Leptospira

- Borrelia

Morphological and ultra structural peculiarities of spirochetes

Microscopy techniques (methods) frequently used for detection

- Treponema
- Leptospira

} dark field microscopy

- Borrelia — any microscopy techniques

Morphological and ultra structural peculiarities of chlamydia

Principal differences between them and other Prokaryotes

Obligate intracellular parasites

Morphological and ultrastructural features of rickettsia and chlamydia

Classification

- Order
  - Rickettsiales
- Family
  - Rickettsiaceae
- Genera
    - Rickettsia
    - Coxiella
    - Rochalimaea

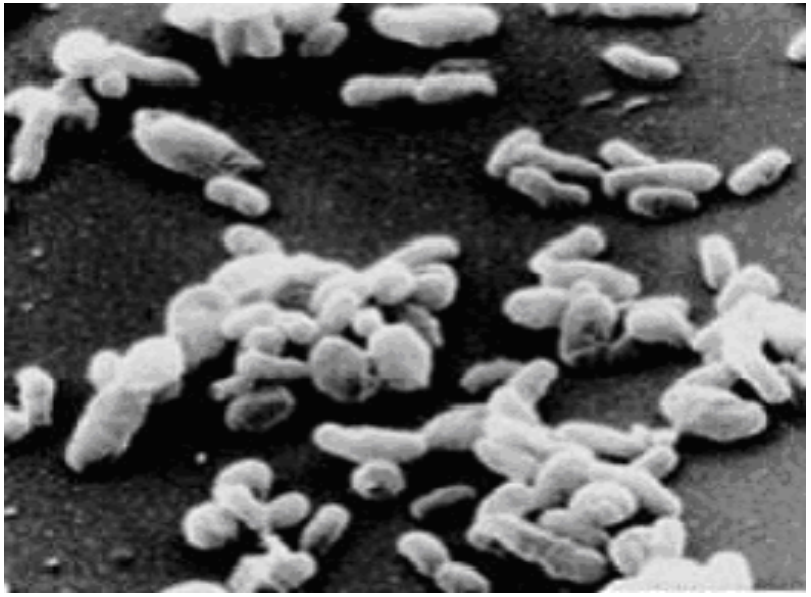
- Order
  - Chlamydiales
- Family
  - Chlamydiaceae

  Genus
  - Chlamydia

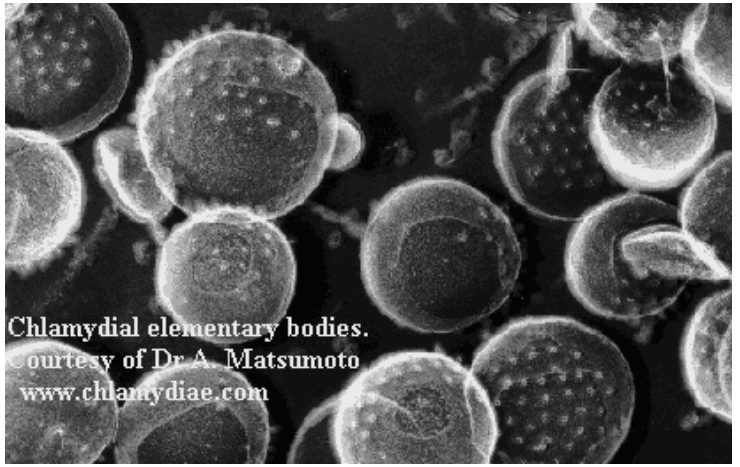
Morphological and ultra structural peculiarities of rickettsia and chlamydia

Shape of bacterial cell

- Rickettsiales
  coccobacteria



- Chlamydiales
  cocci

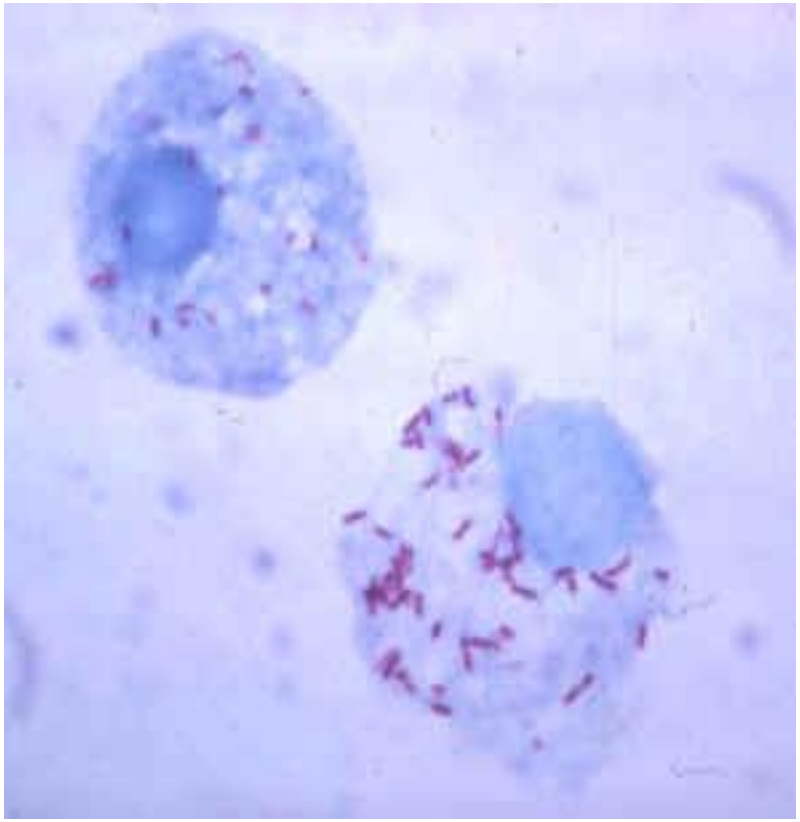


Morphological and ultra structural peculiarities of rickettsia and chlamydia

Localization in the host cell

- Rickettsiales

  Diffusely in cytoplasm and /or in nucleus

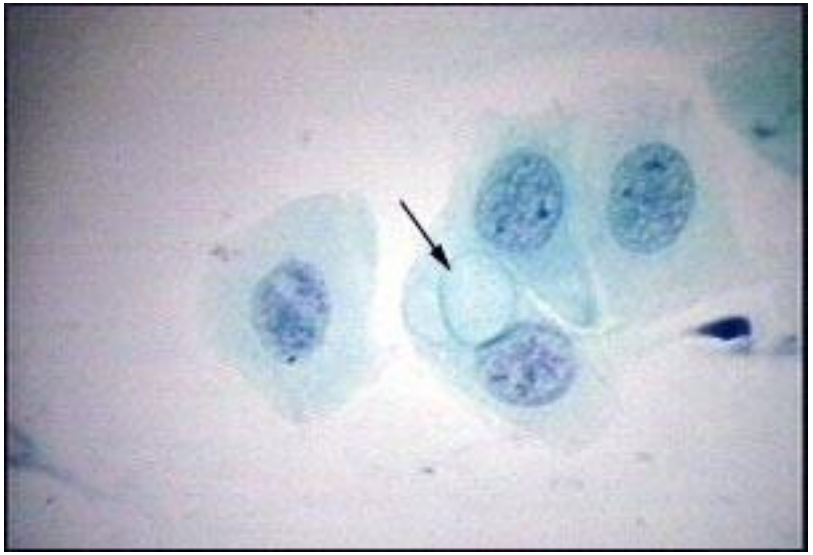


Morphological and ultra structural peculiarities of rickettsia and chlamydia

Localization in the host cell

- Chlamydiales

  Cytoplasm inclusion bodies (microcolonies, covered by coat having origin from the host cell membrane)



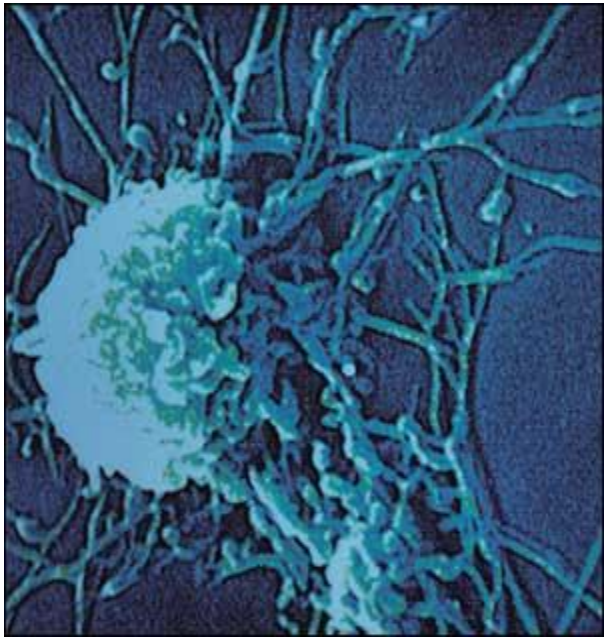
Morphological and ultra structural peculiarities of rickettsia and chlamydia

Staining techniques

- Romanovsky-Giemsa staining
  - Dark blue on the light blue background of the cell
- Zdradovsky staining
  - Pink on the light blue background of the cell

Morphological and ultra structural peculiarities of mycoplasmas

- Principal distinction from other Prokaryotes
  - Lack of the cell wall→ pleomorphic
  - Cell membrane contains sterols
  - Different genome (DNA) composition



Morphological and ultra structural peculiarities of mycoplasmas

- Classification
  - Division
    - Tenericutes
  - Class
    - Mollicutes
  - Order
    - Mycoplasmatales
  - Family
    - Mycoplasmataceae
  - Genera
    - Mycoplasma
    - Ureaplasma

Morphological and ultra structural peculiarities of mycoplasmas

- Study techniques
  - Phase contrast microscopy
  - Electron microscopy

Fungi: classification and taxonomy

Eukaryote - kingdom

- Mycota, divisions:
  - Myxomycota
  - **Eumycota (certain fungi).**

Pathogenic fungi belong to the next classes:

1. **Zygomycetes**
2. **Ascomycetes** } **fungi which have sexual reproduction**
3. **Basidiomycetes**
4. **Deuteromycetes - asexual reproduction - fungi imperfecti**

Structure of fungal cell

- Fungi are eukaryotic organisms
- The fungal cell wall contains polysaccharides presented by:
    - chitin (a structural component of fungal cell wall) which differs from chitin of arthropods (contains less amounts of N)
    - glucans
    - mannans

Fungi: reproduction

- **Sexual Reproduction**

  Sexual reproduction occurs by the fusion of two haploid nuclei (karyogamy), followed by meiotic division of the diploid nucleus.

  The union of two hyphal protoplasts (plasmogamy) may be followed immediately by karyogamy, or it may be separated in time.

- **Asexual Reproduction**

  Asexual reproduction occurs via division of nuclei by mitosis. With the absence of meiosis, other mechanisms associated with the nuclear cycle result in recombination of hereditary properties and genetic variation.

Fungi: morphology

Structurally, fungi exist in two morphological variants:

- Fungi which produce long, branching filaments are multicellular microorganisms and called **moulds.**

  Each filament is called **hypha**. Hyphae could be divided into a chain of cells by the formation of transverse walls – **septa**.

  As the hyphae grow and branch they form a mass called **mycelium**.

  Fungi reproduce by **forming spores (in the case of sexual reproduction) or by forming conidia (asexual fungal spores).**
- Fungi which are unicellular forms (spherical or ovoid) and do not form mycelium are called **yeasts.** They reproduce by **budding**.

Structures of fungi: dimorphism

Phenomenon called dimorphism is characteristic for fungi. They could be presented by:

- filamentous forms (moulds) – usually when grow in the natural environment or in laboratory culture
- unicellular forms (yeasts) – when they grow in the infected tissue

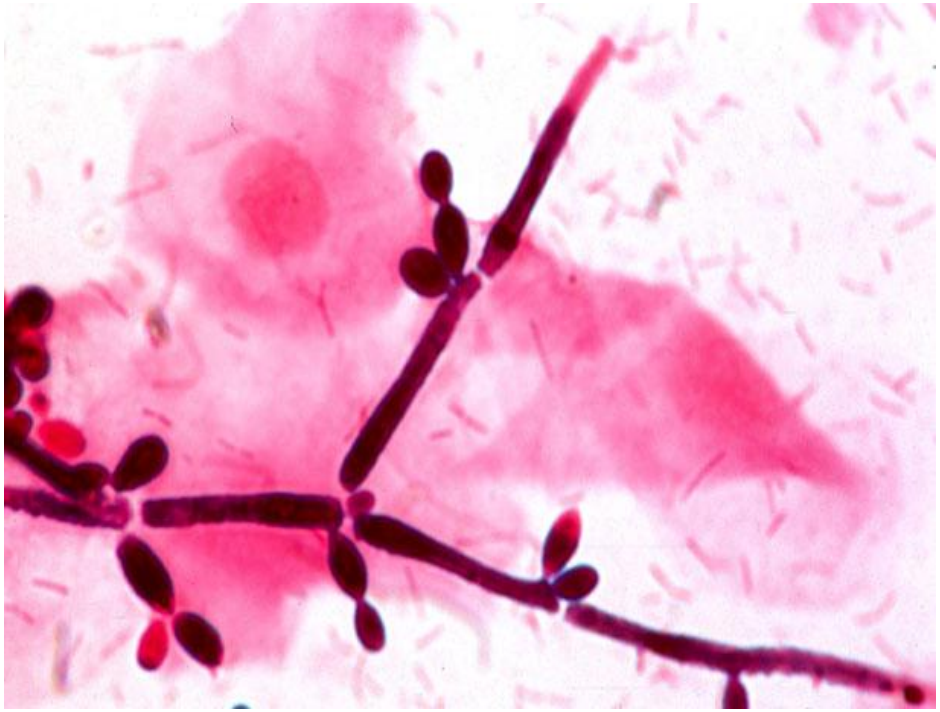
Some fungi are capable to exist in both forms depending upon the environment, nutrients or other conditions. This is phenomenon of the adaptation of fungi to changing conditions of environment.

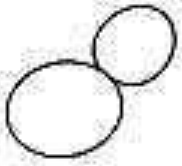
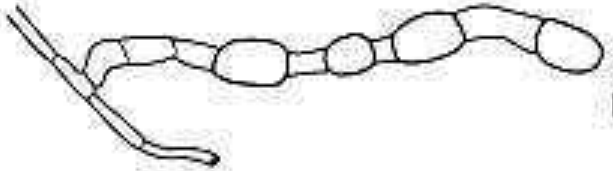
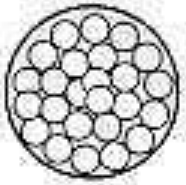
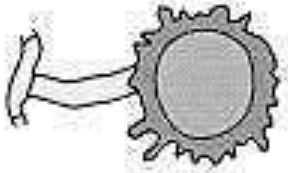
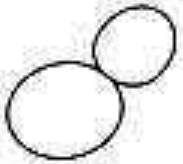
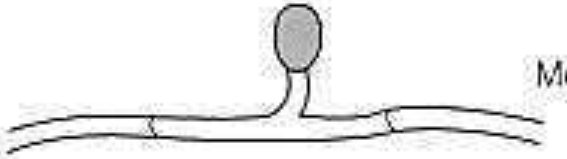
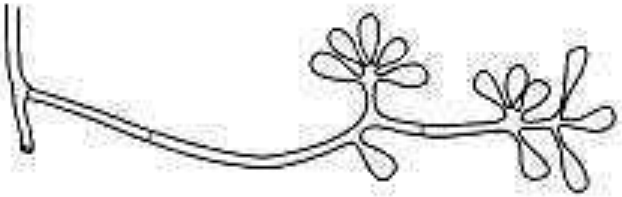
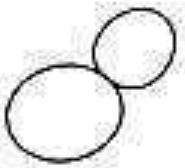
Character of growth of Candida (yeasts) on agar slant

Character of growth of mould on agar medium

Mucor

Candida albicans in the infected tissue



Fungus	In vitro (25° C)	In vivo (37° C)
Blastomyces	 Mold	 Yeast
Coccidioides	 Mold	 Spherule
Histoplasma	 Mold	 Yeast
Paracoccidioides	 Mold	 Yeast
Sporothrix	 Mold	 Yeast

Ziehl-Neelsen staining

- The purposes of application
  - Detection of endospores
  - Detection of mycobacteria

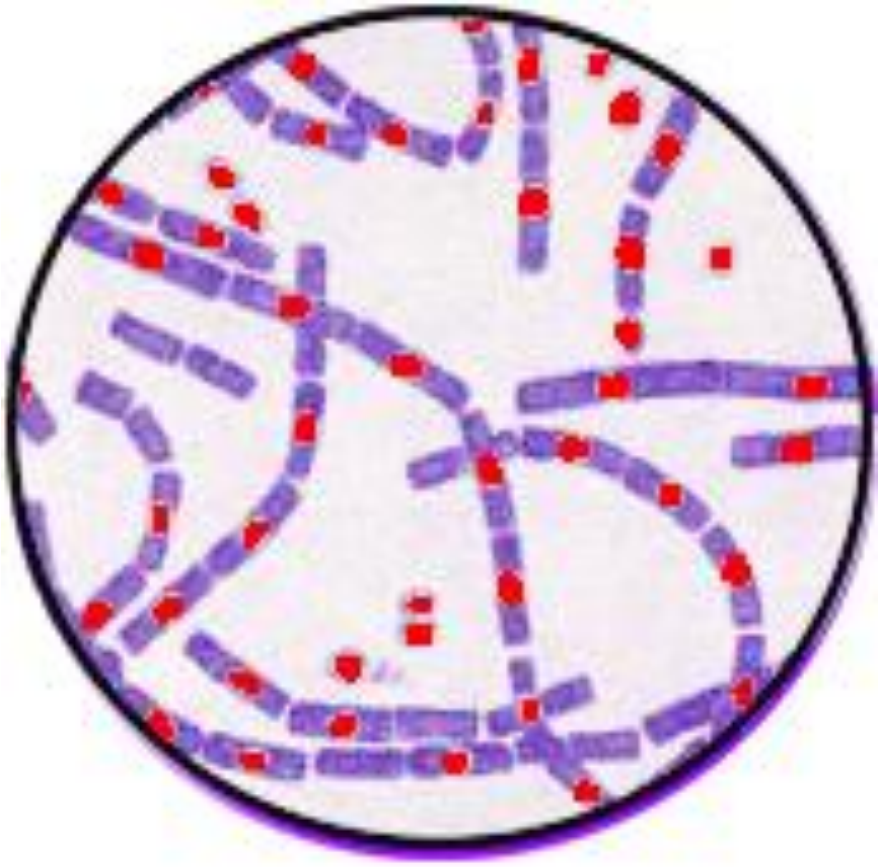
Ziehl-Neelsen staining

- procedure

Stage	Result			
	spores		mycobacteria	
	spore	cytoplasm	mycobacteria	other bacteria
Carbolic fuchsine (with heating)	red	red	red	red
Decolouring with acid	red	uncoloured	red	uncoloured
Additional staining by methylene blue	red	blue	red	blue

Bacillus

Ziehl-Neelsen stain



Physiology of Bacteria

Theme N4

FEATURES OF METABOLISM IN MICROORGANISMS

- Bacteria
  - can use any source of main chemical compounds
  - possess high speed of metabolic processes
  - show high elasticity at the environment
- Viruses
  - do not have their own metabolic enzymes

METABOLISM IN BACTERIA

MAIN ROUTES OF PENETRATION OF NUTRIENTS INTO BACTERIAL CELL

- Without energy consumption (diffusion)
  - ordinary
  - facilitated (involves activity of enzymes – permeases)
- With energy consumption (involves activity of enzymes – permeases)
  - without chemical modification of transferred molecules - active transport
  - with chemical modification of transferred molecules - translocation of chemical groups

THE CLASSIFICATION OF BACTERIA BY THE SOURCE OF CARBON

- Inorganic compounds: CO_2 or carbonates – autotrophic bacteria
- Organic compounds – heterotrophic bacteria
  - compounds of the environment – saprophytes
  - compounds of an alive cell – parasites

Parasites which:

- can also use organic compounds of the environment – facultative parasites (majority of pathogenic bacteria)
- can only use organic compounds of an alive cell – obligate parasites
  - ***Rickettsia***
  - ***Chlamydia***

CLASSIFICATION OF BACTERIA BY THEIR GROWTH FACTORS NEEDS

PROTOTROPHIC BACTERIA

- can synthesize growth factors (nitrogen bases, amino acids, vitamins, lipids, etc) by themselves

AUXOTROPHIC BACTERIA

- cannot synthesize growth factors and require their adding to the growth media

CLASSIFICATION OF BACTERIA BY THE FEATURES OF THEIR ENERGY METABOLISM

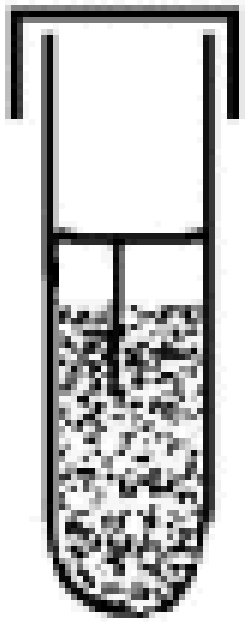
- energy source – the sunlight (phototrophs)
- energy source – oxidation-reduction reactions with ATP synthesis as a result of the reaction (chemotrophs)
  - electron donor
    - inorganic compounds (lithotrophs)
    - organic compounds (organotrophs)
  - electron acceptor
    - external (oxidation)
      - oxygen – aerobic respiration
      - others (nitrate, fumarate) – anaerobic respiration
    - internal– organic compounds of the cell (fermentation)

CLASSIFICATION OF BACTERIA BY THEIR REQUIREMENTS OF THE OXYGEN IN THE AIR

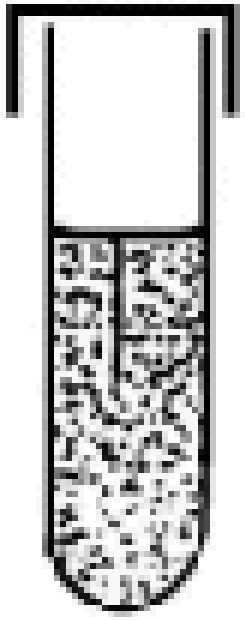
Group	Presence of enzymes, neutralizing toxic products of oxidation in bacteria		In presence of oxygen	Without the oxygen in the air
	superoxide dismutase ($O^{\bullet} \rightarrow H_2O_2$)	catalase ($H_2O_2 \rightarrow H_2O + O_2$)		
Obligate aerobes	+	+	grow	do not grow
Microaero-philes	+	±	$\downarrow O_2$ – grow	do not grow
Capnophiles	+	±	$\uparrow CO_2$ - grow	

An-aerobes	Presence of enzymes, neutralizing toxic products of oxidation in bacteria		In presence of oxygen	Without the oxygen in the air
	superoxide dismutase ($O^{\bullet} \rightarrow H_2O_2$)	catalase ($H_2O_2 \rightarrow H_2O + O_2$)		
Aero-tolerant	+	–	do not grow, but do not perish	grow
Obligate	–	–	perish	grow
Facultative (most of bacteria)	+	+	grow	grow

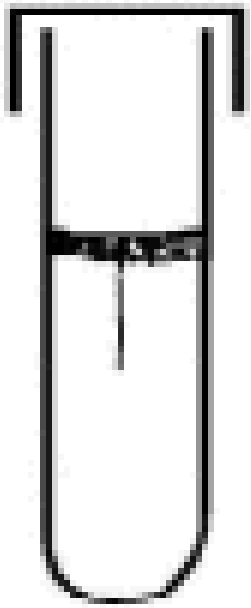
Obligate anaerobes



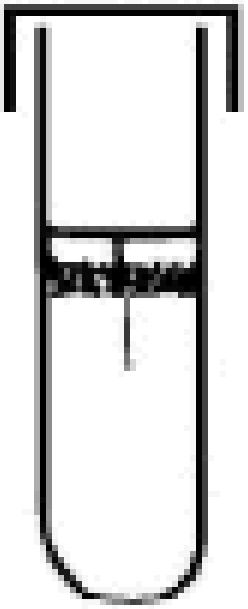
Facultative anaerobes



Obligate aerobes



Microaerophiles



FEATURES OF METABOLISM IN RICKETTSIA, CHLAMYDIA AND MYCOPLASMAS

- Rickettsia

  are incapable of synthesizing some macromolecules necessary for their metabolism (NAD) → obligate intracellular parasites

- Chlamydia
  - are incapable of synthesizing some macromolecules necessary for their metabolism → obligate intracellular parasites
  - are incapable of synthesizing ATP – «energy parasites»
- Mycoplasmas

  are incapable of synthesizing sterols for own cytoplasmic membrane – «membrane parasites»

REPRODUCTION OF BACTERIA AND MAIN PRINCIPLES OF THEIR CULTIVATION

WAYS OF REPRODUCTION OF BACTERIA

- Binary division (simple transverse division)

  most of bacteria:

  – partition develops from CW (cell wall) towards the centre of a cell – G^+

  – cellular strangulation (a cell makes thinner in the middle) – G^-
- Budding
  – Francisella
  – Mycoplasma
- Filamentary forms segmenting
  – Actinomycetes
  – Mycoplasmas
- Exospores

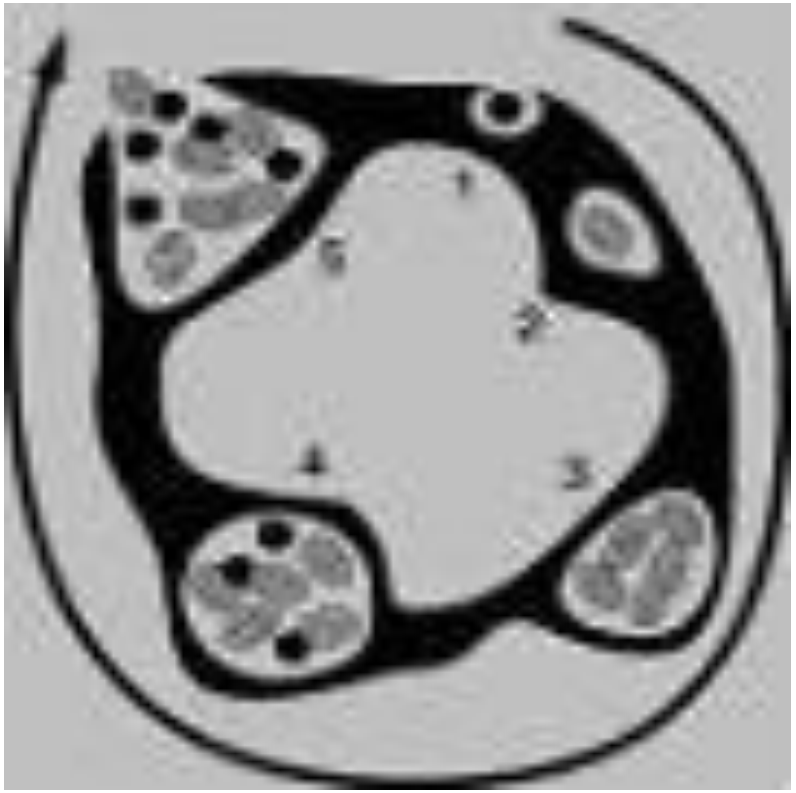
  Streptomycetes
- Particular cycle of division

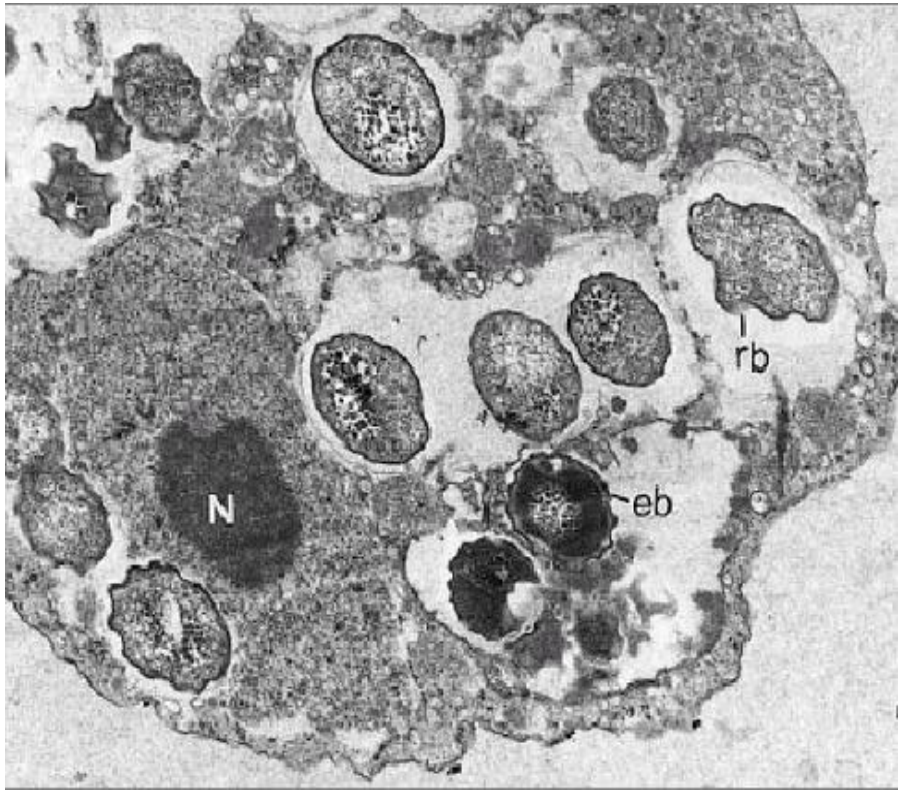
  Chlamydia

THE DEVELOPMENTAL CYCLE OF CHLAMYDIA

Stage	Function
Elementary body	Infectious form (penetration into a host cell by invagination the place of adsorption)
Reticulate (initial) body	Reproductive form (reproduction by binary division → forming a body inclusion – micro colony in the host cell cytoplasm)

THE DEVELOPMENTAL CYCLE OF CHLAMYDIA





CLASSIFICATION OF MEDIA (CULTURE MEDIA)

- in consistency
  - liquid
  - semisolid (0,5% agar)
  - solid (1,5-2% agar, coagulated)

CLASSIFICATION OF MEDIA (CULTURE MEDIA)

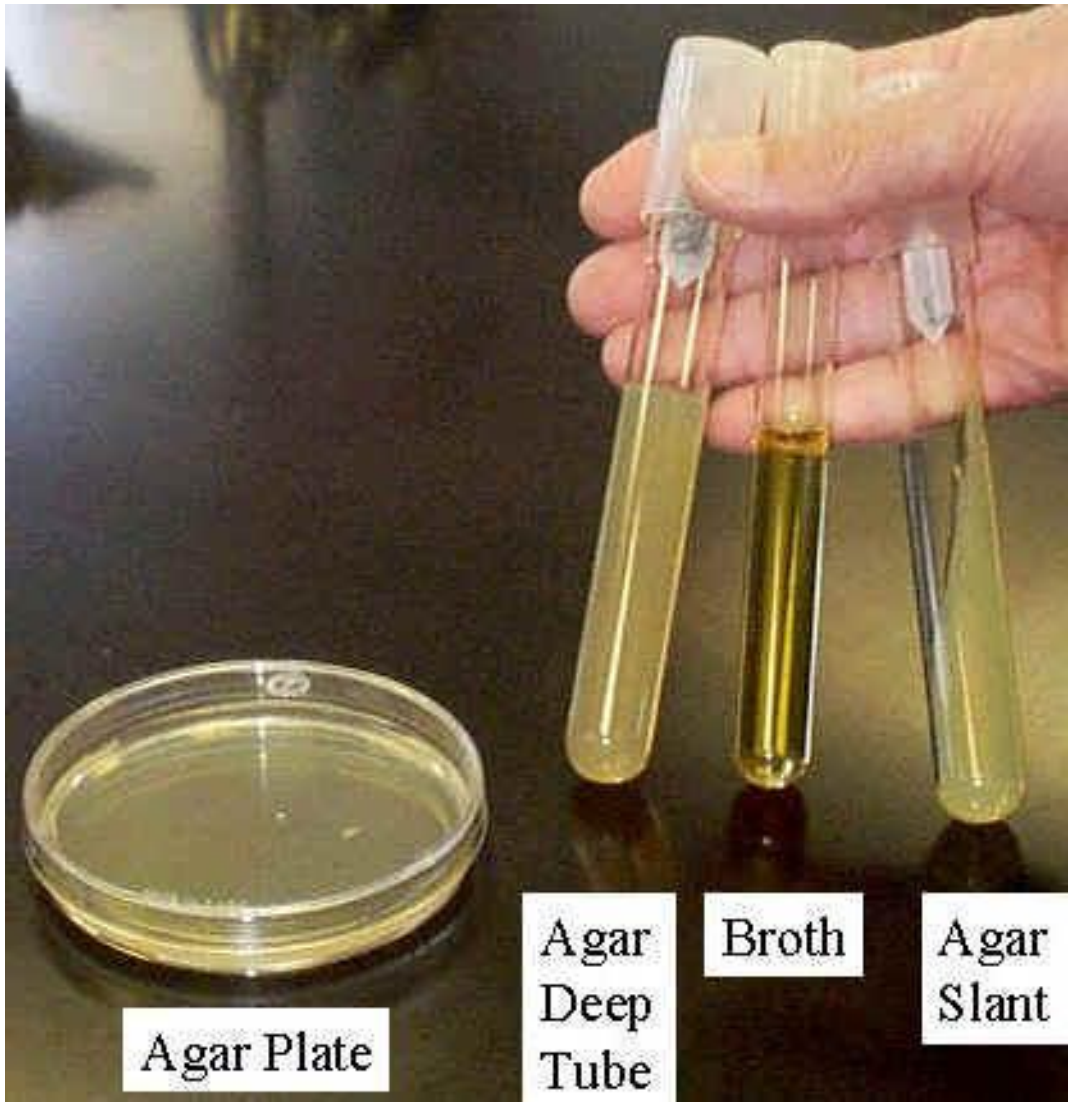
- in composition
  - natural
    - simple
      - meat-peptone broth and agar (MPB and MPA)
      - gelatin
      - milk
      - bits of vegetables
    - complex (compound): simple media+ additional components
  - synthetic

CLASSIFICATION OF MEDIA (CULTURE MEDIA)

- in destination
  - fundamental
    - universal (simple natural)
    - special (compound natural)
  - elective (selective)
  - differential diagnostic
  - conservation

- Agar – is a complex organic substance received from marine algae. It melts in water at 80-86^{o} C and solidifies at 36-40^{o} C
- Coagulated nutrient media – are solid media with serum or high percentage of albumin (from eggs, e.g.) witch condenses by warming during sterilization
- Natural media are prepared on basis of decoctions, extracts from meat, fish, vegetables and other natural products
- Simple natural media are such decoctions or extracts
- Complex (compound) natural media are prepared by adding any matters to simple natural media (coloring agent, sugar, antibiotic, blood, etc.)

- Synthetic nutrient media are prepared by mixing pure chemical substance (salts, as a rule).
- elective (selective, enriched) media are the ones in which only certain species of bacteria grow well, and other species either grow poorly or do not grow at all. Such media are quite often employed in laboratory practice.
- Differential diagnostic media are used to distinguish among analogous bacteria by their fermentative activity or cultural properties.
- Conservation media are used for primary seeding and transportation of the material for diagnostics (specimens); they prevent the death of microbes, but the bacteria cells do not multiply in them.


Agar Plate
Agar Deep Tube
Broth
Agar Slant

BACTERIAL CULTURE REQUIREMENTS

- Nutrient needs
  - simple – the culture grows in universal nutrient media
  - complicated – the culture grows in special nutrient media
- The temperature optimal for cultivation
  - ≈ 37°C – mesophiles
  - 6 – 20°C – psychrophiles
  - 50 – 60°C – thermophiles

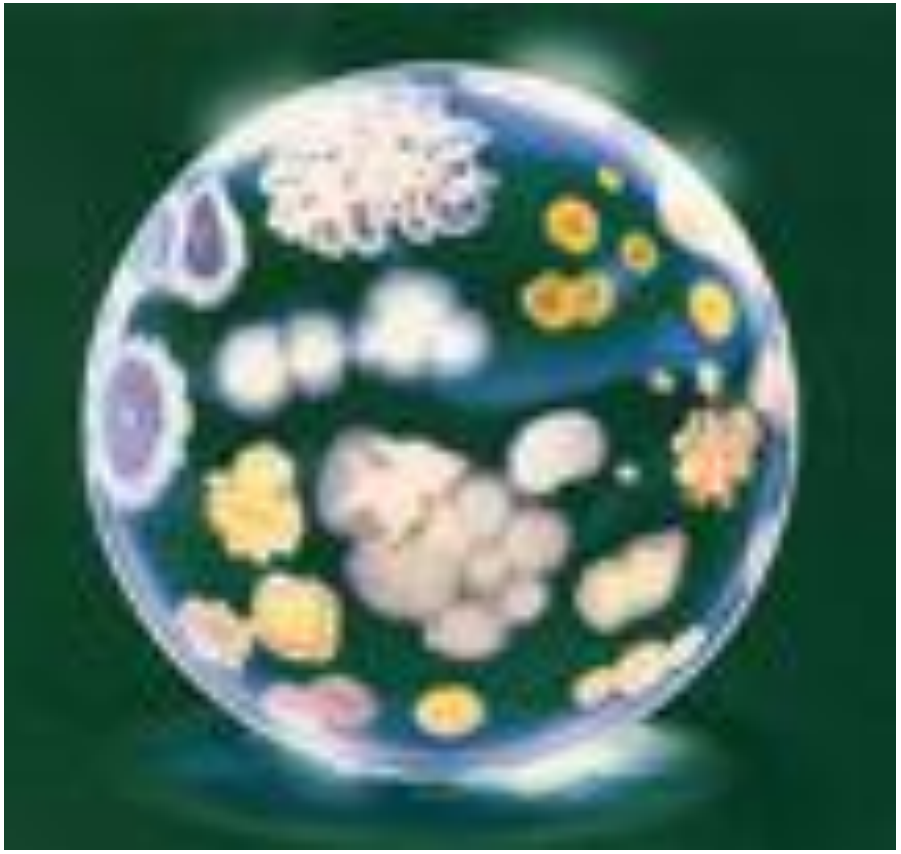
THE CHARACTER OF BACTERIAL GROWTH IN NUTRIENT MEDIA

- Liquid nutrient media
  - a diffuse suspension – the most of bacteria
  - a film (pellicle) – «Koch's bacteria»
  - near-bottom or parietal (near-wall) growth – streptococci
  - pellicle on the surface with thread-like growth resembling stalactites and a flocculent precipitate – Yersinia pestis

THE CHARACTER OF BACTERIAL GROWTH IN NUTRIENT MEDIA

- Solid nutrient media – bacteria form colonies on agar plate:
  - S-shaped («smooth»)
    - cocci
    - G– rods, excepting Yersinia pestis
  - R-shaped («rough»)
    - G+ rods
    - Yersinia pestis

Bacterial growth in liquid medium and on agar plate



Physiology of Bacteria (continuation). Method of Cultivation of Bacteria. Bacteriophages

Theme N5

ANAEROBIC TECHNIQUES USED FOR BACTERIAL CULTIVATION

KITT-TAROZZI'S METHOD OF CULTIVATION OF ANAEROBIC BACTERIA

- a Kitt-Tarozzi's medium
  - broth containing glucose
  - on the surface of the medium – liquid vaseline
  - at the bottom – pieces of animal parenchymatous organs (liver)

METHOD OF GROWING OF BACTERIAL CULTURE

THE PRINCIPLE SCHEME OF THE METHOD: PRELIMINARY STAGE

Soporiferous bacteria isolation

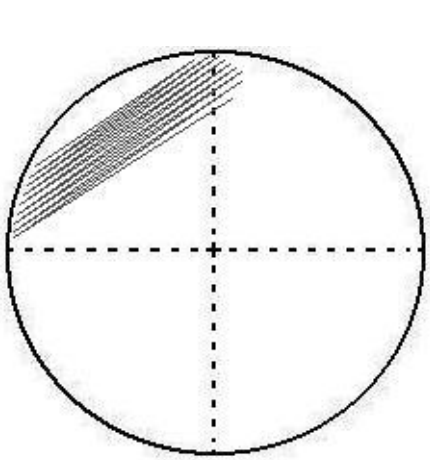
- aerobes and facultative anaerobes
  - the preliminary stage is absent
- anaerobes (obligate and aerotolerant bacteria)
  - microscopic investigation of the specimen (PM – pathological material) smear
  - seeding of the specimen on a Kitt-Tarozzi's medium (into two tubes)
    - one tube is warmed up at 80°C for 15 minutes
    - and another one is native (there is no temperature influence)

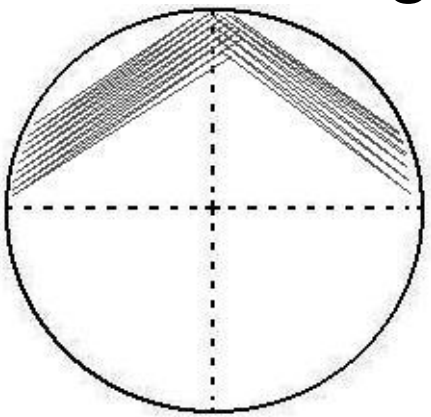
I stage

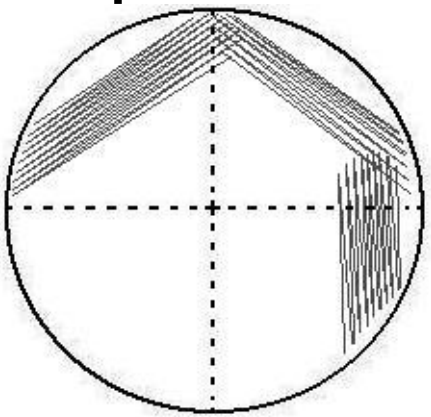
Separate growth establishment (isolation of a pure culture)

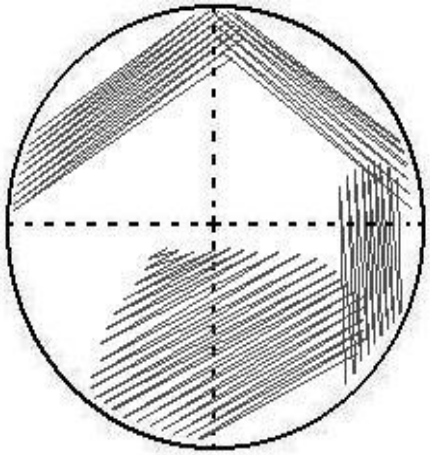
- aerobes and facultative anaerobes
  - microscopic investigation of the smear prepared with use of the specimen
  - seeding of the material of the specimen on an agar plate with use of bacteriological loop (streaking) or with use of spatula (by Drigalsky) to get separate growth – colonies
- anaerobes (obligate and aerotolerant)
  - microscopic investigation of of the smear prepared with use of the specimen
  - seeding of the specimen to get separate growth (by Zeissler or by Weinberg)

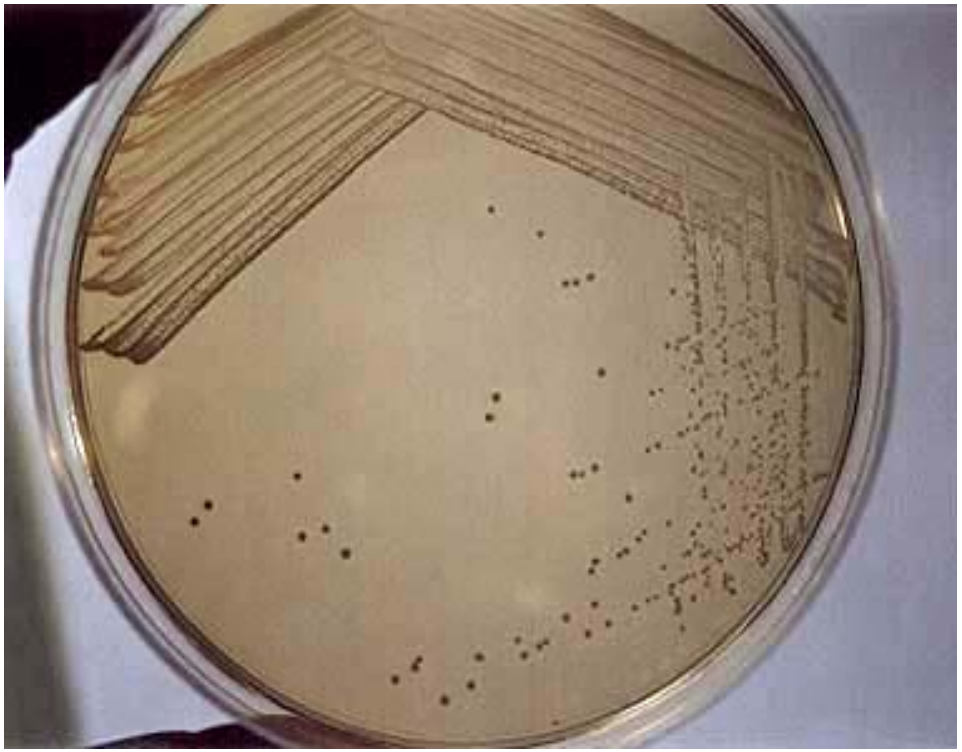
Seeding of the specimen on an agar plate with use of streaking technique











II stage

Accumulation of the pure culture

- aerobes and facultative anaerobes
  - scrutinizing grown colonies
  - microscopic investigation of the smear prepared with use of the material got from the separate colony
  - agglutination reaction of the material got from the separate colony with multivalent sera
  - seeding of the material got from colony on an agar slant
- anaerobes (obligate and aerotolerant)
  - analysis of the colonies grown in agar media
  - microscopic investigation of the smear prepared with use of the separate colony
  - seeding of the colony in Kitt-Tarozzi's medium

III stage

Final pure culture identification

- aerobes and facultative anaerobes
  - microscopic investigation of the smear prepared from pure culture
  - agglutination reaction of the material from pure culture with monovalent sera (serological identification of species and serotype)
  - study of biochemical properties of bacteria from pure culture
  - study of virulence of the bacteria
  - determination of epidemiologic markers
- anaerobes (obligate and aero tolerant)
  - microscopic investigation of the smears prepared with use of the material from pure culture
  - study of biochemical properties of the bacteria
  - detection and identification of exotoxin produced by bacteria from pure culture

CULTURAL FEATURES OF BACTERIA

- nutrient needs
- optimal nutrient medium
- temperature conditions optimal for growth
- aeration conditions optimal for growth
- rate of growth of bacteria
- characteristics of bacterial growth in liquid and solid nutrient media

STUDY OF BIOCHEMICAL PROPERTIES OF BACTERIA
(on the example of enterobacteriae): I stage

- Nutrient (culture media), methods

  Differential diagnostic media:

  – Endo agar
  – Levine agar
  – Ploskirev agar

- The principle of the method:

  utilization of lactose contained in the media by bacteria

  ⇓

  pH shift to acidic region

  ⇓

  change of the colour of the colony

II stage

- Nutrient (culture) media, methods

  Media for accumulation of bacteria and their primary identification:

  – Ressel (glucose+lactose)
  – Kliegler (glucose+lactose+H_2S)
  – Olkenitsky (glucose+lactose+H_2S+urea)

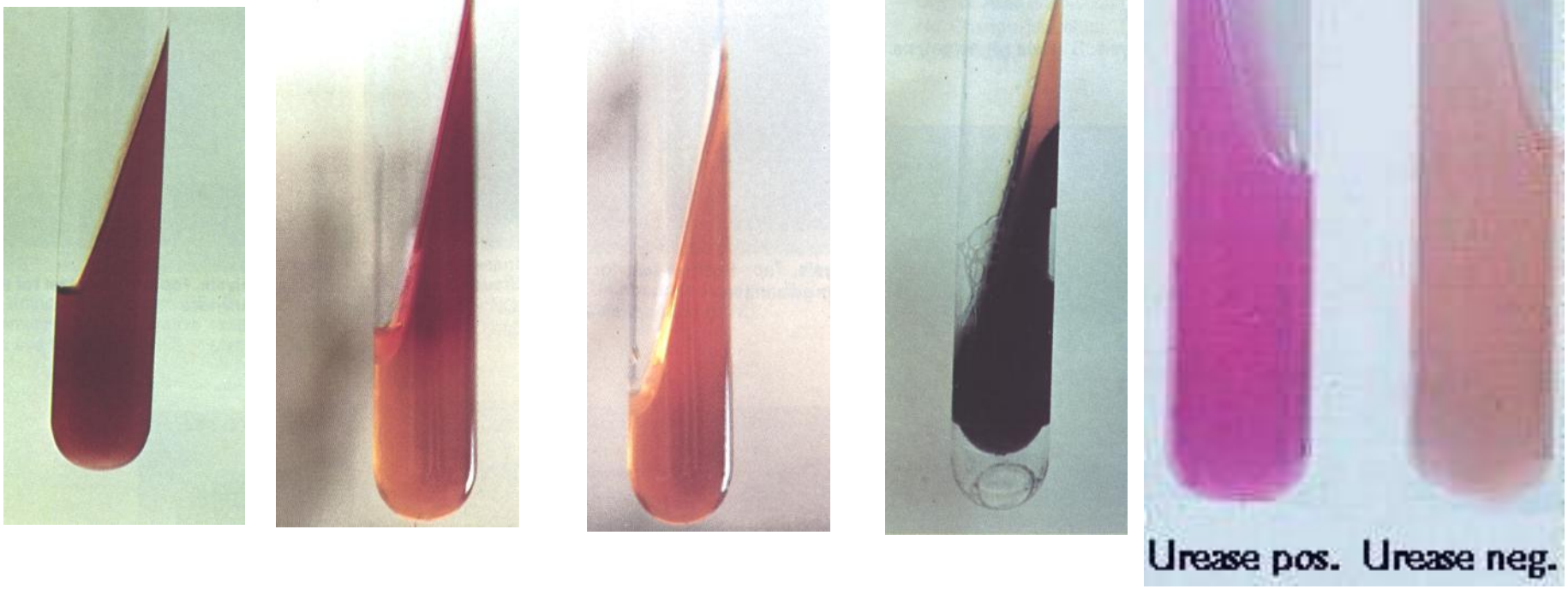
- The principle of the method
  – glucose utilization $\Rightarrow$ colour is changed only in agar stub
  – lactose utilization $\Rightarrow$ colour is getting changed both in agar stab and in agar slant
  – production of H_2S (hydrogen sulphite)$\Rightarrow$ the colour of the media is changing to black
  – urine utilization $\Rightarrow$ the colour of the media is changing to red

Media for accumulation of bacteria and their primary identification

Ressel

Kliegler

Olkenitsky



III stage: determination of carbohydrolytic activity

- Nutrient media, methods

  Hiss media (in a short Hiss row – the semisolid media containing lactose, glucose, mannitol, maltose and saccharose)

- The principle of the method

utilization of carbohydrate contained in the media

⇓

pH shift to acidic region

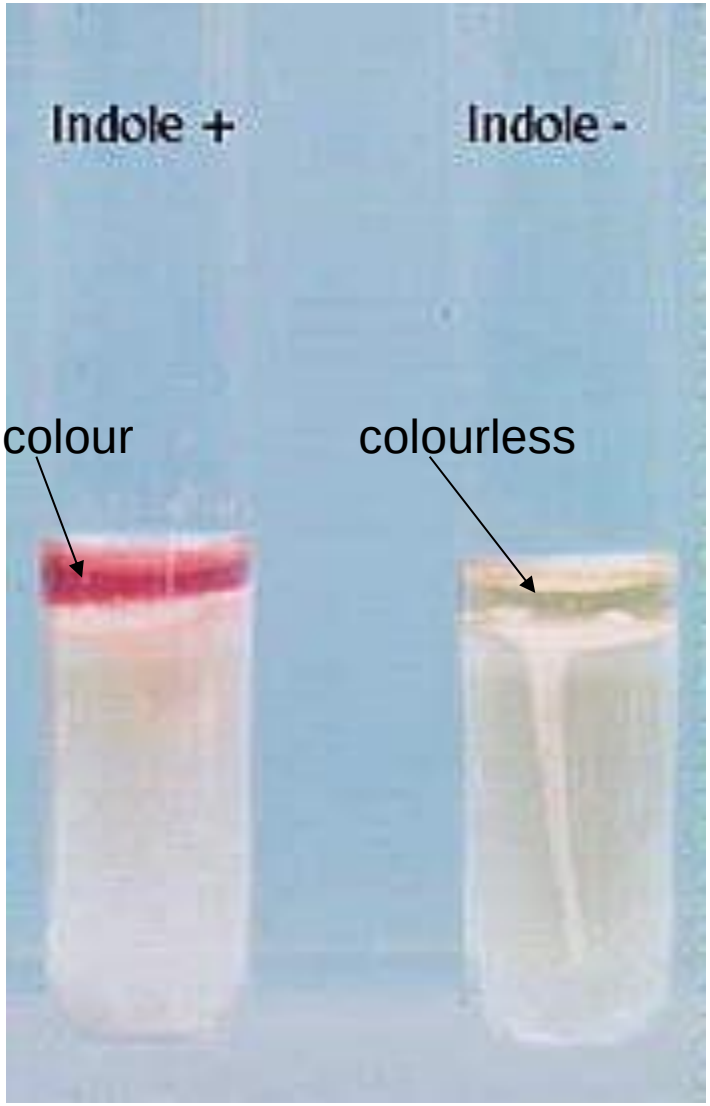
⇓

change in the colour of the medium

III stage: determination of proteolytic activity

- Nutrient media, methods
  - gelatin containing media
  - indole production
  - ammonia production
  - H_2S production
- Visible effect of the positive result of the tests
  - liquefaction
  - the specific reagent turns red
  - litmus paper turns blue
  - see Kliegler and Olkenitsky media

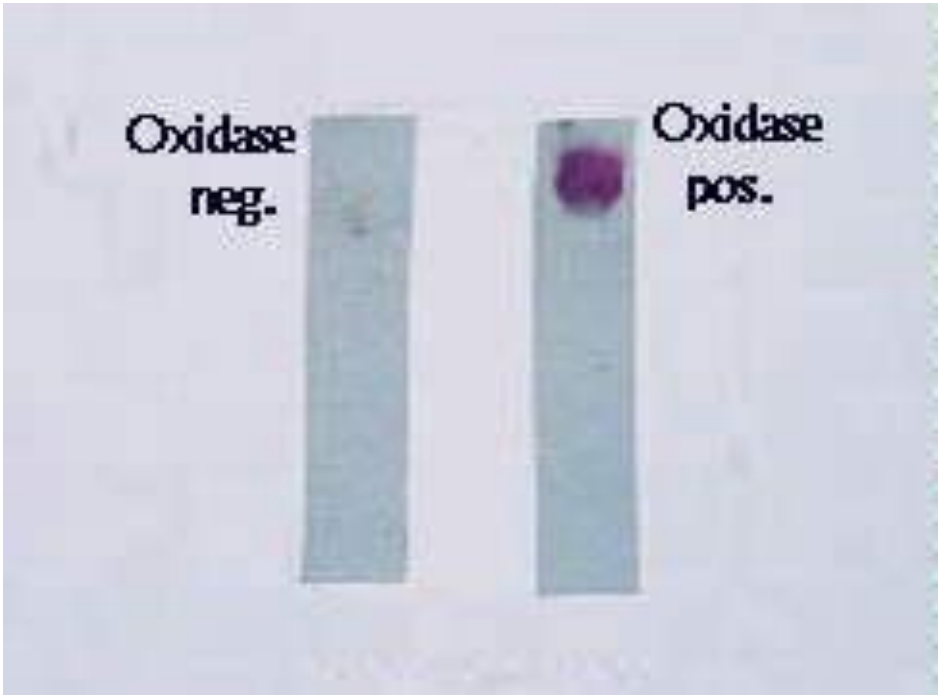
Determination of proteolytic activity: indole production



III stage: determination of the activity of separate enzymes

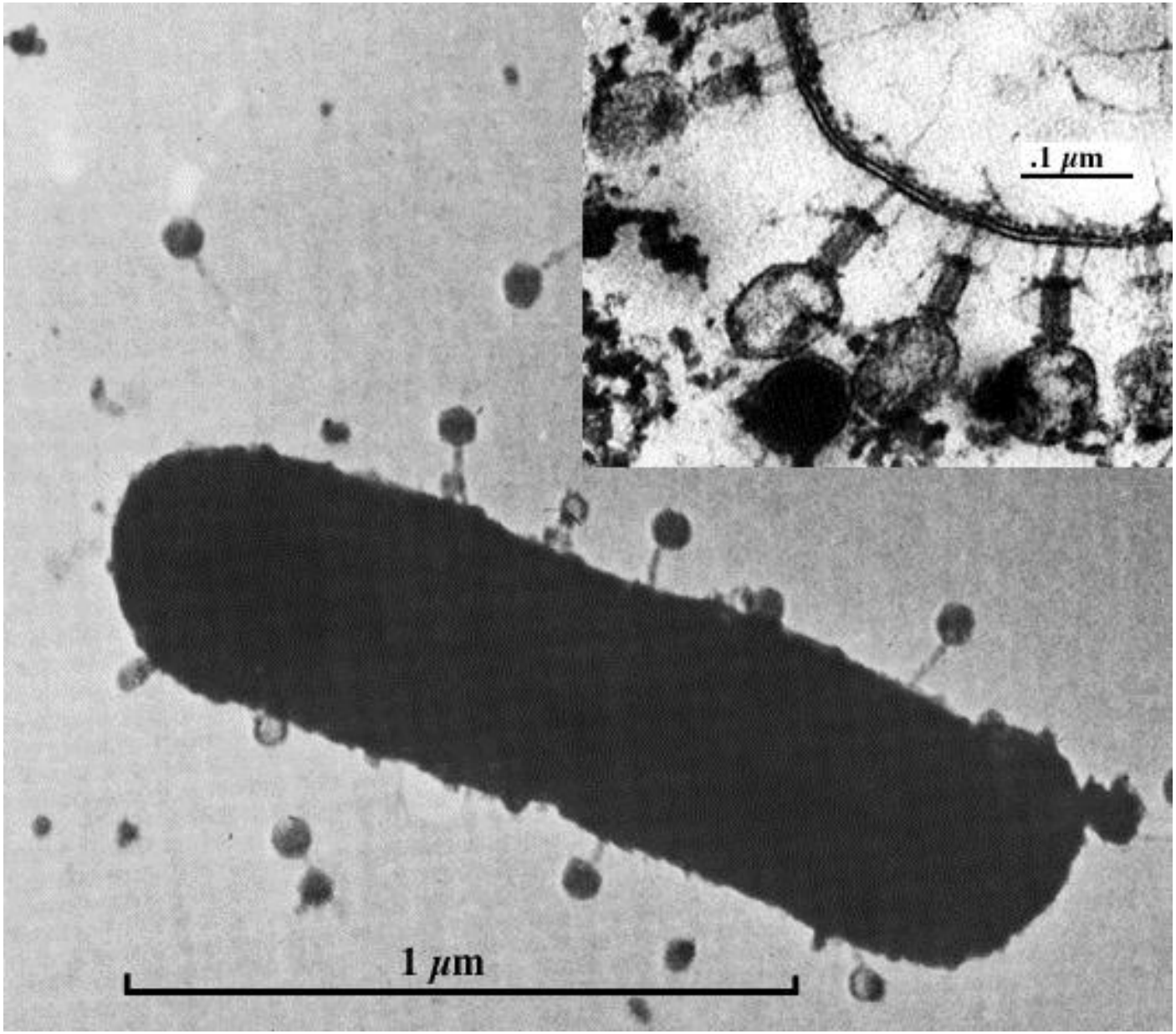
- Nutrient media, methods
  - catalase activity
  - oxidase activity
- Visible effect in the positive result of the tests
  - gas-production when mixing the bacterial culture with hydrogen peroxide on the glass slide
  - appearance of red colour on the test paper strips

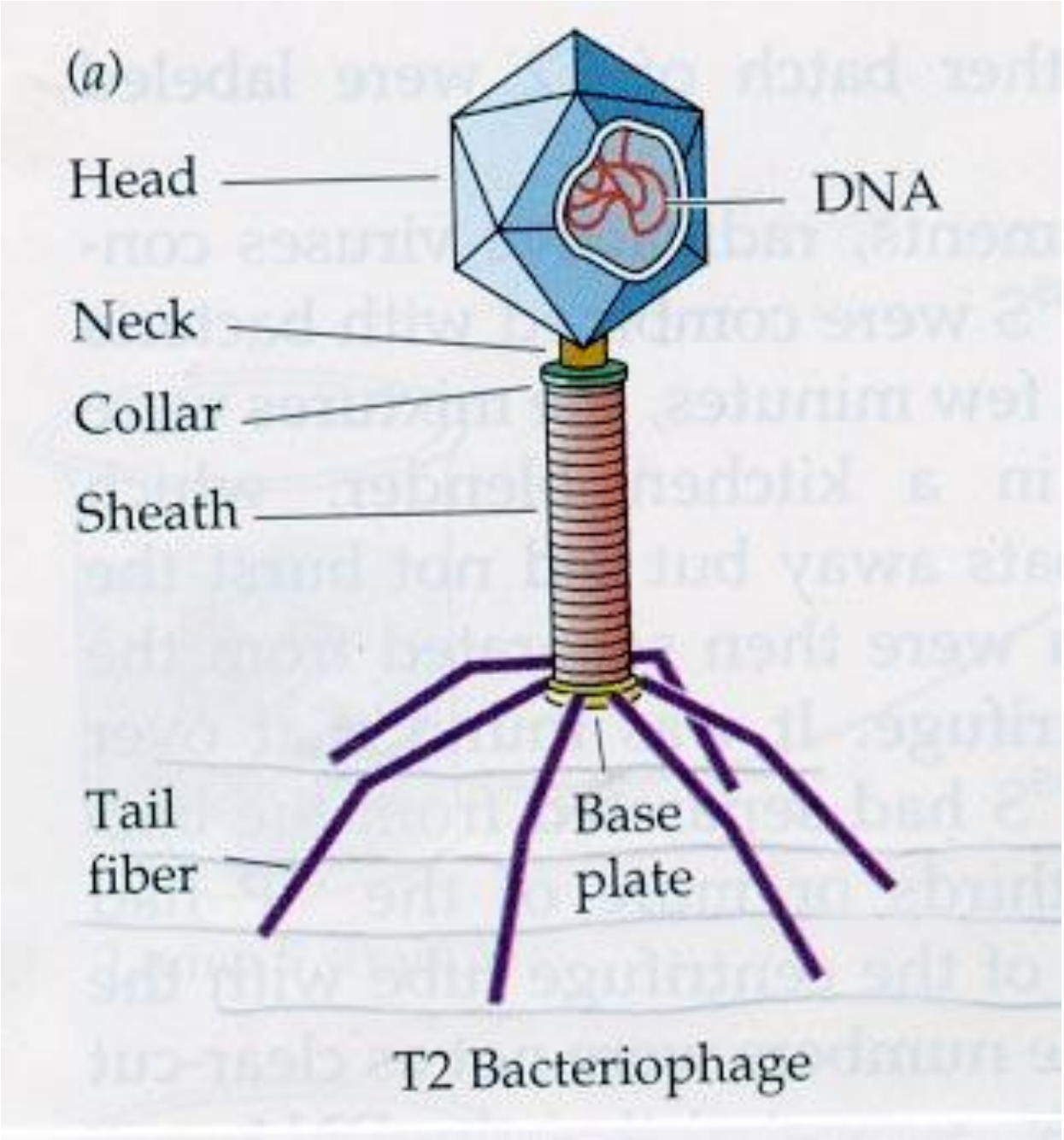

Catalase +
Catalase -

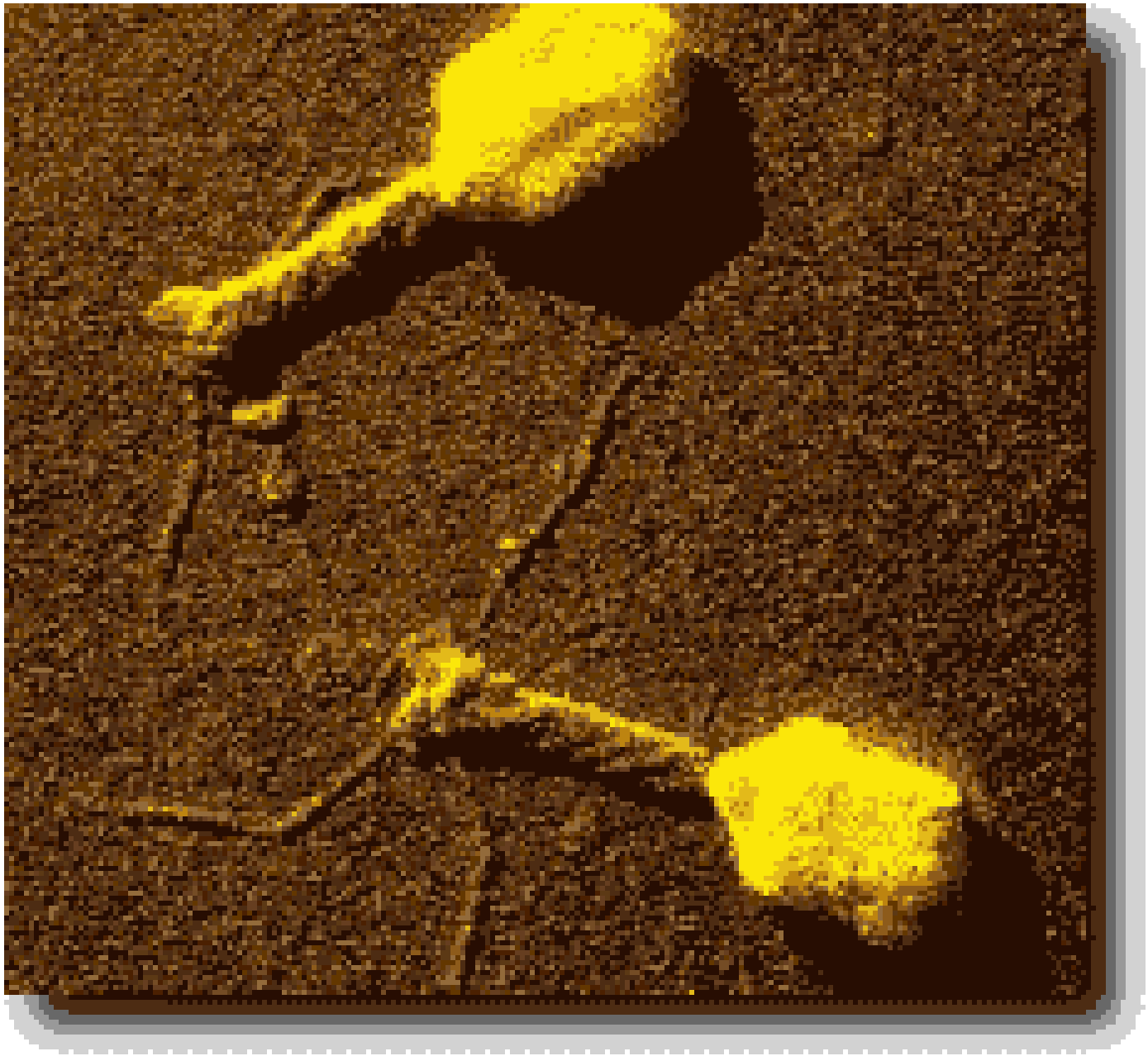

Oxidase
neg.
Oxidase
pos.

Bacteriophages

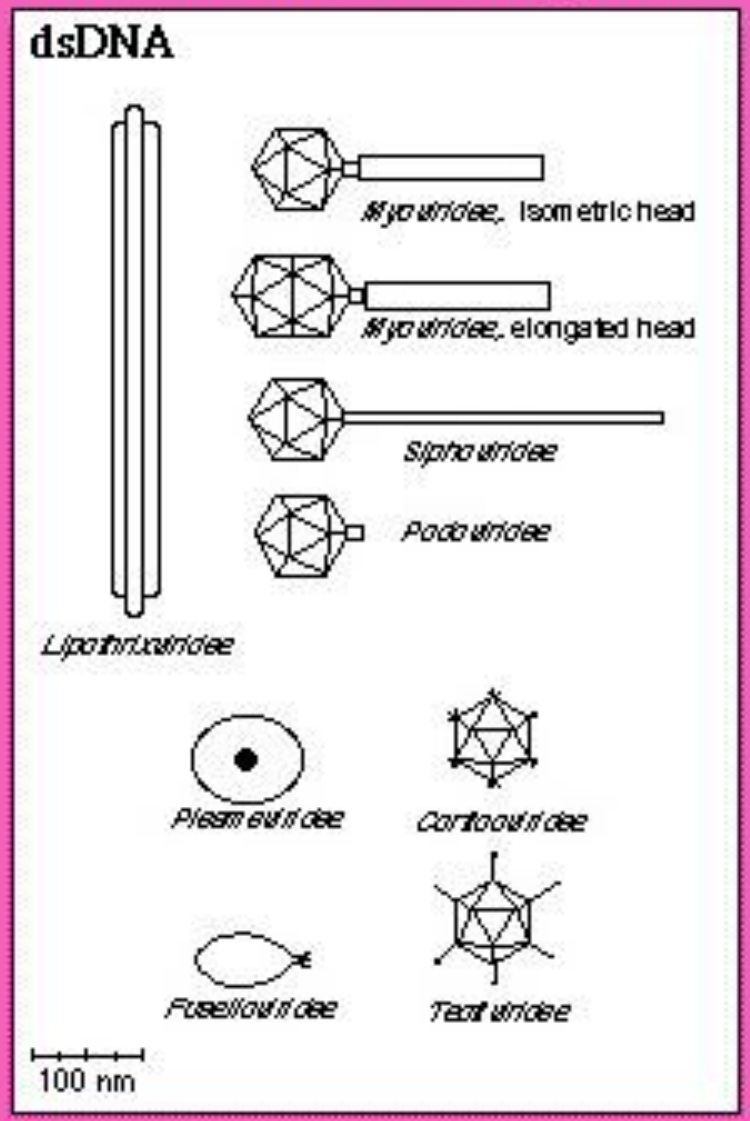
The definition of the term bacteriophage: *bacterial viruses.*
Discovery of bacteriophage - d'Herelle, 1917.
Nomenclature of phages:
it is based on the name of the host which is sensitive to definite phage plus special index.
Structure of phages: nucleic acid (DNA or RNA) + protein


.1 μm
1 μm


(a)
Head
DNA
Neck
Collar
Sheath
Tail fiber
Base plate
T2 Bacteriophage



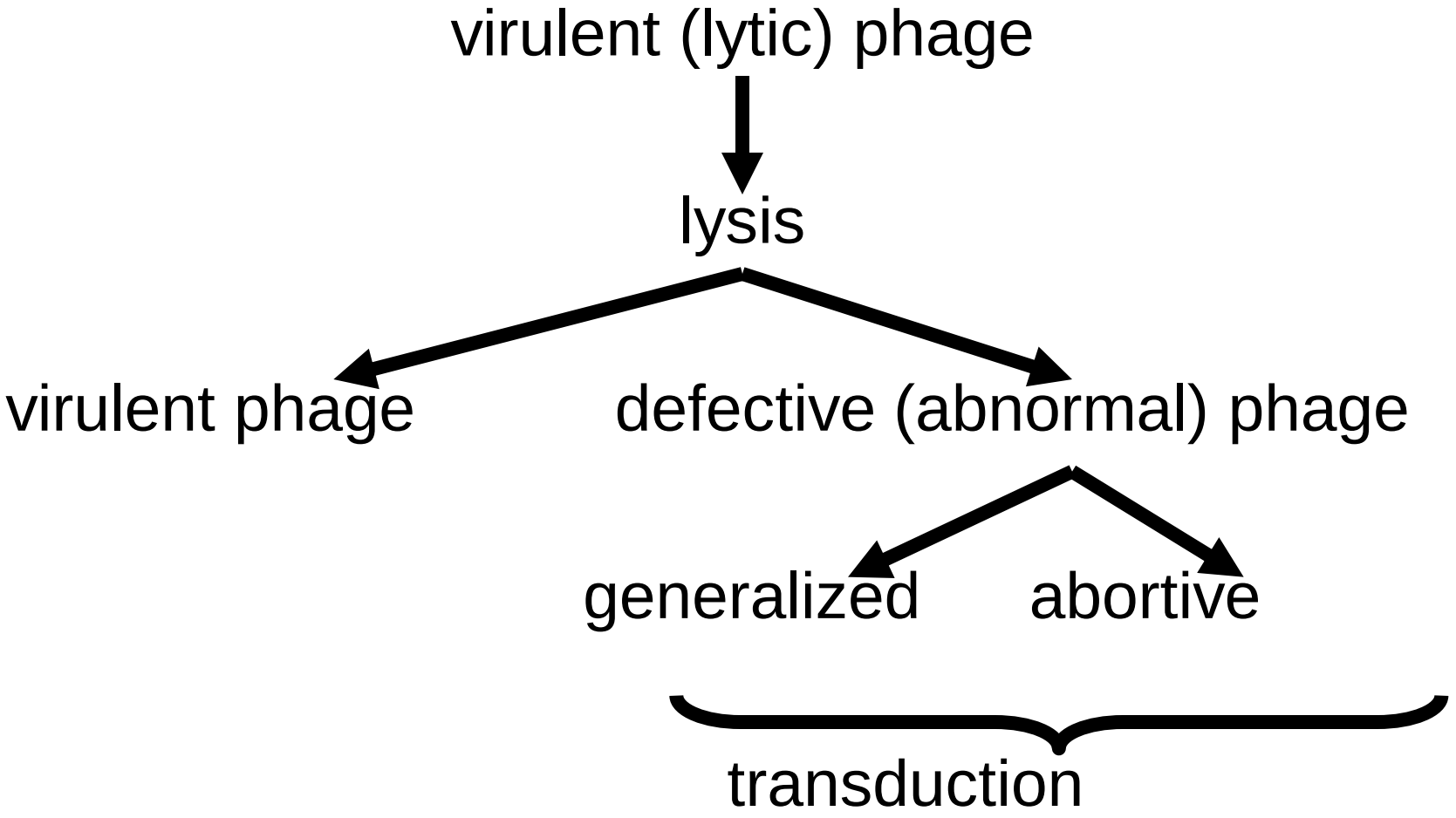
Families of Viruses Infecting Bacteria



CLASSIFICATION OF THE PHAGES BY THEIR LYTIC SPECTRUM (THEIR EFFECT ON BACTERIAL CELL)

- polyphages (polyspesific)
  - infect several species of bacteria
- monophages (monospesific)
  - infect one species of bacteria
- type phages (type specific)
  - infect only part of bacteria belonging to the same species (phage type)

CLASSIFICATION OF PHAGES BY THE RESULT OF THEIR EFFECT ON A BACTERIAL CELL



CLASSIFICATION OF PHAGES BY THE RESULT OF THEIR EFFECT ON A BACTERIAL CELL

temperate bacteriophage

↓

lysogeny

- without a change of bacterial phenotype
- with a change of bacterial phenotype (*phage conversion*)

↓

lysis

↙ ↘

temperate phage

defective phage

↓

specialized transduction

CONSEQUENCES OF INTERACTION OF VIRULENT PHAGE WITH HOST BACTERIAL CELL

adsorption of phage at the specific receptors present on the surface of bacterial CW
(it never occurs on protoplasts' surface)

↓

injection of DNA or RNA genome into the target cell (penetration)
(the protein capsid remains at the surface of the cell)

↓

replication of the nucleic acid of infective phage resulted in appearance of many new copies of the phage genome and in synthesis of phage-specific proteins

↓

assembly of the phage progeny

↓

release of the progeny from the host cell

↙ cell lysis

↘ without killing the host cells
(some filamentous phages)

PRODUCTIVE INFECTION

CONSEQUENCES OF INTERACTION OF TEMPERATE PHAGE WITH HOST BACTERIAL CELL

adsorption of the phage at the specific receptors on the CW
(it never occurs on protoplasts)

↓

injection of DNA or RNA genome into the target cell (penetration)
(the protein capsid remains at the surface of the cell)

↓

integration of the phage genome into the bacterial genome

↓

forming of prophage
(phage repressor blocks transcription of the phage genome)

↓

lysogenic bacterial culture
L Y S O G E N Y
later events which can occur

↓

prophage induction

↓

productive infection

PRACTICAL APPLICATION OF THE PHAGE IN MEDICINE

Phage diagnostics

1. Detection of the bacterial species in a pathological material
   - the evaluation of increase production of the new generations of specific phage in the material
2. Pure culture identification
   - bacterial species determination
     - phage indication
   - bacterial phage type determination
     - phage typing

PRACTICAL APPLICATION OF THE PHAGE IN MEDICINE

Phage therapy

Phage could be used locally (at the infected place)

PRACTICAL APPLICATION OF THE PHAGE IN MEDICINE

Phage prophylaxis of some bacterial infections

- enteric fever
- dysentery

PHAGE INDICATION: METHOD OF “STREAMING DOWN DROP “

spread the investigated bacterial strain over the surface of agar plate to get bacterial lawn

⇩

place a drop of the solution containing monophage to stream down over the bacterial lawn

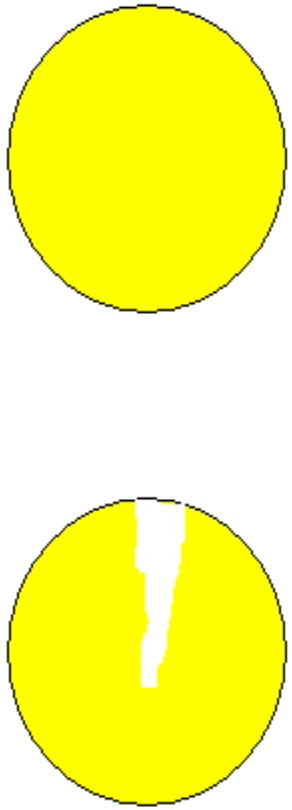
⇩

reveal the bacterial growth over the streaming down area

⇩

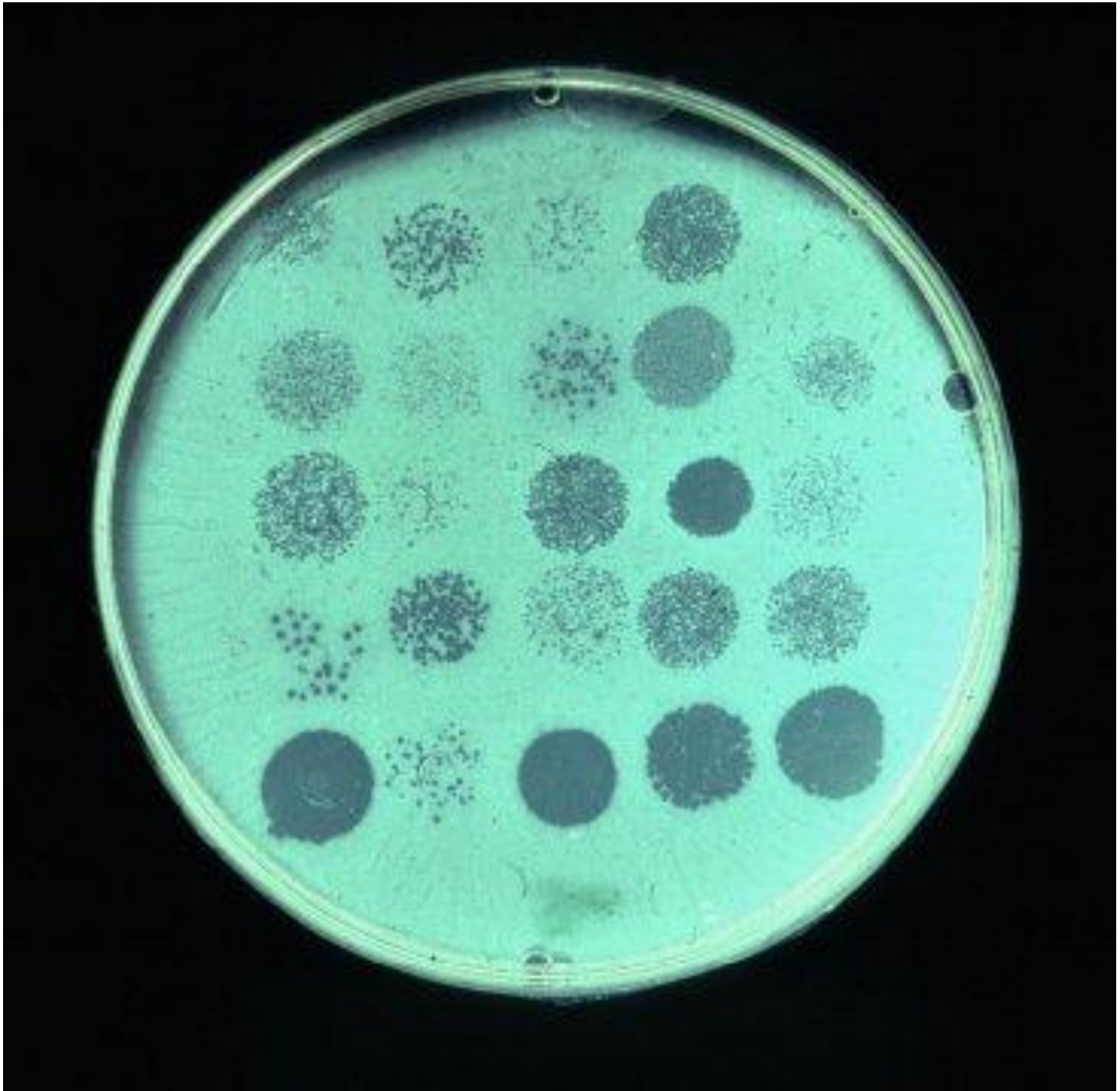
growth – negative result

no growth – positive result



BACTERIA PHAGE TYPING

1. platting of the investigated bacterial strain to get bacterial lawn on an agar plate
2. placing drops of the solution containing type phages
3. incubation
4. revealing of «sterile spots» («plaques») and register phage type of the bacteria = a list of type phages causing lysis of the bacterial strain (appearance of “plaques” on the bacterial lawn)



Genetics of Bacteria

Theme N6

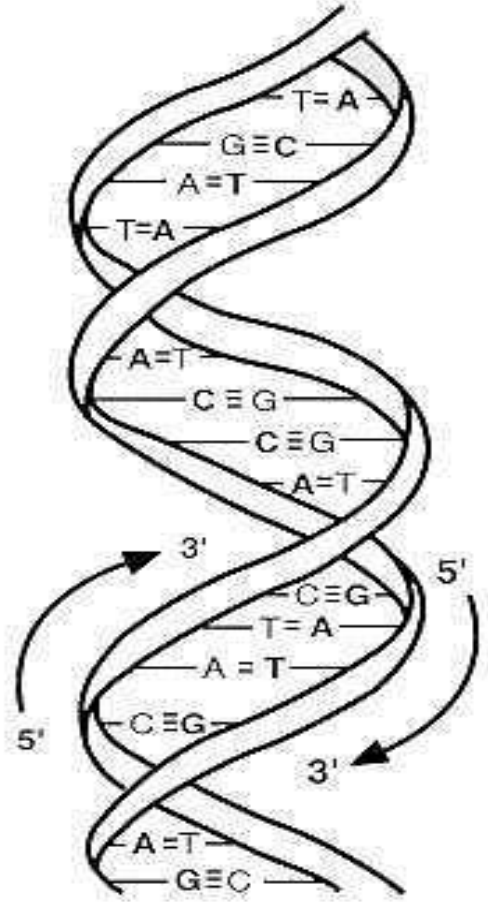
ORGANIZATION OF GENETIC MATERIAL IN BACTERIA

DNA

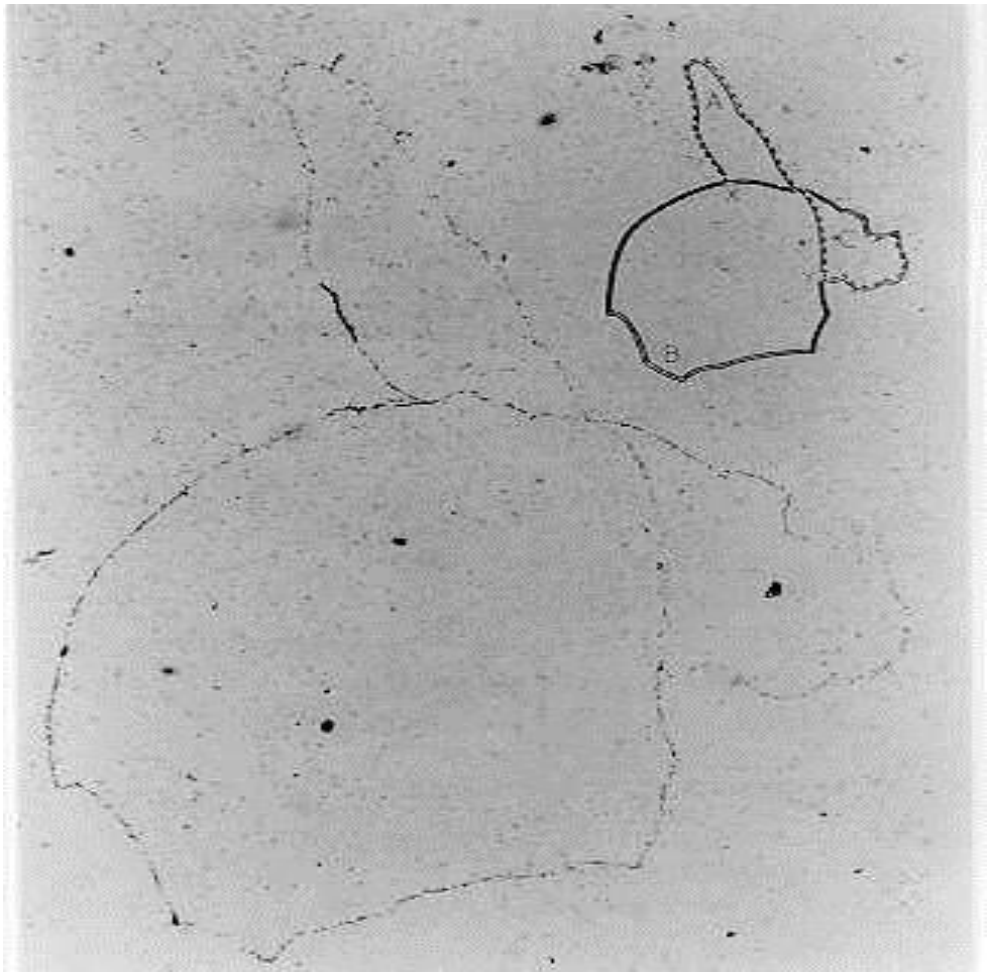
- nucleoid (bacterial chromosome)
- it codes information vital for bacterial cell
- extra-chromosomal factors of heredity
  - they code the information which is not important for life of bacteria

DNA STRUCTURE:

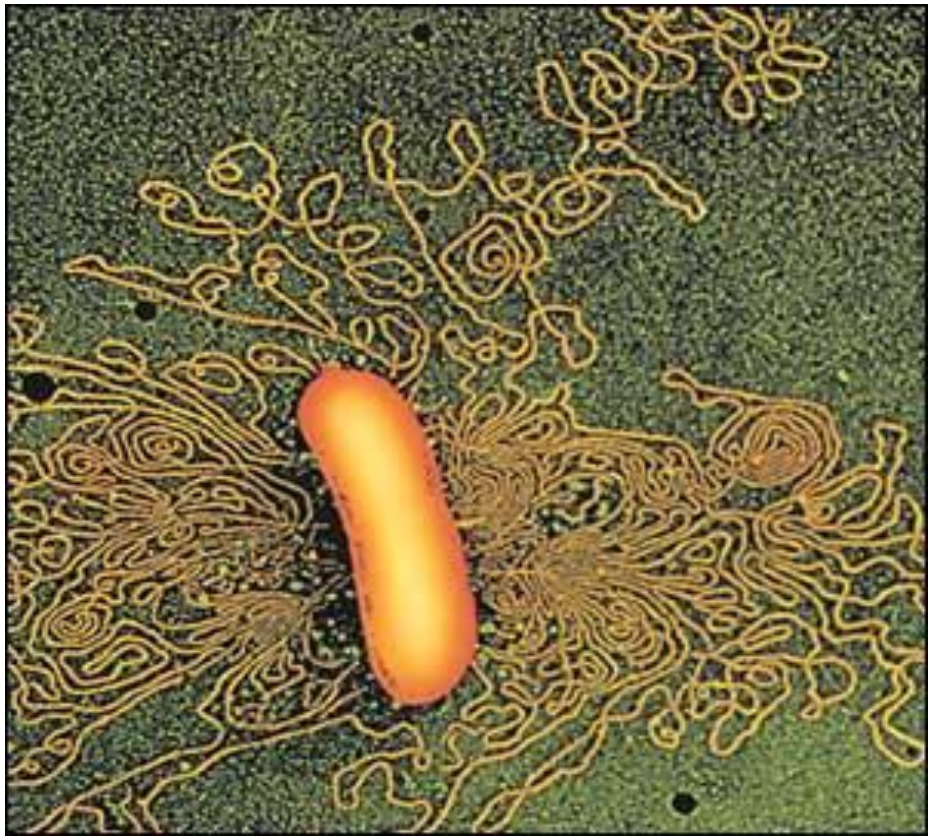
two complementary strands of the DNA double helix



Autoradiograph of E.coli DNA



Electron micrograph of E.coli bacterial cell



EXTRACHROMOSOMAL FACTORS OF HEREDITY

- *autonomous* – called replicons (can replicate themselves as independent units)
  - plasmids
- *non-autonomous* - called not replicons as they can't replicate themselves as independent units but only as a part of replicon (when they are inserted into the nucleoid or plasmid)
  - transposons
  - IS-elements
  - temperate phages

PLASMIDS

- definition of the term
  - small extra-chromosomal genetic elements - autonomous factors of heredity in bacteria
- physical properties
  - they are circular, double-stranded DNA molecules with molecular weight from $3x10^6$ to $1x10^8$ and usually code 5-160 polypeptides
- functions
  1. regulatory –compensate infringements of the function of DNA of bacterial nucleoid
  2. coding – introduce new information into the genotype of bacteria
- possible location
  - autonomous (in cytoplasm)
  - integrated (inserted into the nucleoid)
- presence of tra-operon in the genome of plasmid
  - conjugative plasmids (found in gram-negative bacteria, contain tra-operon which carries the information about their own transfer)
  - non-conjugative plasmids (do not contain tra-operon and can’t determine their own transfer)

PLASMIDS

- **character of the control of the replication of plasmid DNA by the nucleoid**
  - strict control (plasmid replicates synchronically with nucleoid) $\Rightarrow$ produce 1-2 copies per one bacterial cell (large plasmids)
  - weak control (plasmid replicates more frequently then nucleoid) $\Rightarrow$ 10-30 copies per bacterial cell (small plasmids)
- **compatibility of plasmids when they are located in the same cell**
  - $>$ exist more then 20 groups of incompatibility, which include plasmids closely related to each other (2 members of the same group can't coexist in the same bacterial cell because of the competition for a specific attachment to the replication site in the cell membrane)

F-PLASMIDS

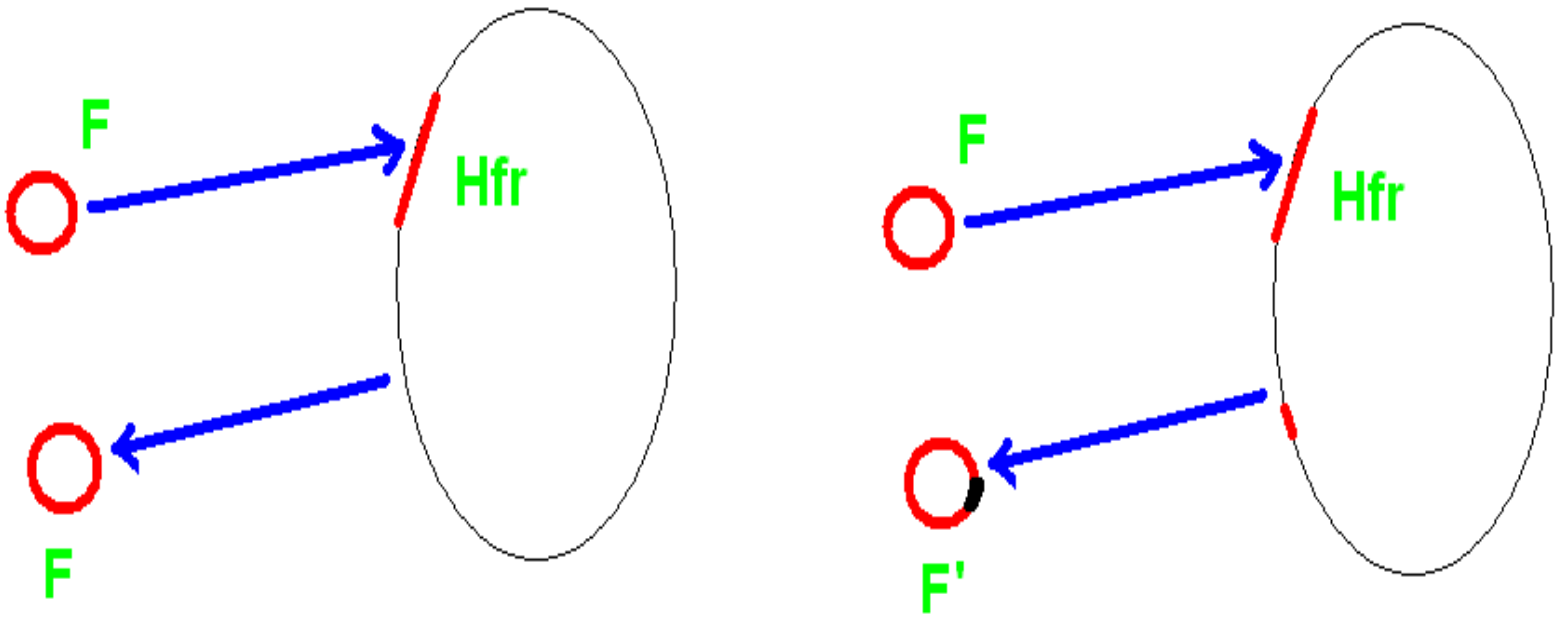
- definition of the term

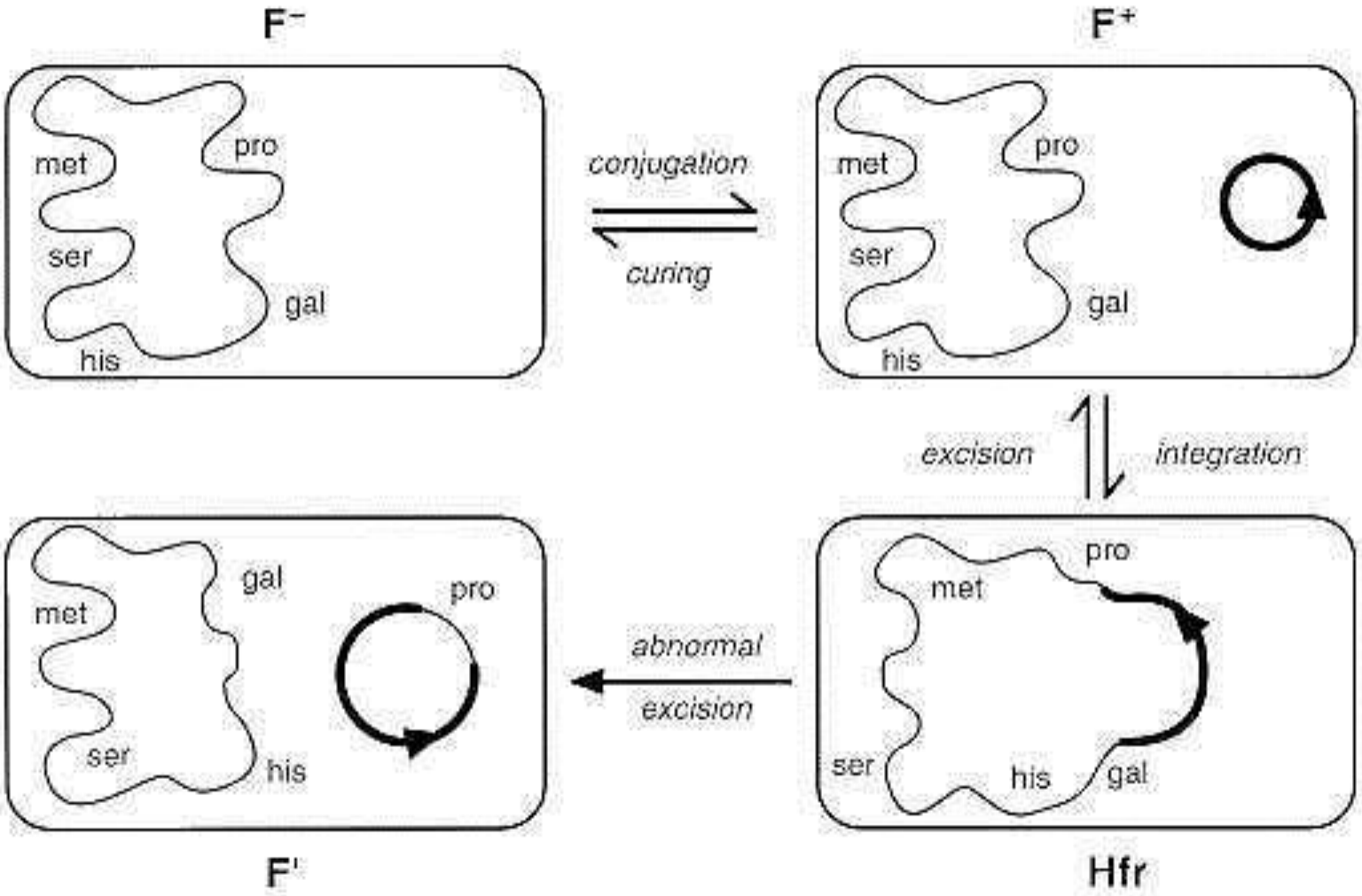
  sex (fertility) factors (contain only tra-operon, any other genes are not presented) mediate bacterial chromosome transfer and synthesis of sex pili

- location in the infected bacterial cell

  1. integrated into bacterial genome
     - Hfr
  2. autonomous in cytoplasm
     - F^+
     - F’

VARIANTS OF THE LOCATION OF F-PLASMIDS




F⁻
F⁺
met
pro
ser
gal
his
conjugation
curing
met
pro
ser
gal
his
excision
integration
gal
met
pro
ser
his
abnormal
excision
pro
met
ser
his
gal
F'
Hfr

R-PLASMIDS

- definition of the term

  resistance factors - plasmids encoding multiple resistance to various anti-microbial agents, such as antibiotics

- composition
  - r-operon (operone) + tra-operon
  - r-operon (operone)
- the ways of transfer of the plasmids from one bacterial cell to another one
  - transduction (plasmids are transferred by phages in gram-positive bacteria)
  - conjugation (gram-negative bacteria)

PLASMIDS OF BACTERIOCYNOGENITY (example of Col-plasmids of E.coli)

- definition of the term
  - plasmids encoding synthesis of colicins (antibiotic - like proteins which are lethal for coliform bacteria)
- composition
  - genes which cause production of colicins by the bacteria
  - tra-operon which determines self-transfer of the plasmid
- peculiarities
  1. rarely integrate into the nucleoid
  2. usually exist in repressed state
  3. after the plasmid derepression bacterial cell synthesizes colicins and dies after that (potentially lethal plasmid)
- biological role
  - decrease of the density of bacterial population when the nutrient media got exhausted
- importance for medicine
  - participate in normalization of natural micro - biocenosis of intestines

TRANSPOSONS

- definition of the term

  short nucleotide sequences (size varies from 2 000 to 20 000 pairs of nucleotides), capable to change the site of the location in DNA molecule and to migrate from one DNA molecule to another one

- location in bacterial cell

1. integrated into the replicon (replicated simultaneously with replicon)
2. autonomous (when located in cytoplasm they take circular shape and do not replicated)

- composition

– contain special terminated sequences - IS (markers of transposon) by which transposon could be distinguished from other DNA segments

– genes encoding synthesis of:

  - toxins
  - enzymes which participate in the development of resistance to antibiotics
  - proteins which participate in other processes

IS-ELEMENTS (FACTORS)

- definition of the term

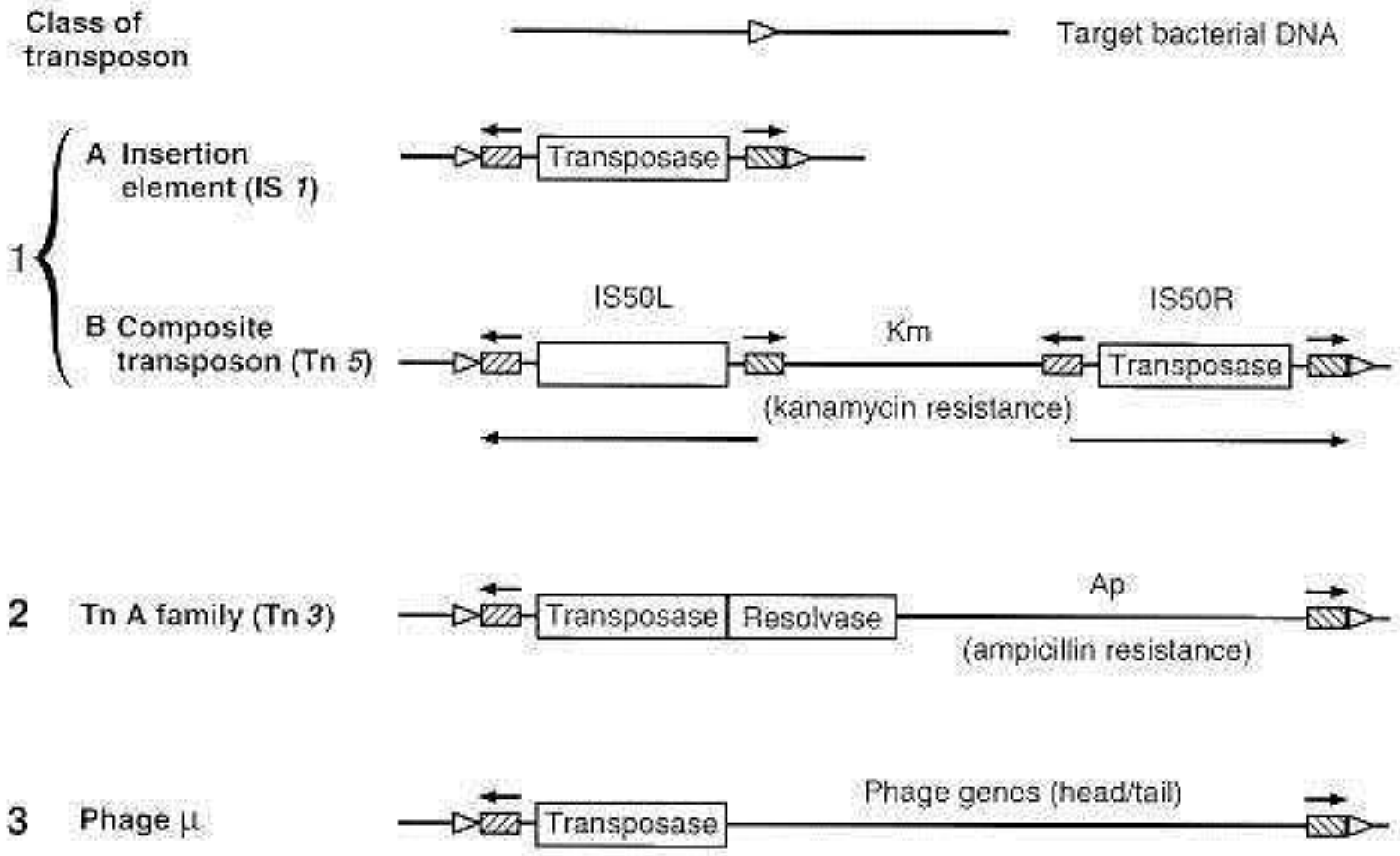
  Insertion sequences - inserts of nucleotide sequences (usually their size is about 1 000 pairs of nucleotides)

- differences in comparison with transposons

1. contain only genes coding transposition
2. never found in autonomous state
3. found in transposons as a repeats inverted with respect to each other's orientation

- functions

1. co-ordination of co-interaction of extra-chromosomal factors of heredity: between transposons, plasmids and during their interactions with bacterial chromosome to provide their recombination
2. regulatory (regulation of the transcription of genes by the mechanism of their «switch on/switch off».
3. induction of the mutations (inversions, duplications which take place by involving 5-9 pairs of nucleotides)


Class of transposon
Target bacterial DNA
1
A Insertion element (IS 1)
Transposase
B Composite transposon (Tn 5)
IS50L
IS50R
Km
Transposase
(kanamycin resistance)
2
Tn A family (Tn 3)
Transposase
Resolvase
Ap
(ampicillin resistance)
3
Phage μ
Transposase
Phage genes (head/tail)

MODIFICATIONS IN BACTERIA

Changes affecting only the phenotype (observable properties) of bacteria

- do not accompanied by changes of DNA structure and thus they are not inherited by the next generations
- are not stable and usually could be lost very quickly

MUTATIONS IN BACTERIA

Definition of the term

Any changes which occur in genotype (the set of genetic determinants carried by bacterial genome) so they involve changes of the primary structure of DNA molecule.

The result of the mutation is loss or change of one or several hereditary features which will be inherited by the next generations of bacteria.

MUTATIONS IN BACTERIA

Classification according to the occurring mechanism:

- spontaneous – difficult or not possible to find the effect of curtain factor (mutagen)
  - mistakes in the function of DNA-polymerase when replication of DNA takes place
  - insertion mutations – occur when extra-chromosomal factors of heredity are inserted into DNA molecule
- induced – produced in the experiment when certain known mutagenic agent (mutagen) is applied

MUTATIONS IN BACTERIA

Classification according to their direction:

- direct – loss or change of the property
- *reverse* (reversions) – restoration of the property
  - true – when restoration of genotype and phenotype takes place
  - suppressive – when we see restoration only of phenotype

SR-DISSOCIATION

- definition of the term

  appearance of R-shaped colonies in pure bacterial culture which normally forms S-shape colonies: phenotypic manifestation of the change of some properties of the bacterial cells

- mechanism

  insertion mutation resulted in the loss of the genes controlling synthesis of carbohydrate chains which are necessary for the formation of LPS component of the outer membrane of the cell wall

- biological importance
  - bacteria producing R-shaped colonies are more resistant to unfavorable physical and chemical factors of the external environment
  - bacteria producing S-shaped colonies are more resistant to phagocytosis and to the effect of antibodies

Significantly complicate isolation and identification of pure culture.

MUTAGENS

Definition of the term:

Chemical substances (for example bromuracil which can be incorporated into DNA in place of thymine) or physical factors (UV causes additional covalent bond formation between neighboring thymines in DNA).

Mutagens cause ***pre-mutative changes*** in DNA structure.

Mutation has occurred:

only in the case when pre-mutative changes are followed by:

- the changes in the function of reparative enzymes
- infringements in the proceeding of the reparation processes (which occur in the case of some mistakes taking place during the work of the reparative systems)

RECOMBINATIVE VARIABILITY IN BACTERIA

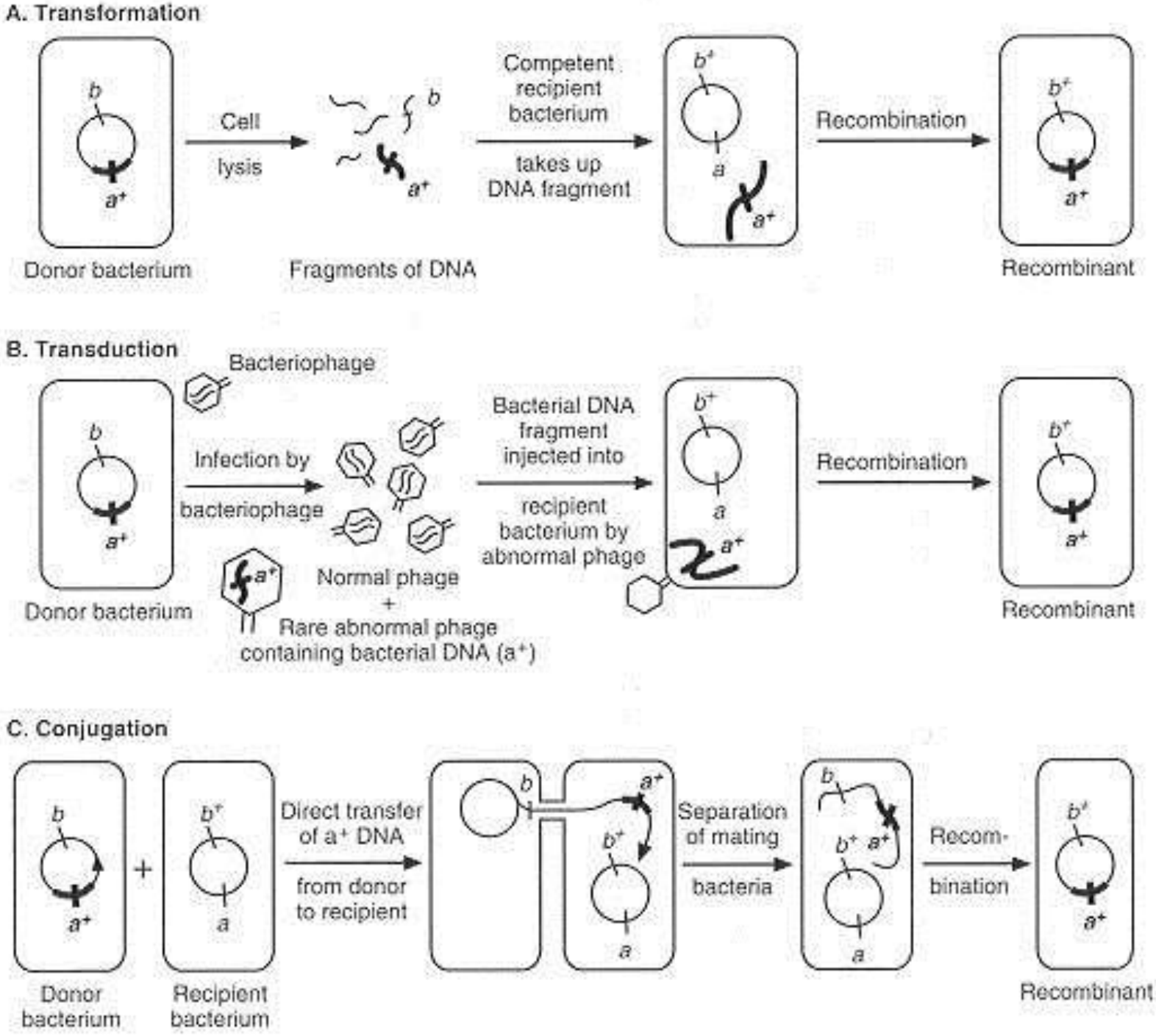
Definition of the term

Changes in DNA structure occurring as a result of integration of the part of DNA of the recipient cell into the DNA of the donor cell

RECOMBINATIVE VARIABILITY IN BACTERIA

Forms of recombinative variability

1. Transformation– direct transfer of genetic material –soluble DNA segments (could be released spontaneously) from donor cell to the recipient one.
2. Transduction – transfer of genetic material (a fragment of donor chromosome) from donor cell to recipient cell by defective bacteriophages:
   - non-specific (generalized transduction) – by virulent phages
   - abortive – by virulent phages
   - specific (restricted transduction) – by temperate phages
3. Conjugation – transfer of genetic material from donor HFR cell to the recipient cell through conjugative pili in gram-negative bacteria.
4. Lysogeny – bacterial genome carries phage genes after infection of bacteria by temperate phage.
5. Phage conversion – appearance of new properties in bacteria as a result of lysogeny.


A. Transformation
b
a+
Donor bacterium
Cell
lysis
b
a+
Fragments of DNA
Competent
recipient
bacterium
takes up
DNA fragment
b+
a
a+
Recombination
b+
a+
Recombinant
B. Transduction
Bacteriophage
b
a+
Donor bacterium
Infection by
bacteriophage
a+
Normal phage
+
Rare abnormal phage
containing bacterial DNA (a+)
Bacterial DNA
fragment
injected into
recipient
bacterium by
abnormal phage
b+
a
a+
Recombination
b+
a+
Recombinant
C. Conjugation
b
a+
Donor
bacterium
+
b+
a
Recipient
bacterium
Direct transfer
of a+ DNA
from donor
to recipient
b
a+
b+
a
Separation
of mating
bacteria
b
b+
a+
a
Recom-
bination
b+
a+
Recombinant

GENE ENGEINEERING IN MEDICAL MICROBIOLOGY

Production of the recombinant vaccine for the prophylactics of hepatitis B

integration of the gene belonging to hepatitis B virus and encoding synthesis of HBs-Ag (surface antigen) into the genome of the yeast cell

⇩

manifestation of the gene

⇩

synthesis of HBs-Ag by the yeast cell

⇩

purification of HBs-Ag

⇩

vaccine containing HBs-Ag which doesn’t contain viral particles or their fragments

METHODS OF GENETICS APPLIED IN MICROBIOLOGICAL DIAGNOSTICS

- content (in percent) of G+C (guanine + cytosine) nucleotides in bacterial genome
- method of molecular hybridization
- polymerase chain reaction (PCR)

METHOD OF MOLECULAR HYBRIDIZATION

Target DNA

⇩

Increase of the temperature

⇩

Separation of the DNA strands at high temperature and high pH

⇩

Attachment of one of the DNA strands to the special filter

⇩

Adding of the one – strand DNA labeled by radio active isotopes

⇩

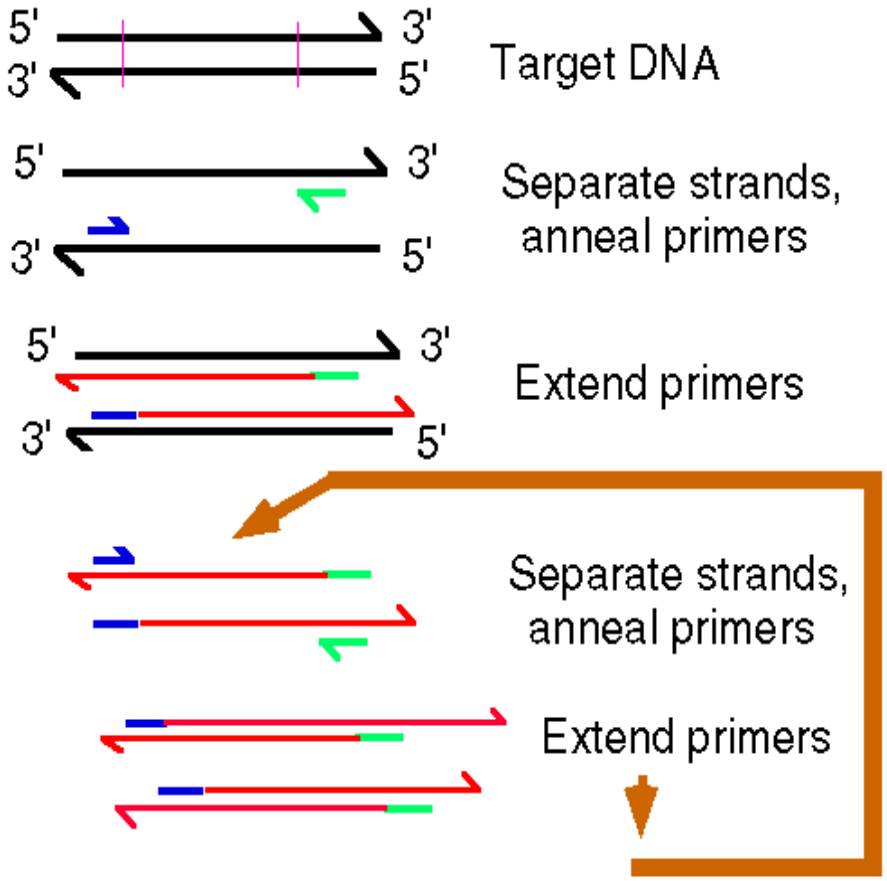
Decrease of the temperature for restoration of the double stranded DNA

⇩

Positive result – radioactivity is registered in the two stranded DNA

Negative result – radioactivity is not registered in the two stranded DNA

POLYMERASE CHAIN REACTION (PCR)



ECOLOGY OF MICROORGANISMS

Theme N7

Definition of the term “ecology of microorganisms”

- section of general microbiology, studying the next relationships of microorganisms:
  - between each other
  - with unanimated objects of environment
  - with macro-organism (human organism).

Ecological niches of microorganisms: soil

Microbial populations (micro-biocenosises) of soil

1. The upper layer of soil is most populated by microorganisms.
2. Survival of pathogenic microorganisms in soil :
   - species of bacteria which don't form spores and viruses – from several days to several months,
   - spores – for years,
   - infectious agents causing botulism, actinomycosis, deep mycosis and mycotoxicosis – usually live in the soil.

Ecological niches of microorganisms: sanitary control of soil

- For sanitary control the bacteria - indicators of fecal contamination of soil (representatives of normal micro-flora of human intestines) are usually used.
- Bacteria – indicators live in the soil during the same period of time as pathogenic intestinal microorganisms. Main bacterium – indicator is E.coli.
- The sanitary standards used for evaluation of fecal contamination of soil:
  - coli-index – number of E.coli cells in 1 gm of soil,
  - coli-titer – mass of the soil (in grams), containing 1 cell of E.coli;
  - microbial number – total numbers of all microorganisms in 1 gram of soil.

Ecological niches of microorganisms: water

Microbial populations of water

1. In subsoil waters only single microorganisms are present.
2. Survival of pathogenic microorganisms in water:
   - Shigella sp., Vibrio cholera and Brucella sp.– from some days to some weeks,
   - enteroviruses, hepatitis A virus, Salmonella sp. and Leptospira sp. – several months,
   - spores – for years.

Ecological niches of microorganisms: sanitary control of water

Bacteria are controlled in water in connection with sanitation measures:

- the sanitation of drinking water,
- sanitation of swimming pools,
- purification of sewage.

Sanitary standards for normal

drinking water:

coli-index: ≤ 3 E.coli cells per 1 litre,

coli-titer: ≥ 300 ml of water per 1 E.coli cell

microbial number: ≤ 100 microbial cells per litre.

THE MICROFLORA OF HUMAN BODY

Characteristics of the micro-flora of human body

Micro-flora inhabiting human body:

- **Obligate**

  (constant = residential = indigenous = autochthonic)

  Includes microorganisms normally occurring in human body.

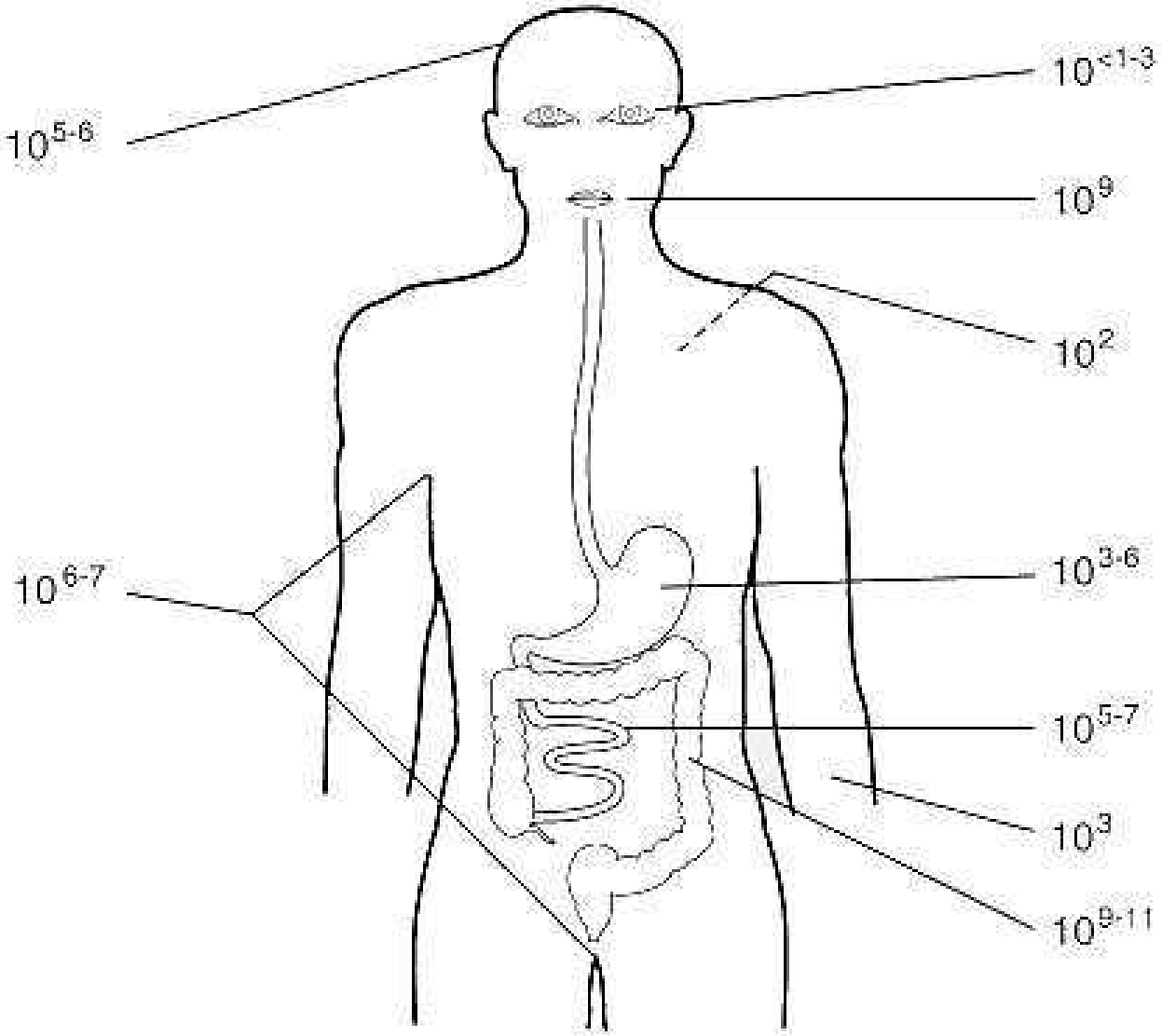
- **Facultative**

  (evidential = transit = allochthonic)

  It's composition is dependent on:

  – entering of microbes from the outside,

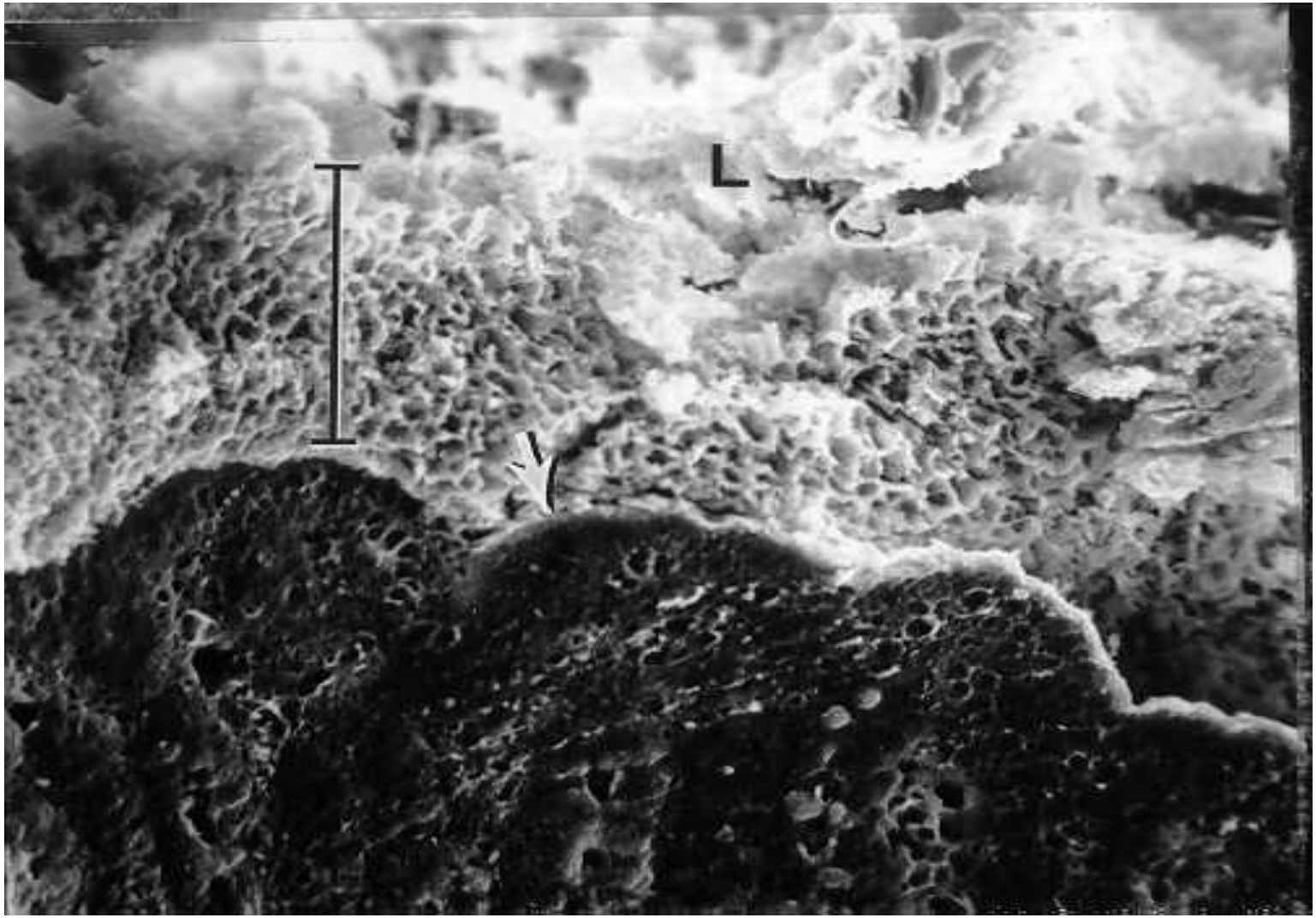
  – state of immune defense of humans.


10^{5-6}
$10^{<1-3}$
10^{9}
10^{2}
10^{6-7}
10^{3-6}
10^{5-7}
10^{3}
10^{9-11}

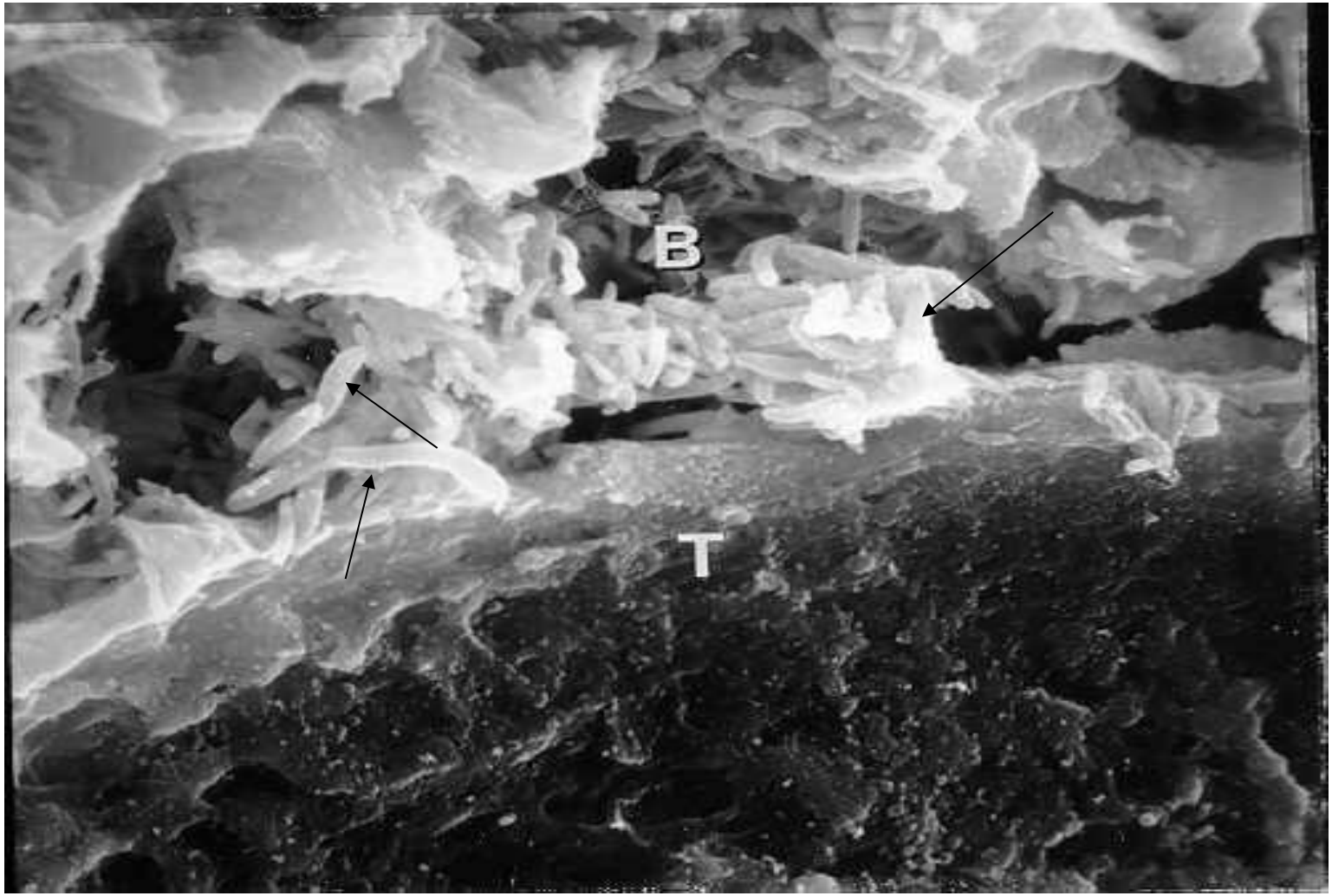
Composition of normal micro-flora of human intestine

1. Predominant:
   - bifidobacteria
   - lactobacteria
   - bacteroides
2. Found in high quantities:
   - E. coli
   - enterococci
3. Found in minor quantities:
   - other representatives of enteric bacteria
   - staphylococci
   - fungi Candida
   - clostridia

Mucous layer of human intestine – biofilm

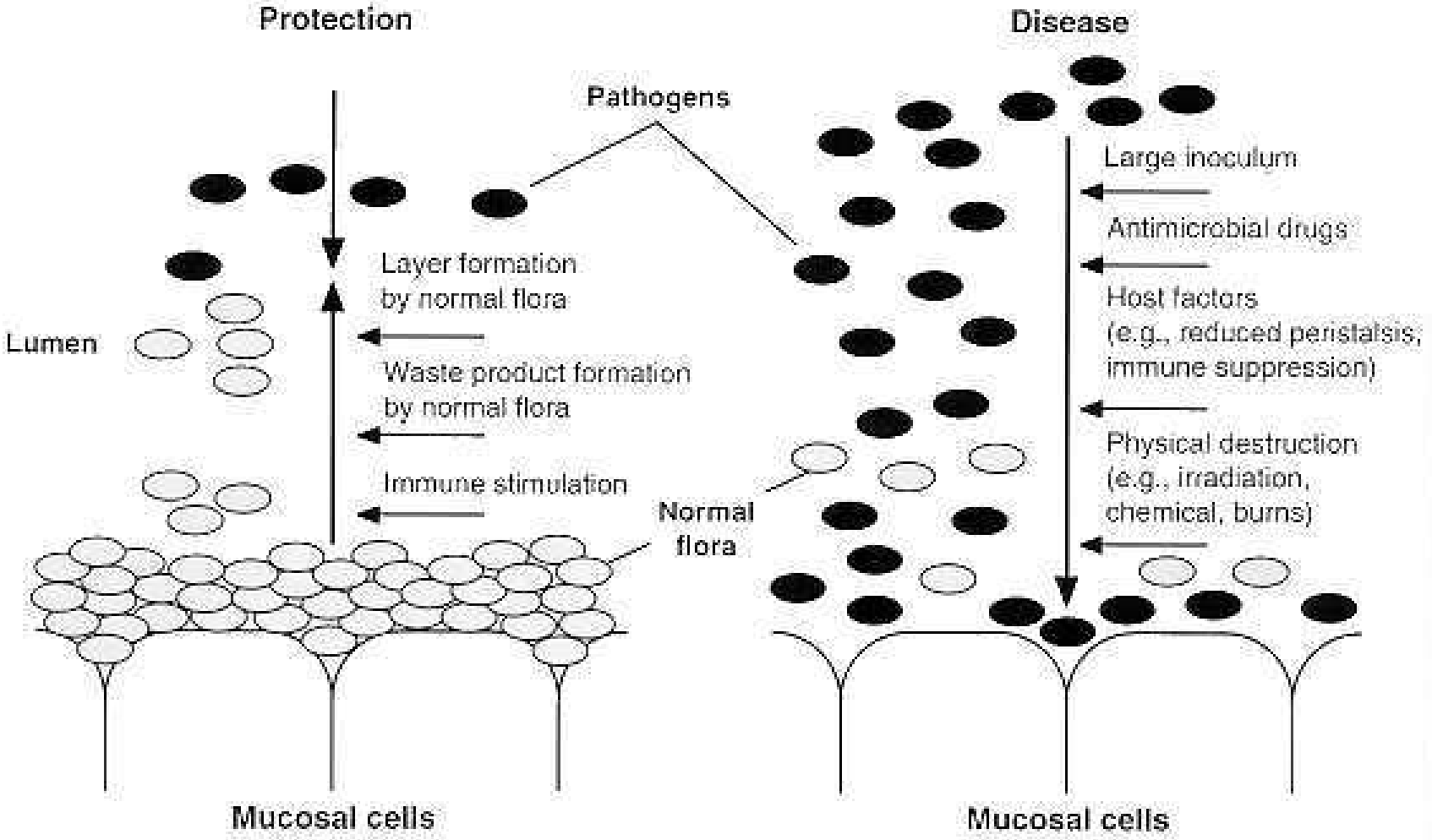


Bacteria – representatives of normal microflora in human intestine



The role of normal micro-flora in human organism

1. Antagonism in relation to pathogenic species (factor of innate immunity).
2. Participation in the processes of digestion in human intestines (maintenance of normal function of gastro-intestinal tract).
3. Activation of the processes of formation, maturation and normal function of immune system.


Protection
Disease
Pathogens
Layer formation
by normal flora
Lumen
Waste product formation
by normal flora
Immune stimulation
Normal
flora
Large inoculum
Antimicrobial drugs
Host factors
(e.g., reduced peristalsis,
immune suppression)
Physical destruction
(e.g., irradiation,
chemical, burns)
Mucosal cells
Mucosal cells

Disturbances in composition of normal micro-flora

Disbacteriosis (disbiosis or dismicrobiosis):

pathological state characterized by qualitative and quantitative disturbances in the composition of microbial populations normally inhabiting human body.

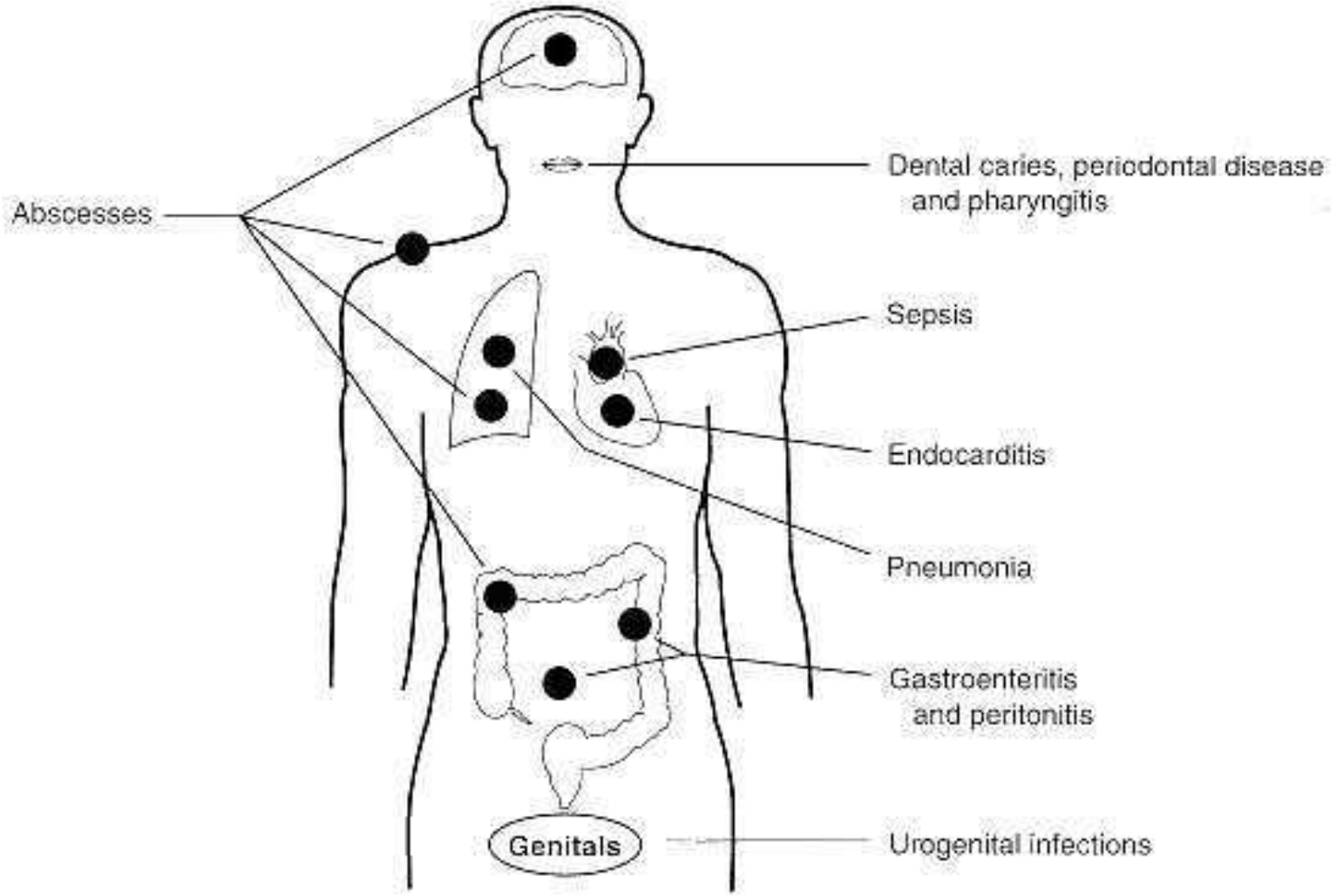
Some factors which can cause disbiosis in intestine:

- physical destruction (irradiation, burns, chemicals, etc.);
- host factors (e.g. reduced peristalsis, immune suppression);
- antimicrobial drugs (antibiotics).

Some approaches to the normalization of misbalance of normal micro-flora in the state of disbiosis

- administration of **eubiotics** (preparations which contain live bacterial strains – normal inhabitants of human intestines):
  - bifidobacteria – bifidumbacterin,
  - lactobacteria – lactobacterin,
  - E.coli – colibacterin,
  - bifidobacteria and E.coli – bificol and others.
- administration of **probiotics** (preparations which stimulate development of normal micro-flora),
- **removal of the factors** which caused the state of disbiosis.

Pathological states which could be caused by representatives of normal micro-flora



INFLUENCE OF ECOLOGICAL FACTORS ON MICROORGANISMS. MICROBIAL DECONTAMINATION.

Influence of physical factors on microorganisms

High temperature
- denaturation of proteins

Low temperature
- damage of the cytoplasm membranes by ice crystals
- inhibition of the microbial metabolism

Unfavorable pH values
- denaturation of enzymes
- disturbances of the function of osmotic barrier

Drying
- loss of the water by the cytoplasm
- damage of the cytoplasm membrane
- damage of the ribosomes

Ultraviolet
- production of thymine dimmers

Ultrasound
- break-down of the components of the cell

Influence of chemical factors on microorganisms

- denaturation of the protein and solubilization of the lipid components of cytoplasm membrane:

  - alcohol
- protein denaturation:
  - phenol and creosol (and their derivatives)
  - halogens (iodine, chloride and their derivatives)
  - aldehydes
  - oxidizing agents (potassium permanganate, hydrogen dioxide, etc.)
  - salts of heavy metals
- damage of structure and disturbance of the function of the cytoplasm membrane
  - detergents (fatty acids, soaps, polymers C_8 – C_{20})

MICROBIAL DECONTAMINATION

Definition of the term

- complete or partial removal of microorganisms from the unanimated objects of surroundings or from the human organism with use of the factors causing direct damage to microorganisms.

Types of microbial decontamination

Microbial decontamination includes:

- decontamination of the unanimated objects of surroundings
  - sterilization
  - disinfection
- decontamination of the live organisms
  - antisepsis
  - chemotherapy

Sterilization

Definition of the term

- complete removal or killing of all microorganisms

Sterilization: methods

- **Use of high temperatures** (heat sterilization):
  - autoclaving (sterilization by hot water steam with use of high pressure which help to increase the temperature of the steam up to 110 - 140°C),
  - dry heat sterilization - sterilization by hot air (the temperature reaches 180°C) for 1 hour in Pasteur ovens (dry-heating chambers). Could be used for sterilization of glass vials and metal equipment
  - fractional sterilization by flowing steam (30 minutes under the temperature of 100°C, with several intervals for one day to cool the material and to enable the spores to germinate).

Sterilization: methods

- **Chemical sterilization**: use of formaldehyde, ethylene oxide, chloroform and other chemical substances.
- S**terilization with use of irradiation:**
  - γ-rays – usually applied at the factories for sterilization of medical equipment
  - UV – applied in practical medicine.
- **Filtration** (mechanical sterilization) – use of bacterial filters, applied for the decontamination of the heat-labile solutions .

Disinfection of unanimated environment

Elimination or reduction of the definite group of pathogenic microorganisms - usually with use of ***disinfectants*** (special chemical substances).

Antisepsis

- **Definition of the term**: inhibition of the growth and propagation of microbes on the intact or injured skin and mucous membranes in the next cases:
  - treatment of the hands of surgeons,
  - treatment of places of surgical intervention,
  - treatment of wounds and mucous membranes.

  In the case if patients have immune deficiency applied methods of antisepsis should give a higher degree of decontamination.

  For this aim chemical substances called *antiseptics* which produce bacteriostatic (or microbiostatic) effect are usually used.

Asepsis

Definition of the term:

- creation of the zone free from any microorganisms (or the zone containing very low numbers of microorganisms) in the next places:
  - patient's areas,
  - rooms where medical manipulations are carrying out: operating theatre,
  - clinical laboratories.

Asepsis

Methods:

- direct
  - sterilisation
  - disinfection
  - antiseptics
- indirect
  - separation

Basics of the Infection. MICROBIOLOGICAL BASICS OF CHEMOTHERAPY OF BACTERIAL INFECTIONS

Theme N8

Basic terms and concepts of the infection

- The infectious process (infection)
  - Physiological and pathological reactions of macro-organism (host), which are initiated and developed as a result of its interactions with pathogenic microorganism
- Epidemiological process
  - The processes of arising and spreading of specific infectious states among the human population when intensity of display of the symptoms is varying from asymptomatic carriage of microbe to manifestation of the symptoms of disease caused by the circulation of agent in particular groups of a population

Basic terms and concepts of the infection

Chain of Infection

The chain of infection includes the three factors that lead to infection:

- the etiologic agent (microbe),
- the method of transmission,
- the host (susceptible human organism).

Basic terms and concepts of the infection

- *The mechanism (method) of transmission of infection:*
  - is the means by which the infectious agent goes from the source of the infection (could be infected organism) to the host (susceptible organism).
  - *The stages of the mechanism of transmission of infection:*

1. Release of the infectious agent from the host organism (infected) to surroundings
2. Presence of the infectious agent in the abiotic (unanimated) and biotic (animated) objects of surroundings
3. Infection of susceptible organism as a result of penetration of the infectious agent into the organism

Basic terms and concepts of the infection

- *The factors of transmission*
  - The elements of surroundings, which provide the transmission of infectious agent from one macro-organism to another one. Such factors could be water, food, air, arthropods, objects of surroundings
- *The ways of transmission*
  - Particular elements of surroundings or their combination which provide entering of infectious agent from one macro-organism to another one in certain conditions of surroundings
- *The portals (place of entry) of entry of the infection*
  - Particular organ or tissue trough which agent is entering inside of the macro-organism

Classification of infections according to the mechanism of transmission, ways of transmission and portals of entry of the infection

Fecal-oral mechanism of transmission

Ways of transmission:

1. alimentary (with food)
2. water
3. contact (including indirect contact)

Portals of entry of the infection – intestines

Air born mechanism of transmission

Ways of transmission:

1. air born (droplet)
2. air born (dust)

Portals of the infection – respiratory tract

Classification of infections according to the mechanism of transmission, ways of transmission and portals of entry of the infection

Blood born mechanism of transmission

Ways of transmission:

1. bite of arthropods (transmissive way of transfer of the infection)
2. by use of contaminated needles, syringes and

   blood - parenteral

1. sexual

Portal of the infection is blood

Classification of infections according to the mechanism of transmission, ways of transmission and portals of entry of the infection

Contact mechanism of transmission

Ways of transmission:

1. wound
2. contact
   - direct contact
   - indirect contact
3. sexual

Portals of the infection – skin and mucous membranes

Classification of infections according to the mechanism of transmission, ways of transmission and portals of entry of the infection

Vertical mechanism of transmission

Ways of transmission:

1. transplacental

Portals of the infection – tissues of embryo

© LSF / Oxford Scientific Films

Dr. J. Deacon, University of Edinburgh School of Biology

Classification of infections according to the nature of infectious agent

- bacterial
- viral
- fungal (mycosis)
- protozoan (invasion)

Classification of infections according to their origin and ways of spreading

- exogenous - caused by microbes entering into human organism from the outside
- endogenous - caused by indigenous (own) micro-flora of human organism
  - autoinfection (variant of endogenous infection – a result of the infection which develops in human organism when translocation of the microorganism from normal (natural) biotope to atypical one takes place

Classification of the infections according to redevelopment of disease caused by the same or different infectious agent

- Secondary (the case of addition to the primary infection another one caused by different microbe).
- Reinfection (the case of repeated infection caused by the same microbe which occurs after recovery).
- Superinfection (the case of repeated infection caused by the same microbe which occurs before recovery).
- Relapse (the case of return of clinical manifestations without additional infection as a result of activation of the infectious agents which survived in macro-organism).

Classification of the infection according to clinical manifestations

- Clinical infection (characterized by marked, characteristic symptoms).
- Obliterated infection (characterized by feebly marked symptoms).
- Atypical (characterized by symptoms which are not typical for the disease).
- Latent or subclinical infection (characterized by almost complete absence of symptoms).

Classification of the infection according to the character of the spreading of the infection and covered territory

- Endemic (found only in definite geographic areas)
- Sporadic (single cases of the disease which are not connected with each other)
- Epidemic (avalanche accumulation of the cases of the disease when all the cases are connected with each other)
- Pandemic (epidemic covering several countries, the whole continent sometimes all human population)

Characteristic properties of the infectious disease

1. Specificity
2. Contagiousity
3. Recurrence
4. Ability to stimulate specific immune response

PATHOGENICITY AND VIRULENCE

Pathogenicity and virulence

- ***Pathogenicity*** – ability (inherited genetic capacity) of microbe to cause infectious process (disease) in sensitive macro-organism (human or animal).
- ***Virulence*** – phenotypic manifestation of pathogenicity – the degree in which microorganism exhibits pathogenic properties.

Characteristics of pathogenicity

- Potentiality
- Polydeterminancy
  - Synthesis of biologically active products by infectious agent such as:
    - proteins
    - carbohydrates
    - lipids
  - Ability of infectious agent to produce
    - toxins
    - enzymes of invasion and aggression
- Specificity
- Virulence

Factors of virulence

- Adhesion
  - The ability of bacteria to attach to the specific receptors on the surface of the cells of macro-organism
- Colonization
  - Propagation of bacteria on the surface of the cells of macro-organism after their adhesion
- Penetration
  - Penetration of bacteria inside of the cells of macro-organism
- Invasion
  - Getting of bacteria into underlining tissues through mucous membranes and connective tissues
- Aggression
  - Withstanding of bacteria against innate and specific immune defence of macro-organism


COAGULASE
TEST
POSITIVE
NEGATIVE

Protein toxins: general characteristics

Protein toxins are exotoxins - metabolites of Gram-positive and Gram-negative bacteria:

- **completely excreting by living bacterial cells and found in fluid medium,**
- **partly excreting,**
- **non-excreting.**

Protein toxins: properties

1. Polypeptides having MW 10,000 – 900,000.
2. Relatively unstable: loss toxicity by heat over 60^0C.
3. High toxic (poisonousness): fatal for laboratory animals in micrograms or less.
4. High immunogenicity - produce strong response of human immune system; stimulate the formation of antitoxin.
5. Specificity of their effect: antitoxin neutralizes toxin.
6. Possess ability to convert into anatoxins (toxoids) – toxin, which lost its poisonousness, but retained its immunogenicity (the property which is typical not for all toxins).
7. Do not produce fever in host.

Protein toxins: classification

- Neurotoxins
  - Affect on the nervous system cells
- Enterotoxins
  - Affect on the cells of digestive tract
- Cytotoxins
  - Block protein synthesis at the subcellular level
- Hemolysins
  - Increase permeability of the outer membrane of erythrocytes resulting in their hemolysis

Endotoxins: differences between endotoxins and protein toxins

Endotoxin – LPS component of the outer membrane of the cell wall in Gram-negative bacteria.

1. Endotoxins are integral part of bacterial cell wall.
2. They are relatively more heat stable.
3. Weakly toxic.
4. Possess weak immunogenicity.
5. Their effect is less specific.
6. They are not converted into toxoids (anatoxin).
7. Often produce fever in host.

Chemotherapeutic agents: definition of the term

Medical preparations (drugs) which selectively inhibit growth of microorganisms in human organism or kill them

The basic characteristics of therapeutic agents

1. Absence of appreciable toxic action on human organism – selective toxicity (toxic only for microorganisms).
2. Different antimicrobial spectrum.
3. Constant formation of drug-resistant forms of microorganisms.

The most important groups of chemotherapeutic agents and the mechanism of their effect

- Antibiotics
- Sulfanilamide preparations
  - antimetabolites of folic acid interfering with its synthesis
- Organic and inorganic compounds of metals, sulfur, etc.
  - inactivation of enzymes of microorganisms
- Preparations of compounds of nitrofuranum
  - infringement of bioenergetic processes in bacterial cell

The most important groups of chemotherapeutical agents and the mechanism of their effect

- Antifungal preparations
  - Polyene antibiotics
    - infringements of integrity and function of fungal membranes containing sterol, act by binding to membrane sterols, make a pore in the membrane and the contents of the fungus leak out
  - Pyrimidine derivatives
    - infringements of synthesis of nucleic acids
  - Imidazole derivatives
    - interfere with the synthesis of lipid components of fungal membranes: infringements of synthesis of ergosterol

The most important groups of chemotherapeutical agents and the mechanism of their effect

- Antiparasitic preparations
  - Metronidazole (Trichopolum)
    - Suppress growth of microorganisms and kill them as a result of inhibition of DNA synthesis in:
      - protozoa
      - anaerobic bacteria
      - spirochetes

ANTIBIOTICS

Antibiotics: definition of the term

Chemotherapeutic substances which include naturally occurring, semisynthetic and synthetic drugs and exhibit the ability to inhibit the growth of microorganisms or to kill them selectively

Classification of antibiotics according to the source of their isolation

1. Produced by fungi – penicillin (fungus Penicillium) and cephalosporins (fungus Cephalosporium).
2. Produced by actinomycetes – 80% of all antibiotics are produced by Streptomyces.
3. Produced by bacteria (Bacillus, Pseudomonas).
4. Produced by animals – lysozym.
5. Produced by plants – phytoncides.
6. Synthetic antibiotics – quinolones or fluoro-quinolones.

Classification of antibiotics according to the method of their production

1. Naturally produced – naturally occurring antibiotics.
2. Chemically synthesized – synthetic antibiotics.
3. Produced with use of combined method – semisynthetic antibiotics.

Classification of antibiotics according to the mechanism of their action

1. Disturb synthesis of cell walls (β-lactams).
2. Disturb function and synthesis of cytoplasm membrane (polymyxin , polyenes).
3. Inhibit biosynthesis of protein – the most numerous groups of antibiotics (aminoglycosides , tetracycline, macrolides).
4. Antibiotics that inhibit structure and synthesis of nucleic acids:
   - ► DNA (quinolones)
   - ► RNA (rifamycins)

Classification of antibiotics according to the spectrum of their antimicrobial activity

1. Narrow spectrum of antimicrobial activity
   – affect on individual species or groups of species
2. Broad spectrum of antimicrobial activity
   – affect on many species of microorganisms

Classification of antibiotics according to the result of their antimicrobial influence on microorganisms

1. Bactericidal (microbicidal)
   – kill bacteria (microorganisms)
2. Bacteriostatic (microbostatic)
   – inhibit growth of bacteria (microorganisms) but don’t kill them

Complications of antibiotic therapy

Effect on macro-organism

1. Toxicity:
   - ► direct toxic effect (organotropic),
   - ► aggravation phenomenon (Hertz-Hamer phenomenon).
2. Disbiosis:
   - ► secondary endogenous infections, cause by conditional-pathogenic micro-flora,
   - ► increase of sensitivity to pathogenic microbes.
3. Immunopathological reactions:
   - ► allergic,
   - ► immune deficiency.
4. Teratogenic action.

Complications of antibiotic therapy

Effect on microorganism

1. Appearance of atypical forms , which are difficult to identify (for example – L-forms lacking cell wall).
2. Formation of drug resistance:
   - ► 1 to 3 years after introduction of new antibiotic the first resistant microbes appear,
   - ► 10 to 20 years of application of new antibiotic complete resistance to the drug is usually formed.

Principles of rational antibiotic therapy

1. Microbiological principle – to administrate an antibiotic only after obtaining results of antibiogram

   Use of antibiotics for prevention of disease and administration of antibiotics before getting of the result of antibiogram is proved only in the case of treatment of patients who:

   - have tumors
   - get cytostatic preparations and immunodepressants and have granulocytopenia and fever.

Principles of rational antibiotic therapy

2. Pharmacological principle – following the instructions concerning:
   - dosage,
   - methods of introduction,
   - duration of antibiotic therapy,
   - knowledge of pharmacokinetics of a preparation,
   - possibility of combination of the preparation administered with others,
   - application of combined therapy in the case of prolonged treatment.

Principles of rational antibiotic therapy

3. Clinical principle – administration of antibiotics only when it is very necessary to improve the condition of the patient.

Principles of rational antibiotic therapy

4. Epidemiological principle – to take into account resistance of microorganisms to antimicrobial agents in:
   - given branch of hospital,
   - whole hospital,
   - geographical region.

Principles of rational antibiotic therapy

5. Pharmaceutical principle – to take into account period of validity and instructions for storage of a drug.

The rules of preference and limitations in use of antibiotics in clinic

► administration of antibiotic is strongly recommended:

- infections caused by streptococci (sore throat, scarlet fever, etc)

►administration of antibiotic is expedient:

- acute respiratory infections complicated by pneumonia, sinusitis, etc
- acute intestinal infections in the case when the faeces contain blood (resembling dysentery)

►administration of antibiotic is strongly prohibited in the case of:

- acute respiratory infections
- acute intestinal with diarrhoea when the infectious agent is not identified, especially in children of any age
- fevers , leucocytosis when bacterial infections are not proved

Mechanisms of resistance of bacteria to antimicrobial agents

TABLE 11-2 Mechanisms of Resistance

Alteration of target
Modification to insensitivity to inhibitor
Reduction in physiologic importance of target
Synthesis of new target enzyme that duplicates function of inhibited target
Prevention of access to target
Efflux of more drug than enters cell
Failure of modified drug to enter cell
Inactivation of agent
Destruction of the agent
Modification of the agent so it fails to bind to target
Failure to convert an inactive precursor agent to its active form

Mechanisms of resistance of bacteria to antimicrobial agents

1. Primary (natural, specific resistance)
2. Secondary (acquired resistance)
   - ► mutations in genes or transfer of genes controlling synthesis of:
     - cell wall
     - cytoplasm membrane
     - ribosomal proteins
     - transport proteins
   - ► transfer of r-genes (which control⇨ inactivation and modification of antibiotics or infringements of their transport into the cell) by:
     - R-plasmids (multiple antibiotic resistance)
     - transposones (resistance to one antibiotic)

Measures preventing development of resistance of microorganisms to antimicrobial agents

1. To apply antibiotics strictly under indications.
2. To avoid application of antibiotics with the preventive purpose.
3. After 10 to 15 days of antibiotic therapy necessary to change the preparation.
4. Whenever possible to use antibiotics possessing a narrow spectrum of antimicrobic activity.
5. Through certain time to make change of used antibiotics not only in branch, hospital, but also in region.
6. To limit application of antibiotics in veterinary medicine.

Antimicrobial susceptibility tests

The Kirby-Bauer disk diffusion method

The plate with standard medium is inoculated by streaking the entire surface by bacterial inoculum

⇓

Standard commercial paper disks containing known amounts of antibiotics to be tested placed on the surface of agar

⇓

Incubation of the plates to let bacteria to grow

⇓

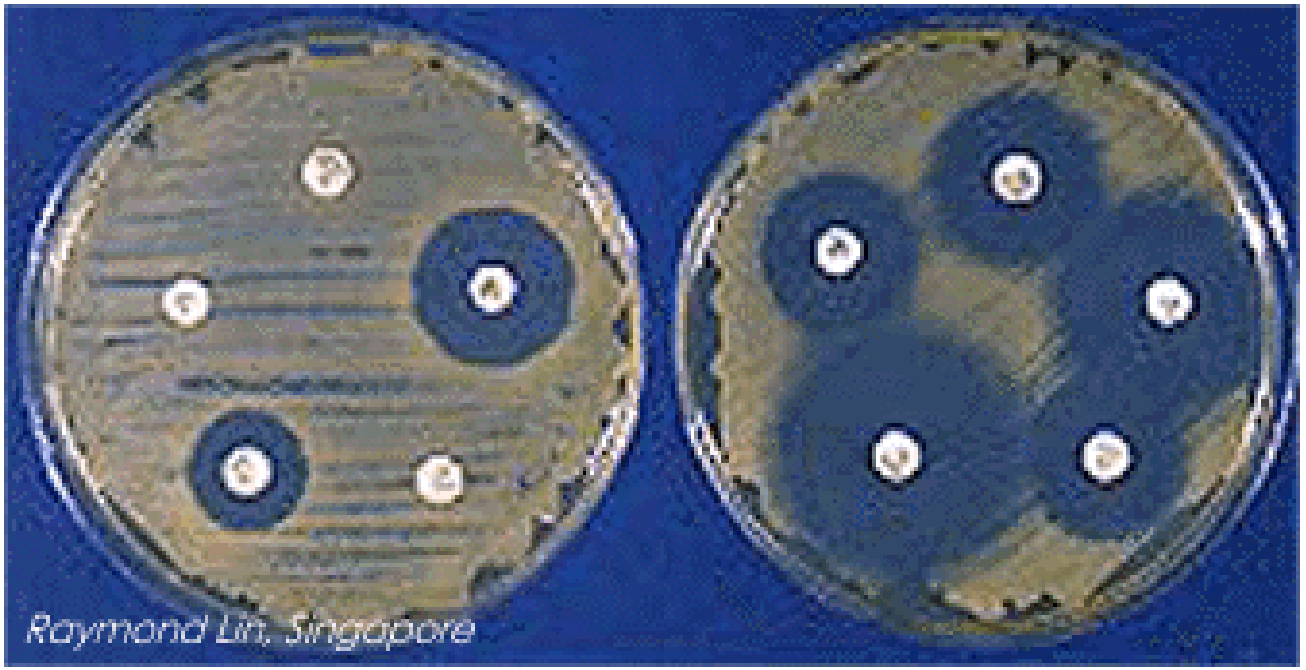
The diameter of the zone of inhibition of bacterial growth produced by the drug is measured for each disk

⇓

Conclusion about susceptibility of tested bacterial strain to each antibiotic has been made (called antibiogram) and the strain is designed as:

- susceptible
- intermediately susceptible
- low susceptible
- resistant

The Kirby-Bauer disk diffusion method



Broth dilution techniques

Twofold serial dilutions of the antimicrobial agent are prepared in the broth dispensed in test tubes (usually 8 tubes)

⇓

Each test tube is inoculated with the strain tested (inoculum usually contains 10^6 cells/ml)

⇓

Incubation

⇓

Determination of ***MIC*** (minimal inhibitory concentration) which is the lowest concentration of antibiotic that inhibits bacterial growth as determined visually by the lack of turbidity (bacteriostatic effect)

⇓

Broth dilution techniques

⇓

Inoculation of drug-free solid agar medium with 0.01 ml of broth from each test tube that showed no growth in the ***MIC*** determination

⇓

Incubation

⇓

The ***MBC*** (minimal bactericidal concentration) is determined by the absence of growth seen on the drag-free agar medium inoculated from the tube with maximal dilution of antibiotic that showed no growth in MIC determination