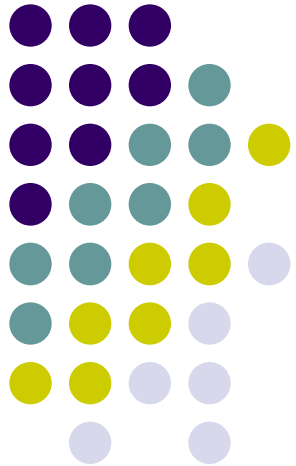
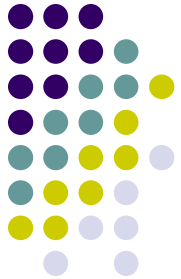
Physiology of microorganisms. Nutrition, growth, reproduction, respiration. Bacterial cultivation.

Bacterial genetics. Bacterial variation. Molecular-genetic assay.



Genetics is the study of heredity and variation of microorganisms.



BACTERIAL gynome:

- **Chromosome:**

Double-stranded circular DNA free located into cytoplasm

Number of genes: from 400 to 4,000

Range of genes: haploid

- **Extrachromosomal elements**

Extra chromosomal genetic elements

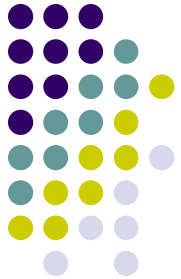
1. Plasmids
2. Transposons
3. IS – elements (inserted sequences)

Plasmids are extra chromosomal circular DNA molecules, including up to **40 genes** and capable of autonomous replication.

Transposons is linear segment of DNA including up to **10 genes.** Each transposon usually encodes properties that confers survival advantages.

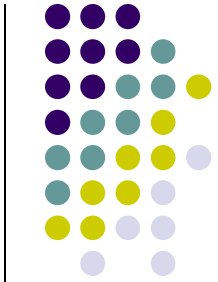
Insertion sequences (IS element) are the simplest type of transposable element. They take part in regulation of gene activity and encode the enzymes necessary for site-specific recombination.

Functions of extrachromosomal elements



- **Encoding function** (plasmids, episomes and transposons)
- **Regulating function** (plasmids and transposable elements)
- **Repairing function** (plasmids, episomes and transposons)

Classification of plasmids



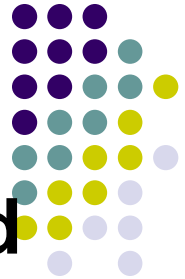
Due to ability to be transmitted during conjugation:

1. **Transmissible (conjugating) plasmids**
2. **Nontransmissible (nonconjugating) plasmids**

Due to localization plasmids are divided into:

1. **Independent replicons (localized into cytoplasm and capable of autonomous replication)**
2. **Episomes (integrated plasmids)**

Classification of plasmids



Due to encoded property they are classified into:

- **Resistance plasmid (R-factor) consists of r-determinants (r-genes) and resistance transfer factor (RTF). RTF is responsible for conjugational transfer, and r-determinants encode resistance to antibiotics.**
- **F-plasmids (F-factor). It is a transfer factor that contains genetic information necessary for the synthesis of the sex pili and for self transfer.**

Virulence plasmids

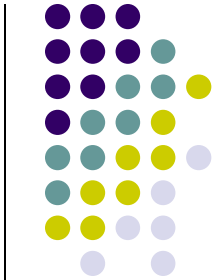
Virulence plasmids may include genes responsible for synthesis of exotoxins, adhesins, or invasion factors.

Enterotoxin-coding plasmid (Ent-factor) is present into enterotoxigenic E. coli.

Col-plasmid contains genetic information about colicins produced by E.coli. Colicins belong to bacteriocins.

Other plasmids: Hly – factor, biodegradation plasmids, cryptic plasmids

Variations of microorganisms

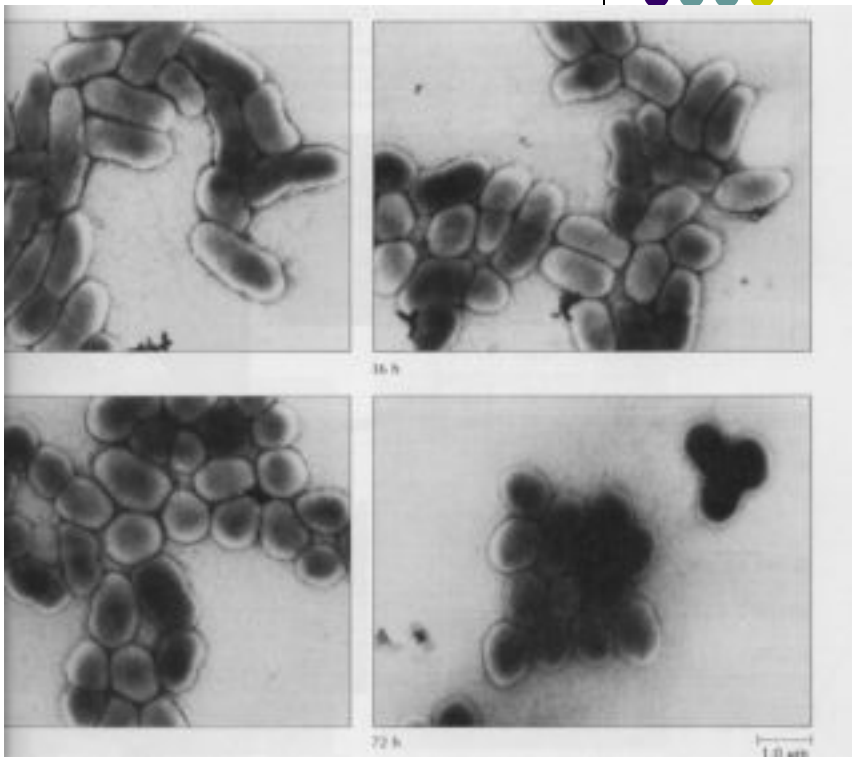


- **Nonhereditary variations** are phenotypic variations (**modifications**).
- **Hereditary variations** are genotypic variations. They are stable and may occur by **mutation or by genetic recombination.**

The **genotype** is the sum total of genes that make up the genetic apparatus of a cell.

The **phenotype** is the physical expression of the genotype in a given environment.

Phenotypic variations



Morphological changes :

- **Formation of unstable L-forms**
- **Formation of involution forms**
- **Arising of changed cells at**

presence of heavy metal ions in the medium. This phenomenon is called heteromorphism.

- **Inability to synthesize some**

additional organoids (capsule, flagella, inclusion body) under definite conditions

Biochemical variation may arise after addition into the culture media some substrates which are split with induced (adaptive) enzymes

Phenotypic variations

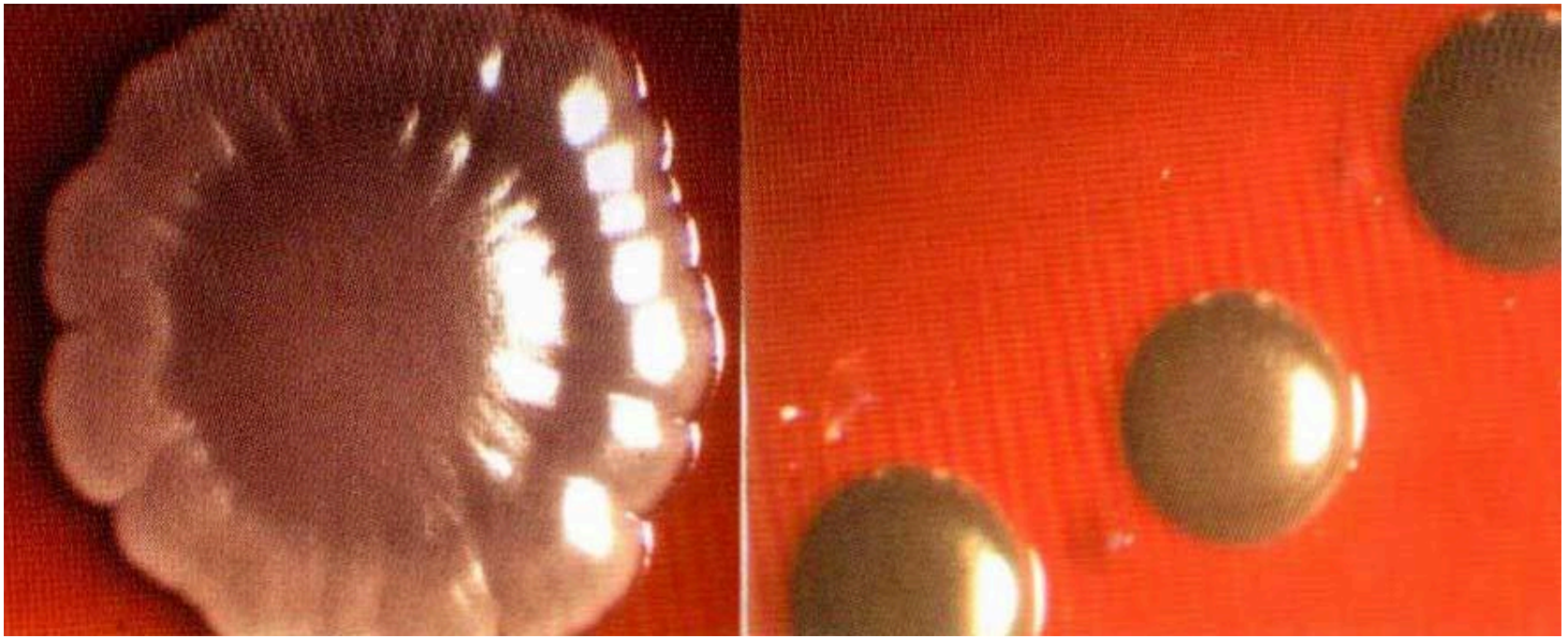
Dissociation is a type of unheritable variation arising into microbial population and characterizing with changes in cultural properties, antigenic structure and biochemical activity of the microorganisms.

Isolated pure culture may grow with different types of colonies which are named as:

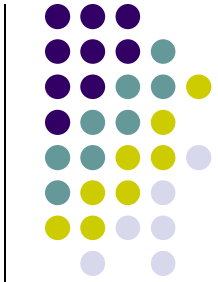
S-form (smooth, entire edge, convex). As a rule it is formed by virulent form of human pathogen with typical morphology, antigen structure (O-Ag) and biochemical activity

R-form (rough, filamentous or another edge, flat or umbilicated). It is isolated from treated patients and possesses changed properties.

R and S colonies of C.diphtheriae



BACTERIAL MUTATION



is a random heritable, directed or undirected variation caused by an alteration in the nucleotide sequence at some point of the DNA of the cell.

They may be due to addition, deletion or substitution of one or more bases (point mutation).

Gene mutations are due to insertion, deletion, duplication, translocation of some nucleotides

Genome mutations arise after translocation, insertion, deletion of some genes

Classification of mutations

1. **Spontaneous mutations result from replication errors, or DNA changes that cause replication errors. They are induced by biological mutagens (replication enzymes, transposable genetic elements and converting phages).**

Their rate is 10^{-6} -10^{-8} per bacterium per division

2. Induced mutations are provoked by mutagens experimentally

a. Chemical mutagens: nitrous acid, alkylating agents, acridine dyes, 5-bromuracil and 2-aminopurine.

b. Physical mutagens : ultraviolet light and radiation (X-rays, γ-rays)

Their rate is 10^{-4} -10^{-5} per bacterium per division

Variation caused by mutation

1. Changed biological properties of the germ

a) Pathogenicity. Attenuated bacterial strains are used to vaccine creation

b) Resistance to radiation

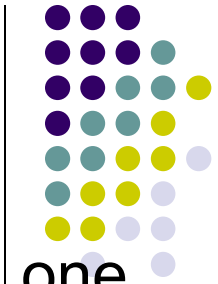
c) Resistance to antibiotics

d) Variation in nutrition requirements.

e) Variation in enzymatic activity

f) Changed antigenic structure and others

Genetic recombination

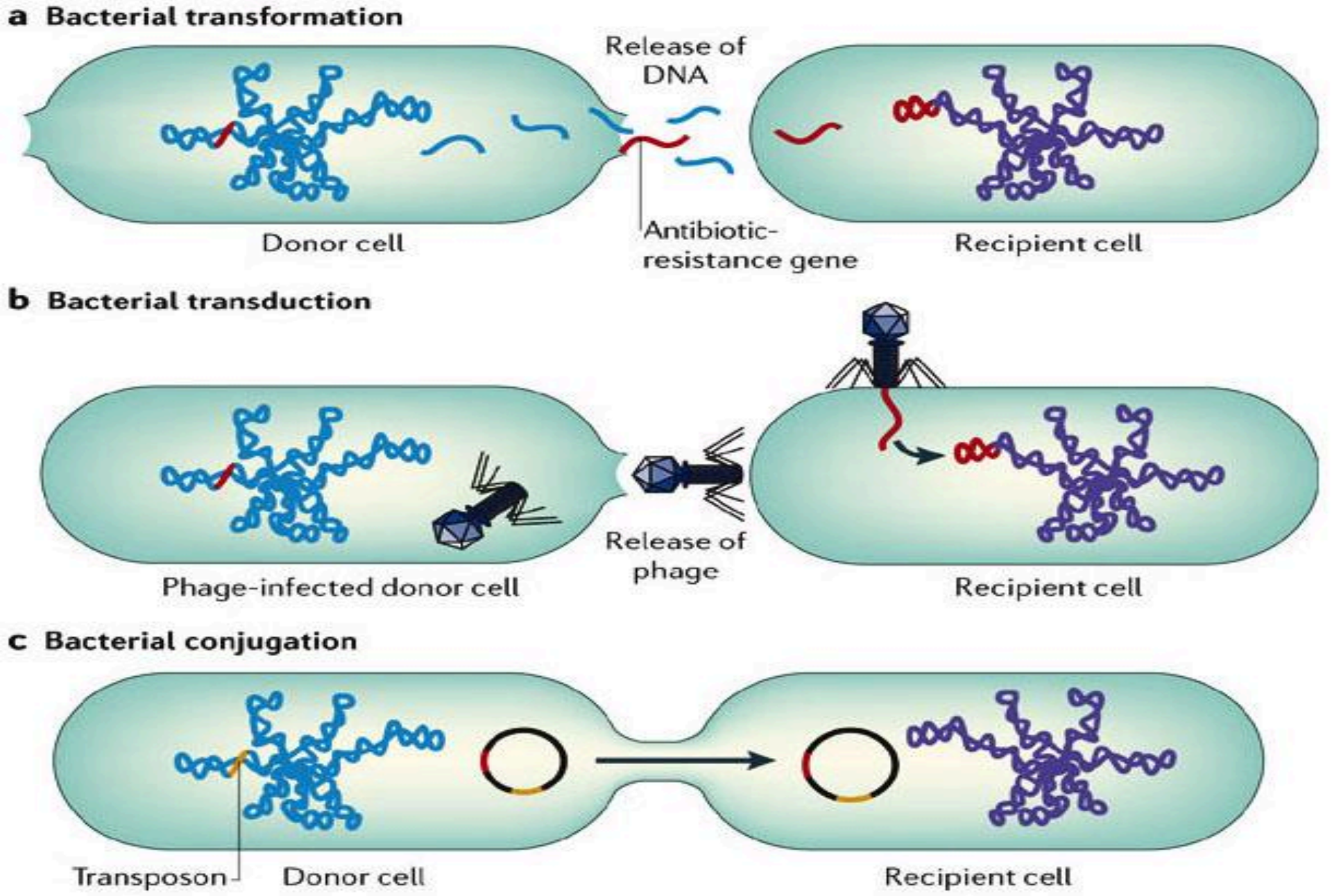


Recombination is transfer of chromosomal genes from one cell (donor) to another (recipient).

Recombination leads to new combinations of genes into chromosome.

There are three main types of bacterial gene transfer:

- transformation,
- transduction,
- and conjugation.



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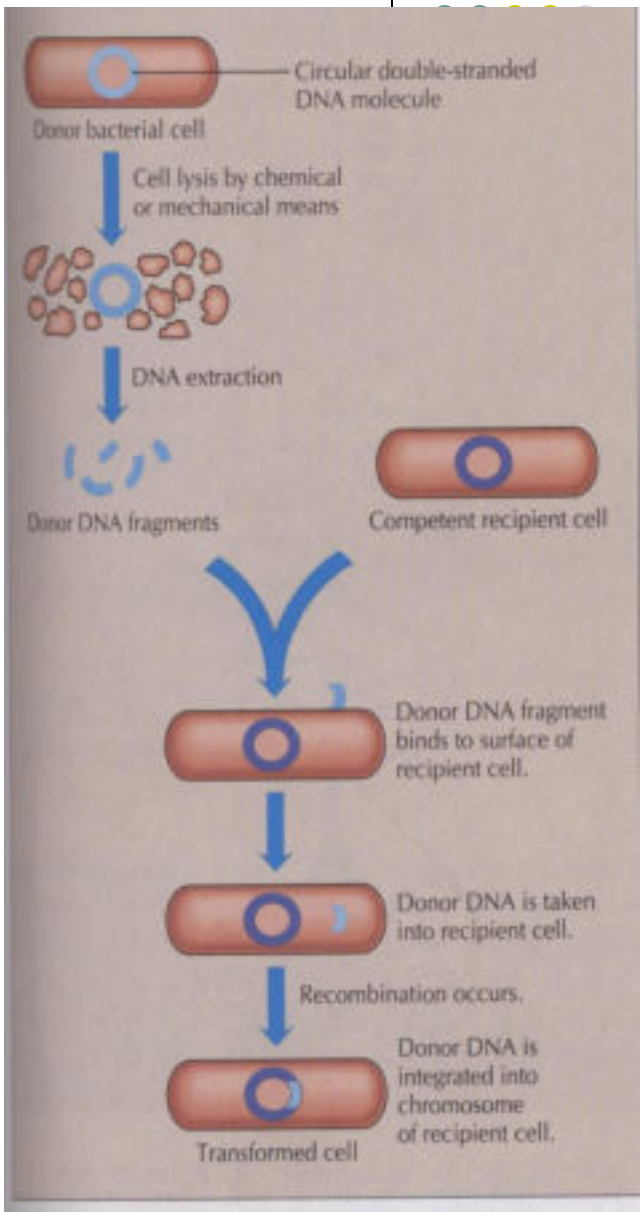
Transformation is a transfer of genetic information through the agency of free DNA

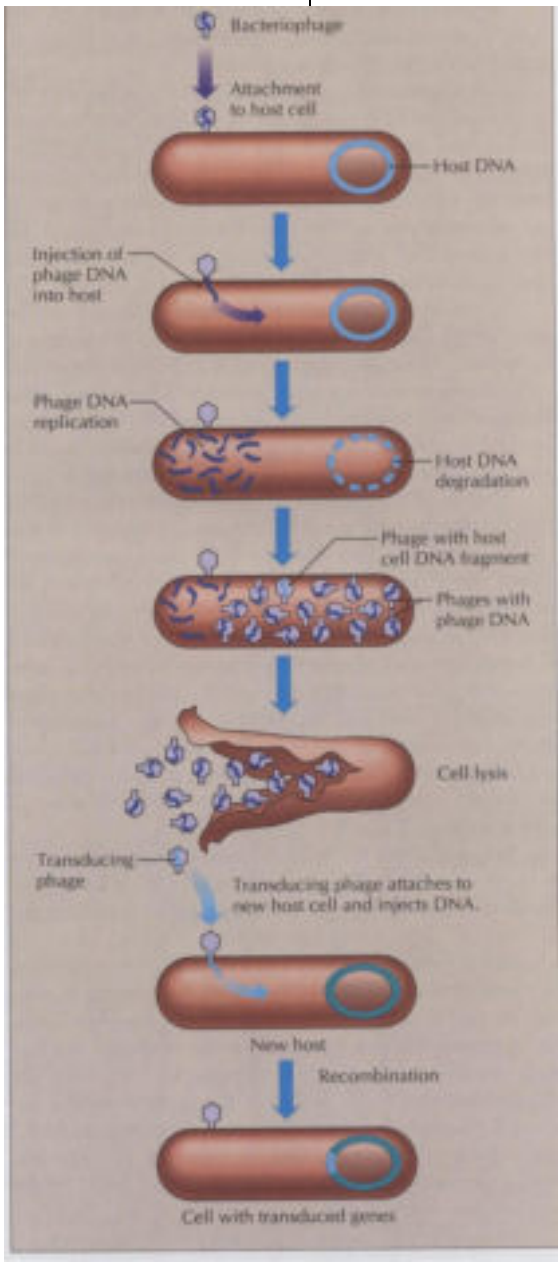
In transformation, a recipient cell acquires genes from donor genome with DNA molecules in the surrounding medium.

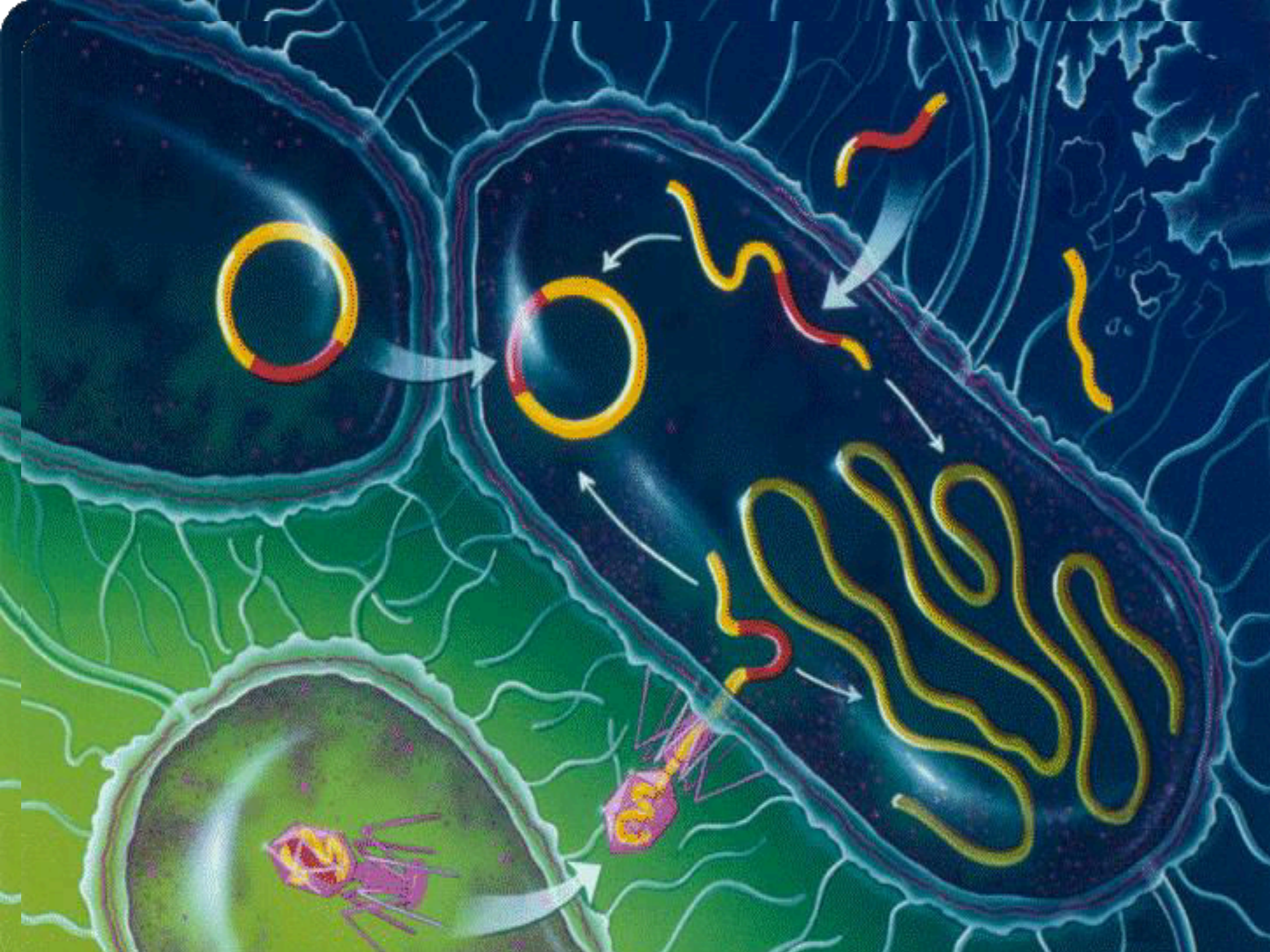


Transduction

In transduction, DNA from a donor cell is carried by a virus (bacteriophage) to a recipient cell.

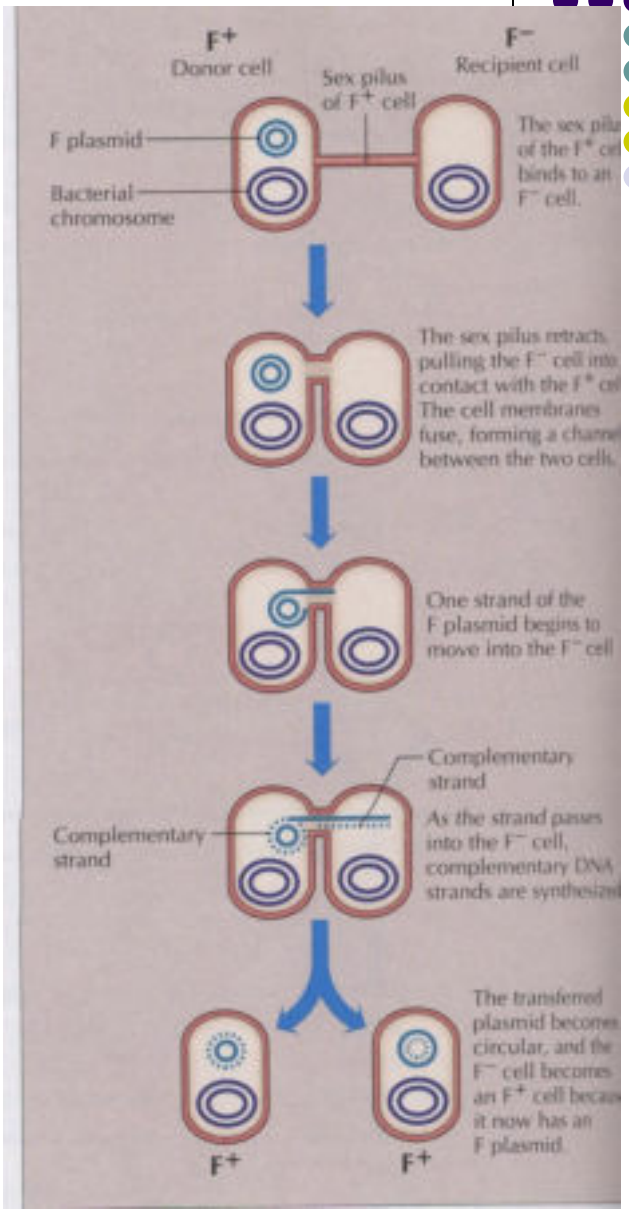
It may be specific, non-specific and abortive.

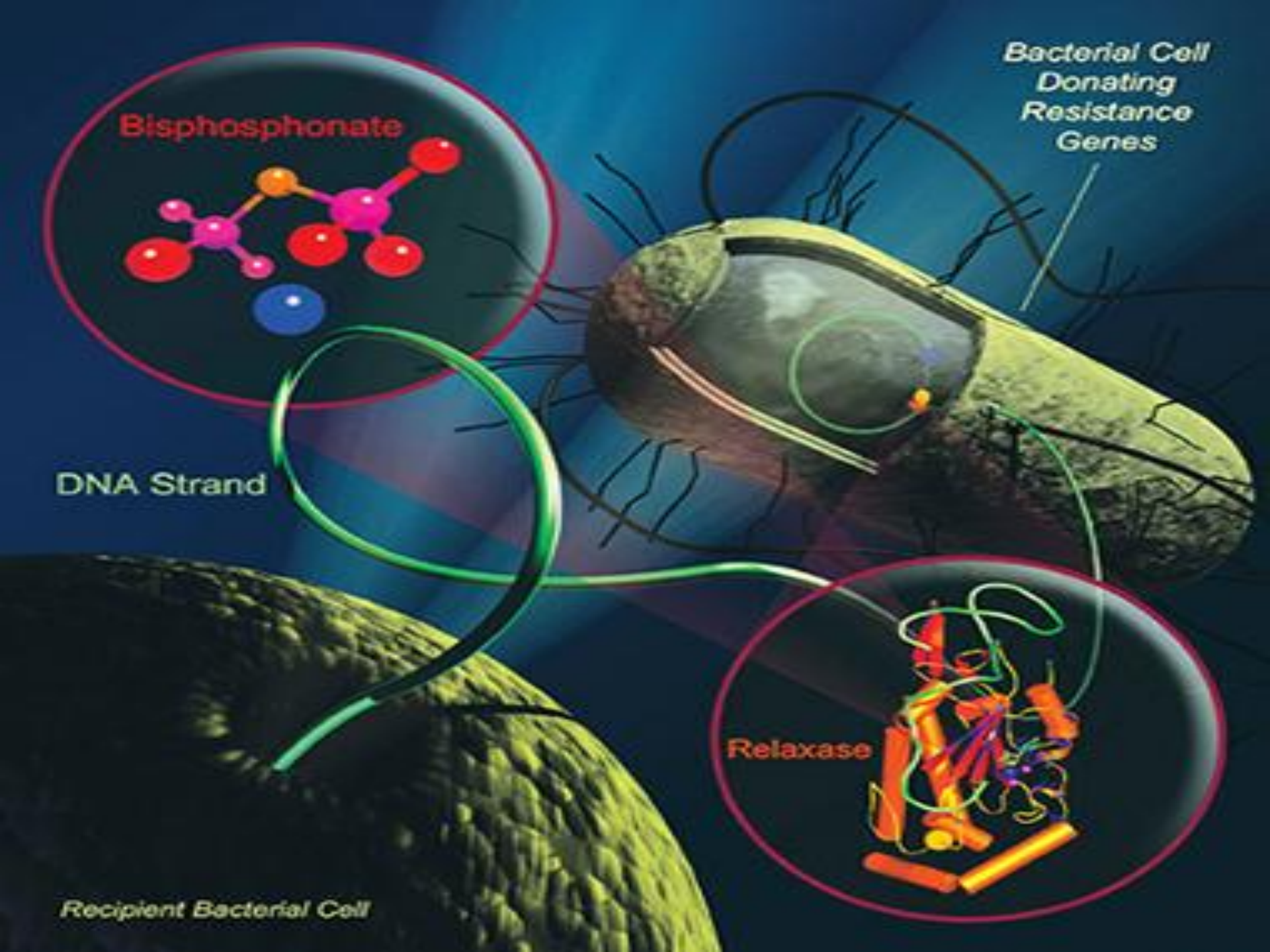




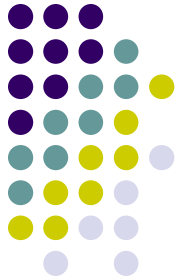
Conjugation

- is dependent on cell-to-cell contact; it may involve transfer of a plasmid, such as the F plasmid in E. coli.
- The F factor contains the genes for the specialized pilus, called a sex pilus, used in conjugation.
- Bacteria with the F factor in plasmid form are referred to as F+ (“male cell”)
- Bacteria without F-factor is marked as F- (“female cell”)




Bacterial Cell
Donating
Resistance
Genes
Bisphosphonate
DNA Strand
Relaxase
Recipient Bacterial Cell

Applications of the molecular genetics

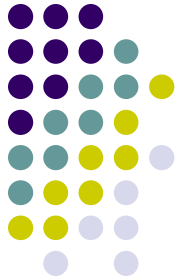


Molecular genetics is concerned with the analysis and manipulation of DNA using biochemical and microbiological technique

Applications of the molecular genetics:

- Genetic engineering or recombinant DNA technology
- Synthesis of the specific DNA probes for hybridization test with DNA from collected material (molecular genetics diagnostic test is named southern blot technique)
- Polymerase chain reaction (PCR)
- Genetic mapping and others

Molecular genetic assay in laboratory diagnostics



Molecular methods allow to compare determined bacterial proteins; to estimate nucleic acid base composition; to reveal specific combination of the DNA or RNA molecules proper for definite pathogen

- **The most widely used molecular methods are next:**

1. **Analyses of the DNA or RNA in the test sample (southern and northern blotting respectively)**
2. **Polymerase Chain Reaction (PCR)**

Southern blotting

Southern blotting allows to determine specific fragments of the DNA in the examined sample. Procedure consists of several steps:

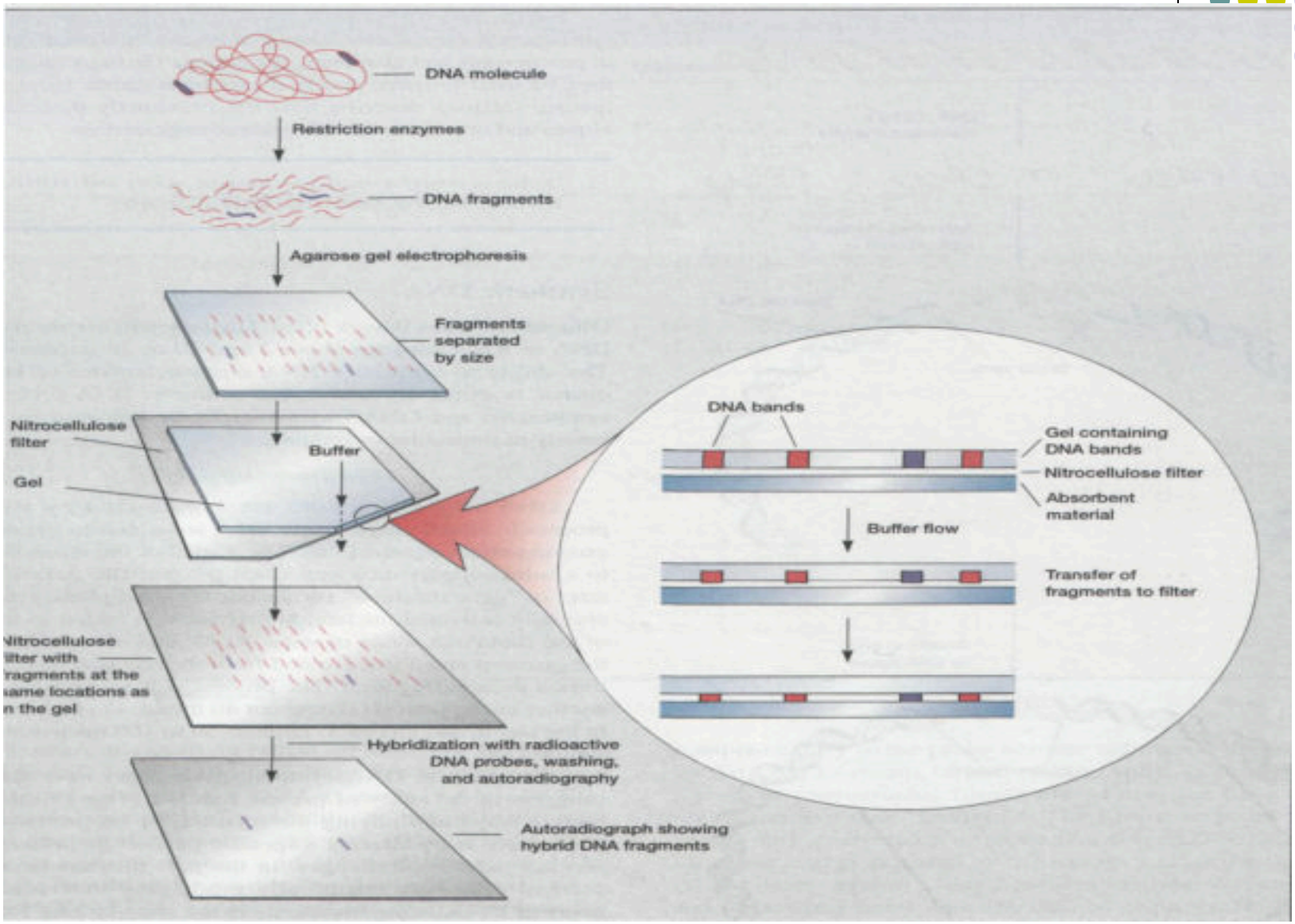
1. **DNA fragments are obtained by restriction enzymes and separated in the gel**
2. **DNA fragments are transferred from gel to nitrocellulose by blotting and converted to the single-stranded form by denaturation**

Southern blotting

3. Then they are treated with radioactive single stranded DNA probes (highly specific probes)
4. If homogenous DNA is present on the membrane, it will hybridize with radioactive probes to form radioactive double-stranded fragments which can be detected

Nothern blotting is the equal procedure for analyses of the RNA fragments obtained from the specimen

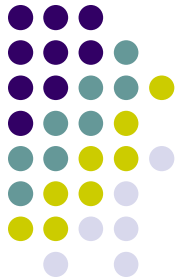
Southern blotting



The Polymerase Chain Reaction (PCR)

- It is a rapid automated method for the amplification of the specific DNA sequences (or genes)
- PCR consists of the several cycles of the sequential DNA replication.
- The cycles are repeated several time in the termocycler (in average 20-50 cycles)until enough number of the DNA copies will be obtained

The Polymerase Chain Reaction (PCR)

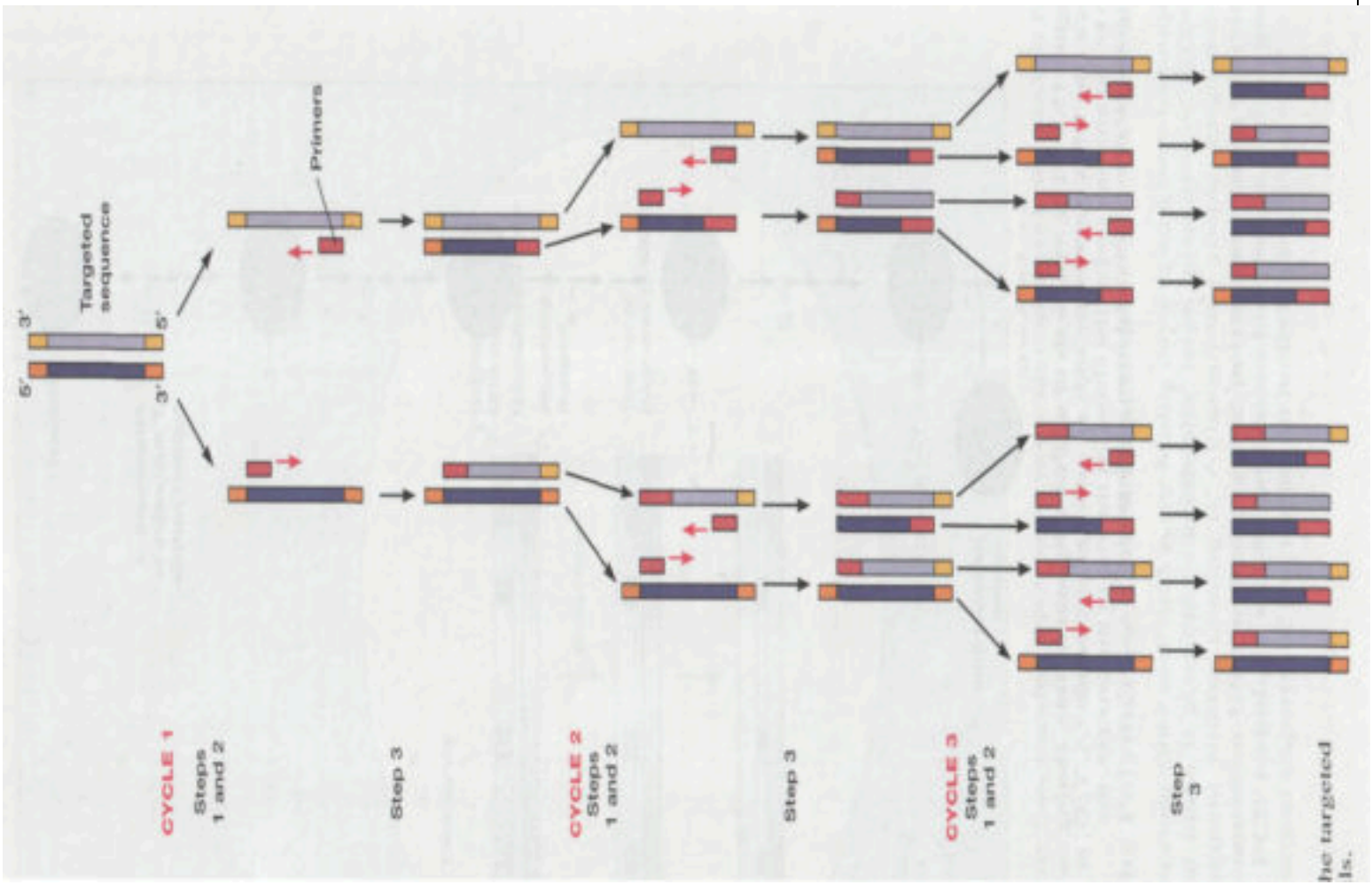


- **Each cycle** consists of the three steps:

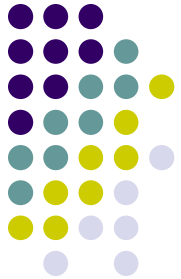
1. **Heat denaturation** of the sample DNA to single strain at 94^0C for 15 sec
2. **Annealing of the sequence specific oligonucleotide primers** to the flanking region of the DNA segment
3. **Extension of the primers by DNA polymerase** to form new double-stranded DNA across the segment by sequential addition of nucleotides

The last two steps take 60 sec and are made at 68^0C

The Polymerase Chain Reaction (PCR)



Genetic engineering



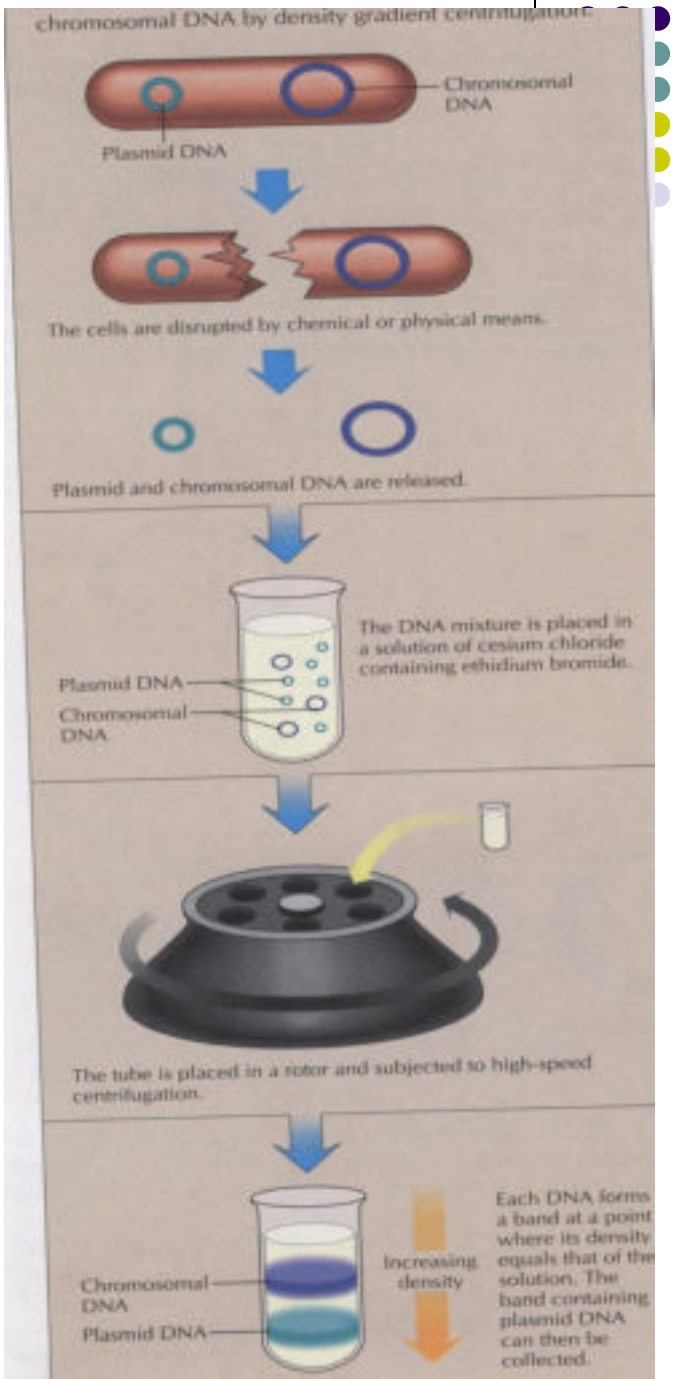
It is a transfer of the genes from one cell to another using a gene carrier called a vector in vitro.

Plasmids have usually been used as vectors because they are easily isolated and manipulated, and do not have to be integrated into the bacterial chromosome in order to be replicated by the recipient cells.

The steps in recombinant technology will be next:

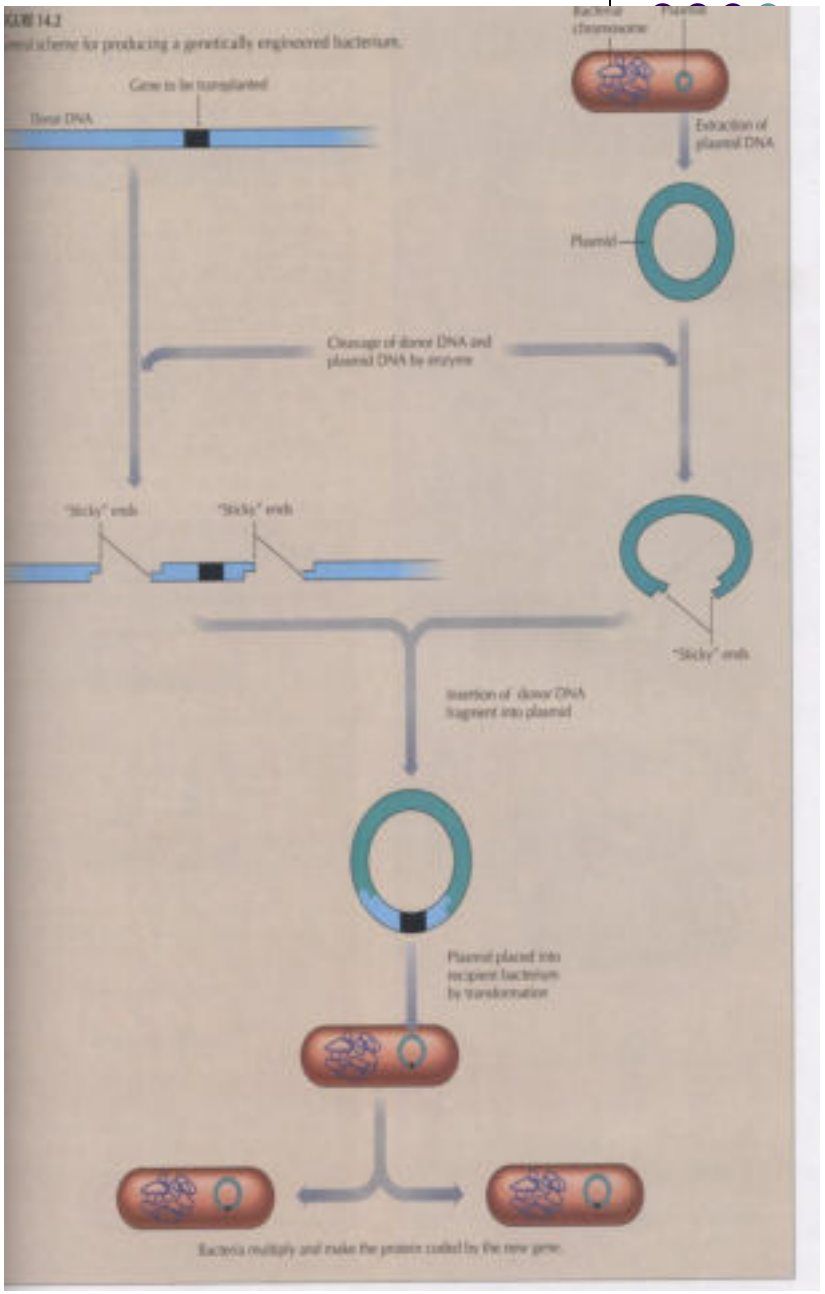
(a) DNA containing the desired gene can either be isolated from donor cells or synthesized by laboratory procedures

(b) The plasmid vector is obtained from the bacterial species into which the donor gene is to be placed



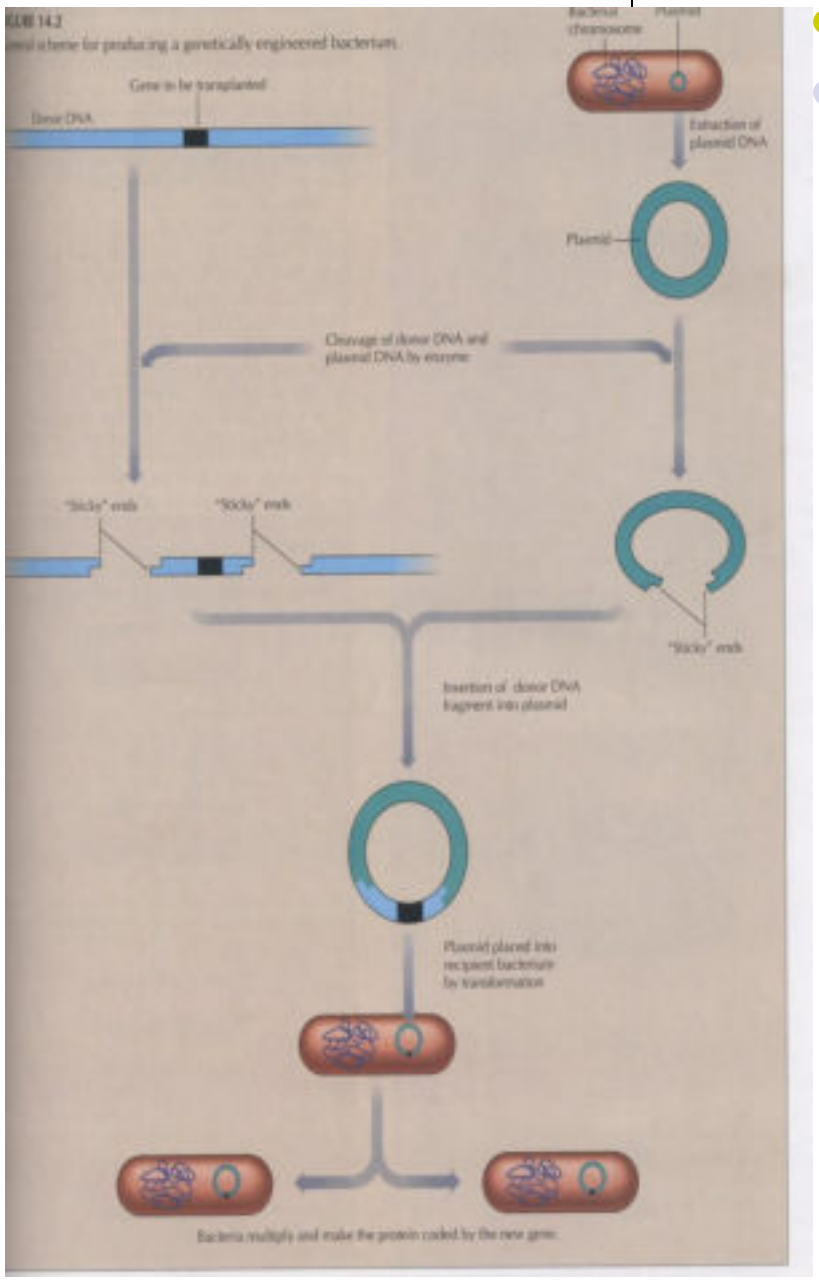
(c) **Both the donor DNA and the plasmid DNA are then treated with the same restriction endonuclease.**

(d) **The sticky ends of a donor DNA fragment form hydrogen bonds with the complementary sticky ends of the plasmid DNA, and DNA ligase is used to repair the break in the sugar-phosphate backbone.**

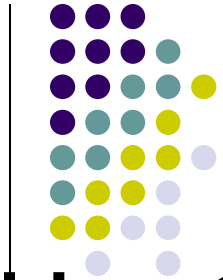


(d) **The resulting recombinant plasmid is then incorporated into a recipient bacterium by means of a technique such as transformation, transfection or electroporation .**

(e) Colonies of those bacteria that can express the new gene are then propagated and the gene product is extracted from the cultures.



Biotechnology

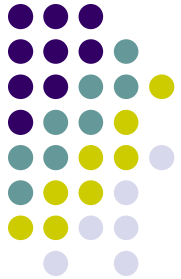


It is an industrial exploitation of a recombinant strain

Medicines obtained from producer strains of bacteria and yeasts

- **Human insulin:** Humulin,
- **Human growth hormone:** Humatrope, Protropin
- **Human interferons:** Roferon, Intron

Medicines and diagnostic preparations



- **Tissue plasminogen activator** can dissolve blood clots at the site of their formation.
- **Interleukin-2** promotes activation and multiplication of leucocytes and enhances the host's ability to resist infection.
- **Vitamins of group B, vitamin C**
- **Recombinant vaccines for prophylaxis of hepatitis B, malaria**
  - Recombivax HB, Engerix-B: hepatitis B vaccine
  - HIbTiter: haemophilus B conjugate vaccine
- **Virus antigens for diagnostic kits**

Physiology of microorganisms studies biochemical processes of cell which are responsible for provision of energy and chemical compounds for growth and reproduction

Chemical composition of microbial cell :

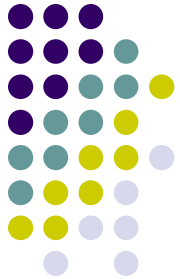
- Water is about 80-90%
- Dry remainder (mixture of organic and non-organic chemical compounds) is about 10-20%

Macroelements or macronutrients: C,N, O, H, P, S

Main minerals: Na, Mg, K, Ca, Fe

Trace elements : Mn, Mo, Zn, Cu, Co, Ni

Classification of bacteria due their nutrient sources



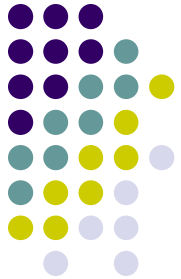
- According to carbon and nitrogen source:

1) **Autotrophs (lythotrophs), which** can use CO_2 and inorganic nitrogen compounds as their sole or principal source of essential elements.

2) **Heterotrophs** (organotrophs) Organisms that use reduced, preformed organic molecules as both carbon and nitrogen sources

- **Saprophytes**
- **Parasites** (facultative and obligate)

Classification bacteria according to source of energy:

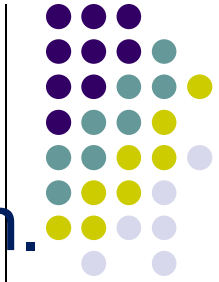


- **Phototrophs** use light as their energy source;
- **Chemotrophs** obtain energy from the oxidation of chemical compounds (either organic or inorganic).
  - Chemoorganotrophs
  - Chemolytotrophs

Ways for energy accumulation:

1. **Biological oxidation (aerobic and anaerobic respiration)**
2. **Fermentation**

Nutrition of bacteria



Bacteria possess a holo-phyto-type of nutrition. They absorb nutrients in a dissolved state by the whole surface.

Mechanisms of nutrient transport into microbial cell:

- Independent of energy

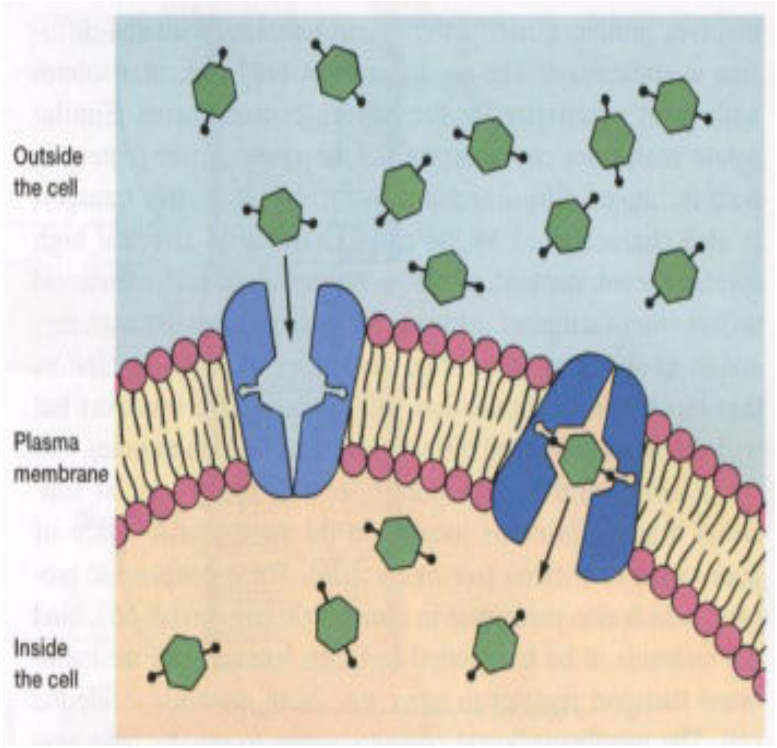
1) passive diffusion
2) facilitated diffusion

- Depending on energy:

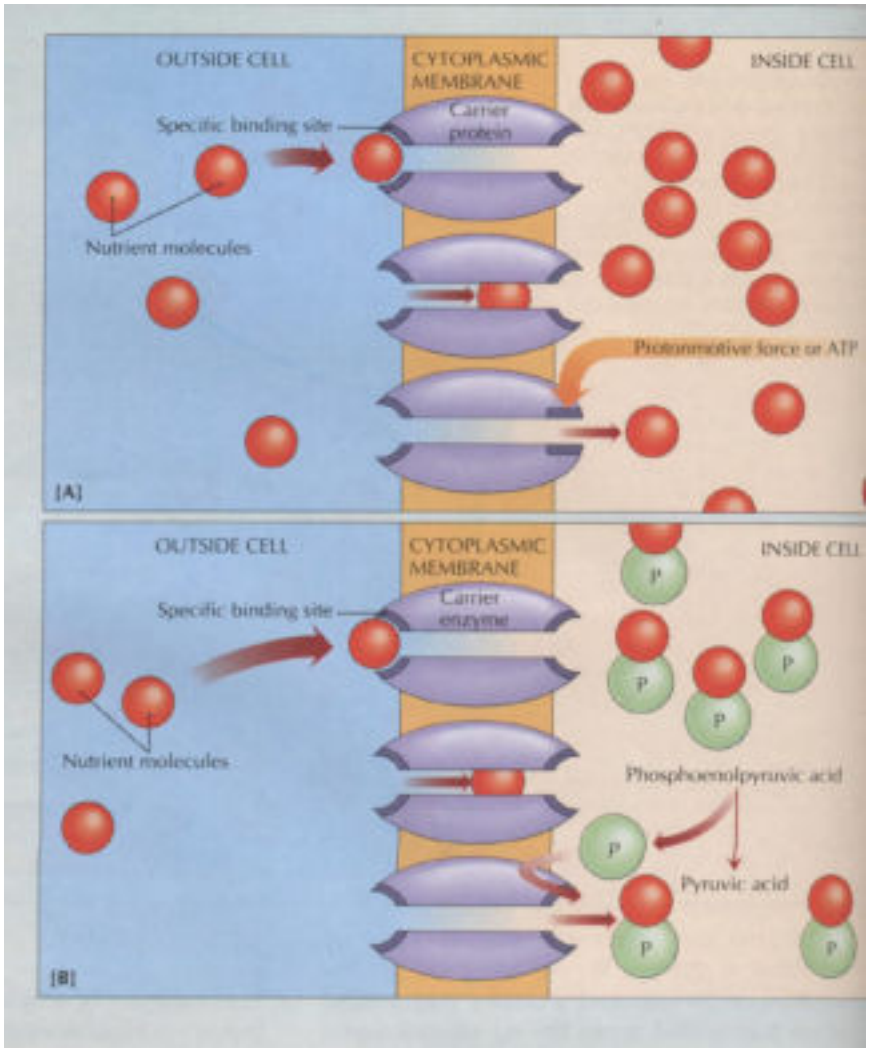
1) Active transport
2) Group translocation through phosphorylation.
3) Ion transport

Different types of transport through CPM

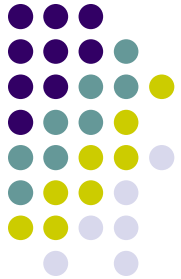
Facilitated diffusion



Active transport



Classification of bacterial enzymes

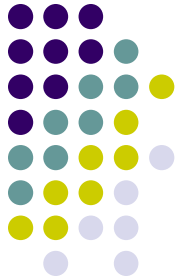


- **Adaptive (induced) enzymes** occur only in the presence of the corresponding substrate.

  Most of them are **exoenzymes**.

- **Constituent (essential) enzymes** are constantly found in the cell irrespective of the presence of a catalyzing substrate. As a rule, these compounds are **endoenzymes**

Classification of bacterial enzymes



Due to split substrate:

- Proteolytic enzymes. They split polypeptides, oligopeptides and amino acids. Such final products as hydrogen sulphide, indole and ammonia may be revealed with indicators
- Sugarlytic enzymes. They split carbohydrates either to acid or to acid and gas. Acidity may be revealed with special indicator
- Lipolytic enzymes

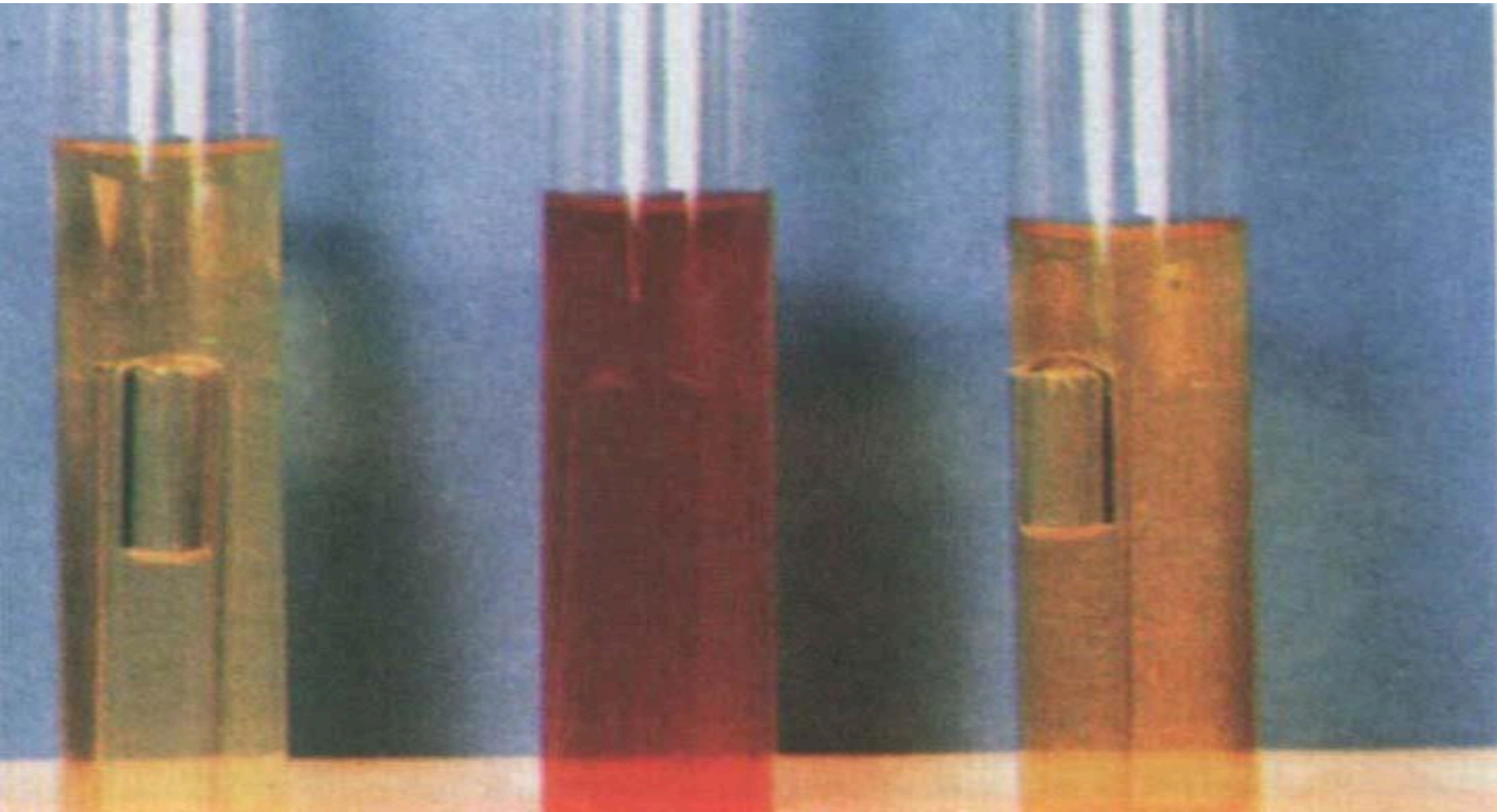
Another group includes virulent enzymes

Determination of biochemical properties in special nutrient media (Hiss media)

on left: reaction is positive

on right: reaction is weak-positive

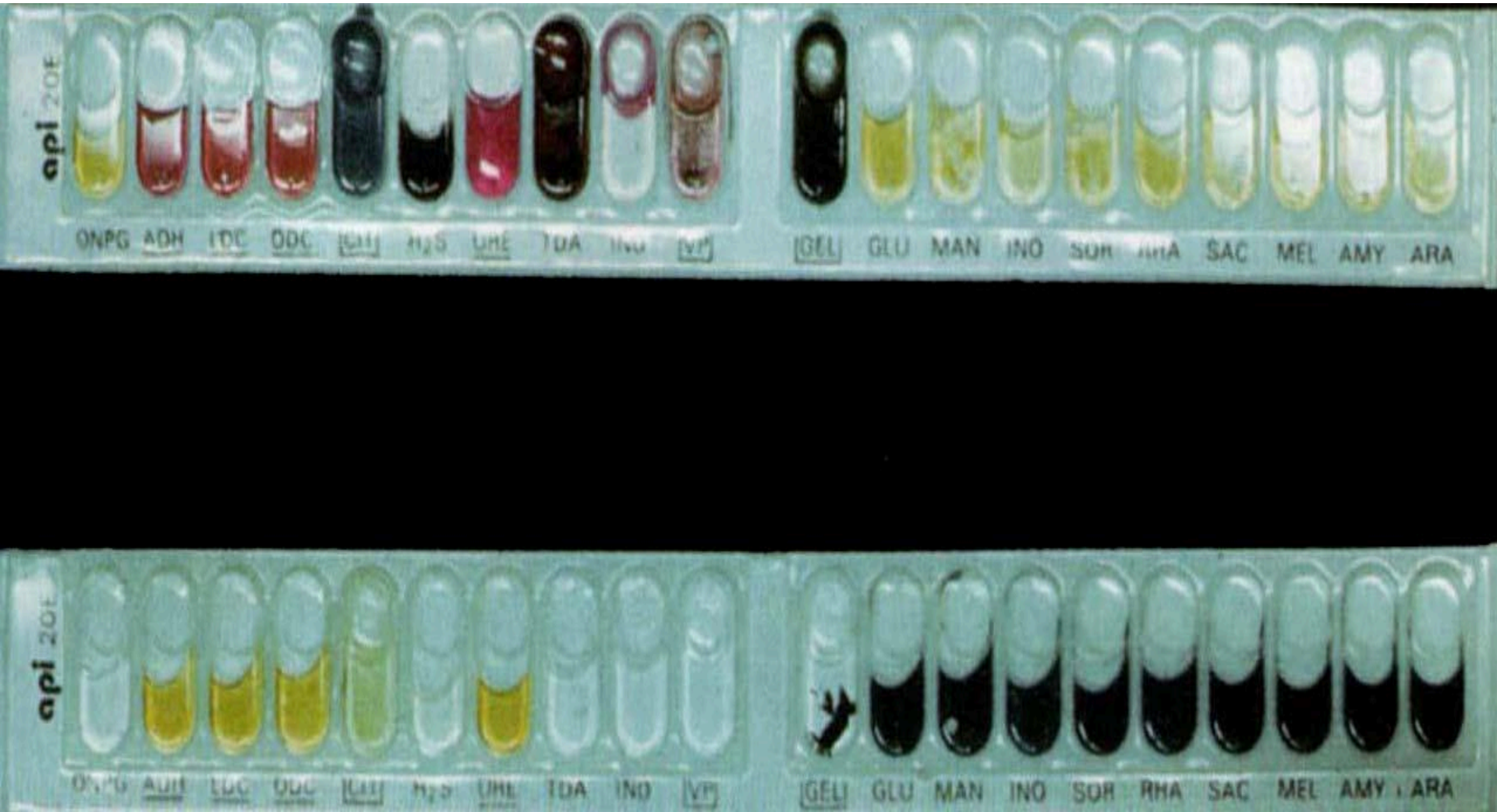
on center: one is negative



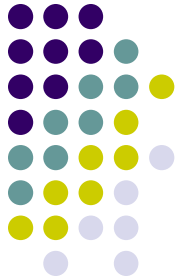
Express test API 20E for biochemical bacteria identification:

on top: positive test

on bottom: negative test

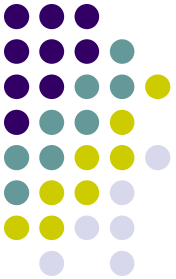


According to type of respiration bacteria may be subdivided into:



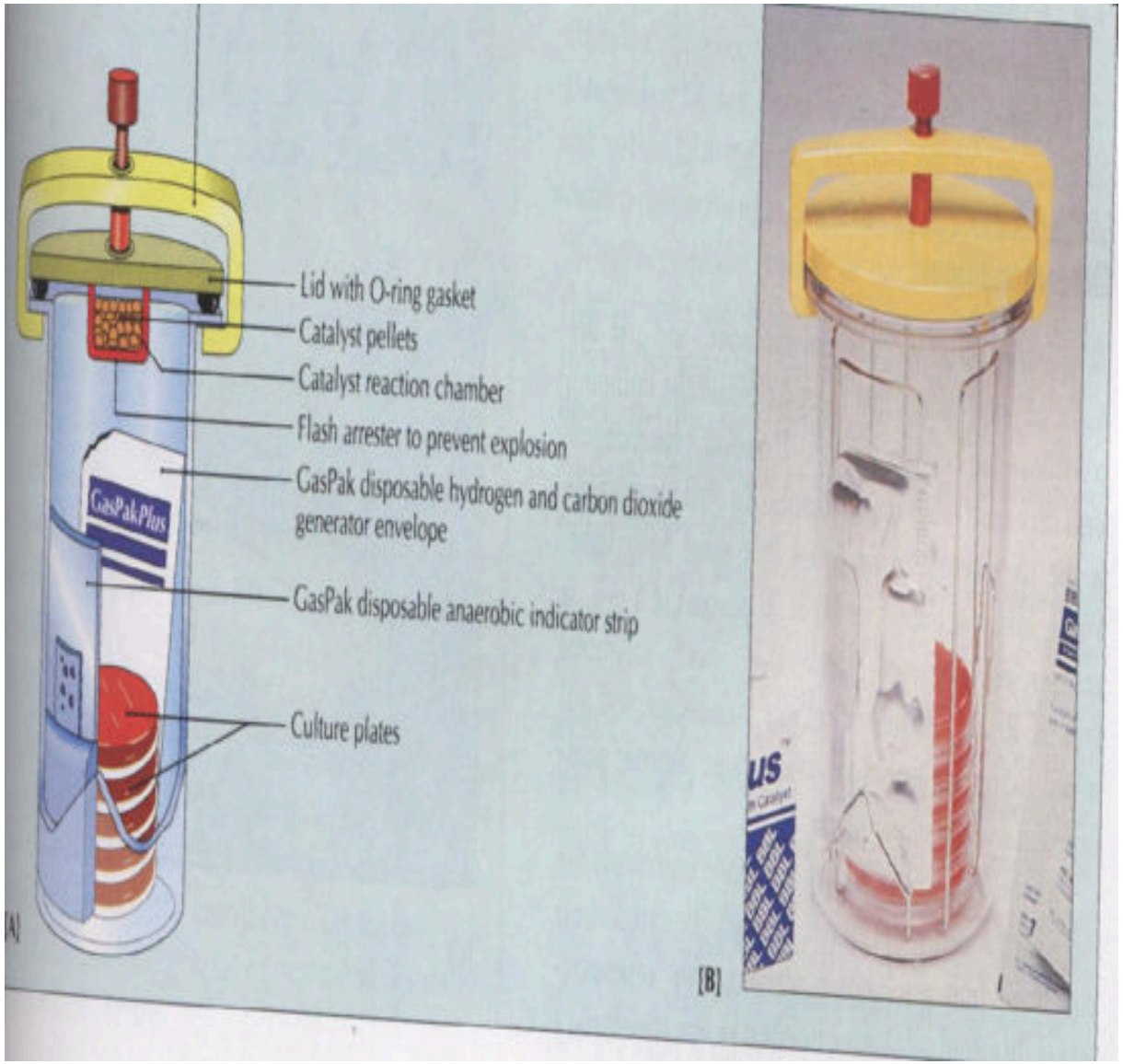
- **Obligate aerobes** which develop well in an atmosphere containing 21 % of oxygen.
- **Facultative anaerobes** which can reproduce even in the absence of molecular oxygen (It is typical for the most part of medical important germs).
- **Obligate anaerobes** for which the presence of molecular oxygen is a harmful growth-inhibiting factor (causative agents of tetanus, botulism, anaerobic infections, etc.).

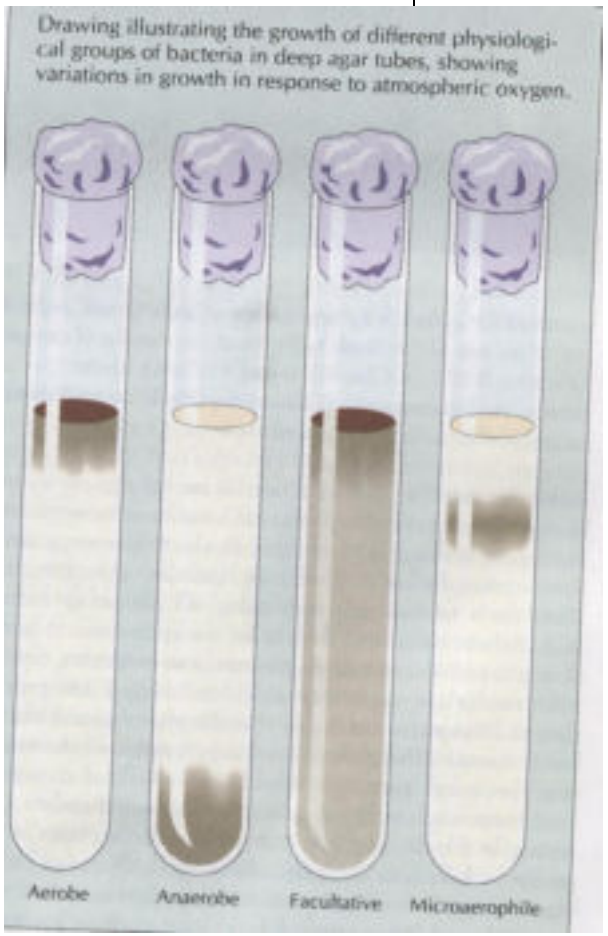
Methods for creation of anaerobic conditions



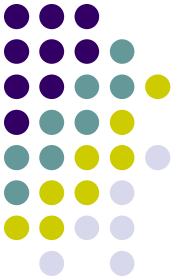
- Mechanical method is usage special chamber (anaerostat) from which air is removed and another gas is pumped in
- Chemical method : bacteria are cultivated with substances which adsorb an oxygen
- Gas container with regenerative substance
- Biological methods: aerobs and anaerobs are seeded in medium and they are cultivated in the same time
- Cultivation within nutrient media

Methods for creation of anaerobic conditions





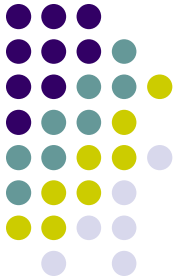
Bacterial cultivation



Requirements for propagation:

- Optimal nutrient media (ordinal, special, enrichment, selective, differential)
- If necessary, growth factors addition
- Optimal pH (7.6-7.8)
- Optimal temperature (37°C)
- Aerobic or anaerobic environment (according to type of respiration)

Growth Factors

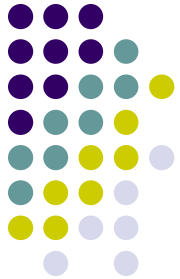


- **Vitamins**
- **Purine, pyrimidine**
- **Amino acids**

Auxotroph is a microorganism which requires addition of growth factors into nutrient media

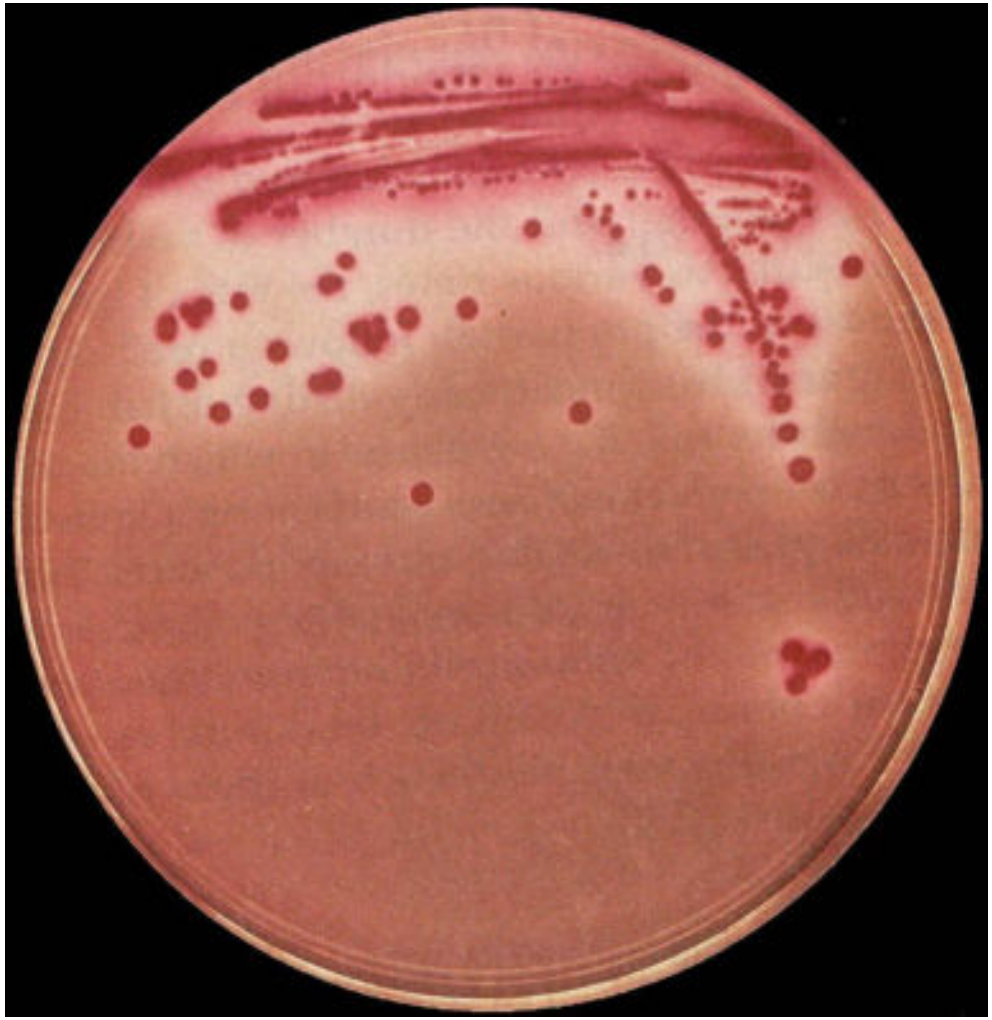
Prototroph is a microorganism which produces growth factors from natural components itself

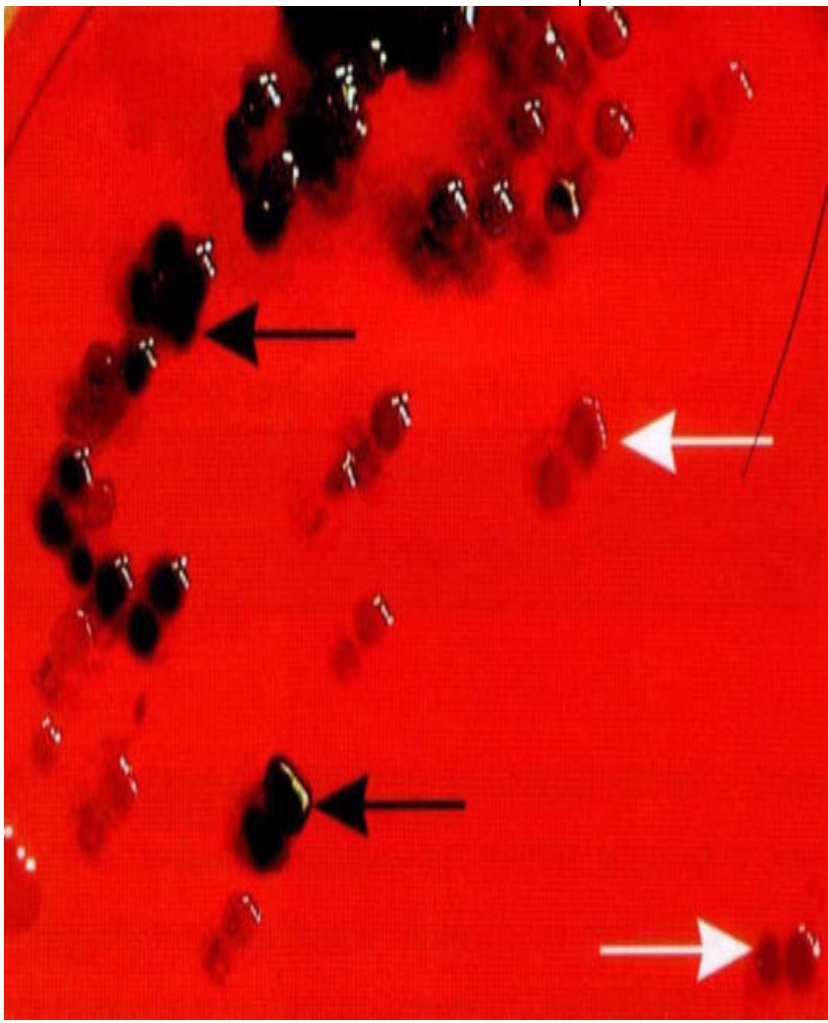
Classification of culture media



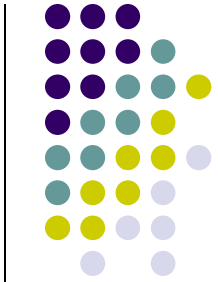
- **According to consistency : liquid (broth), solid (agar), semisolid**
- **According to origin: natural, synthetic, semisynthetic**
- **According to purpose of use:**
  - **Basic nutrient media (MPA, MPB)**
  - **Special media: differential-diagnostic, selective, enriched, for biochemical activity detection, for transport, etc.**

Differential-diagnostic media





Culture properties of bacteria



- Bacteria grow into a fluid media with formation:

-opacity or turbidity (**diffuse suspension**)

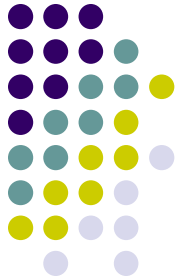
-film or pellicle

-deposit or precipitate

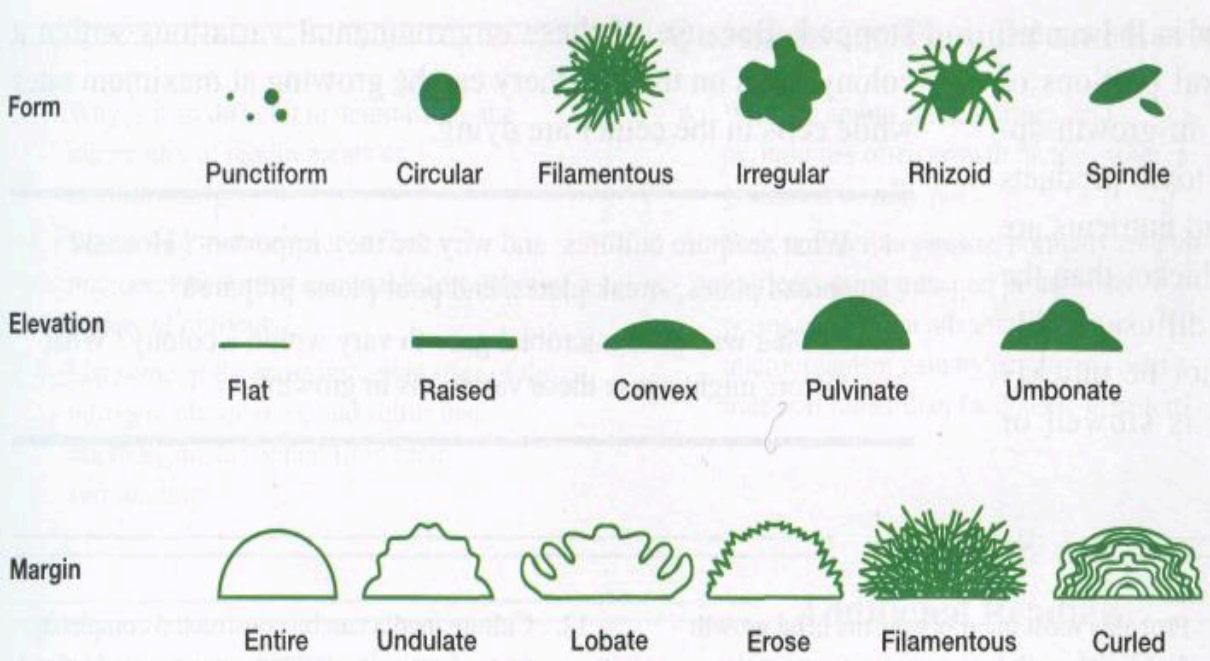
- combination of these forms

- Growing bacteria form a colony on the solid media
- **Colony is a clone of cells originating from a single bacterial cell, which has grown on or within solid medium.**

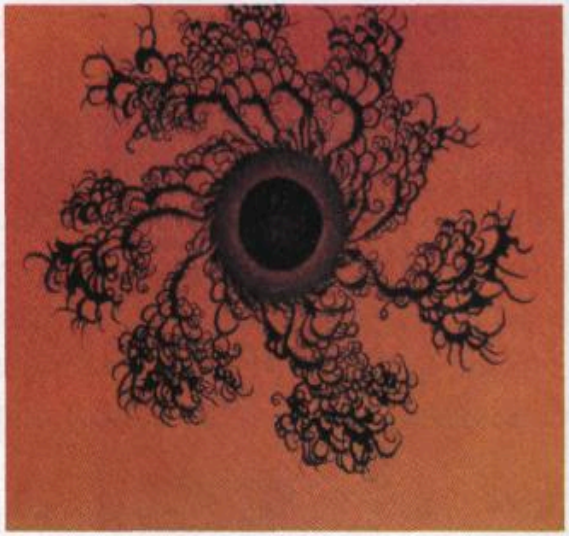
Features of bacterial colonies



- shape: circular, irregular, or rhizoid;
- size in millimeters;
- elevation: effuse, elevated, convex, concave
- margins – beveled or otherwise
- surface – smooth, wavy, rough, granular, etc
- edges – entire, undulate, curled
- colors
- structure – opaque, translucent, transparent
- consistency – friable, membranous or viscid
- emulsifiability



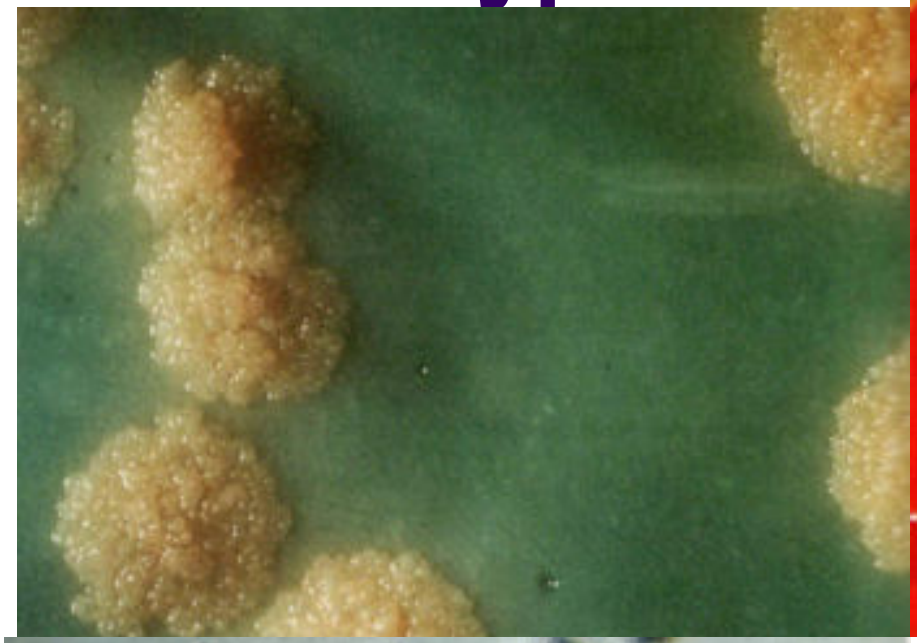
(a)

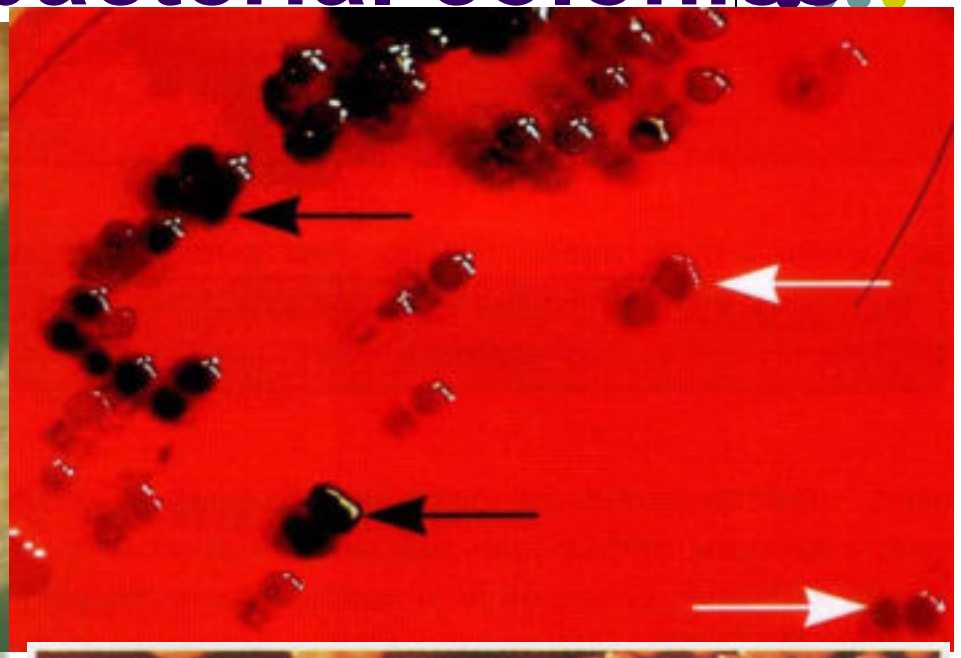


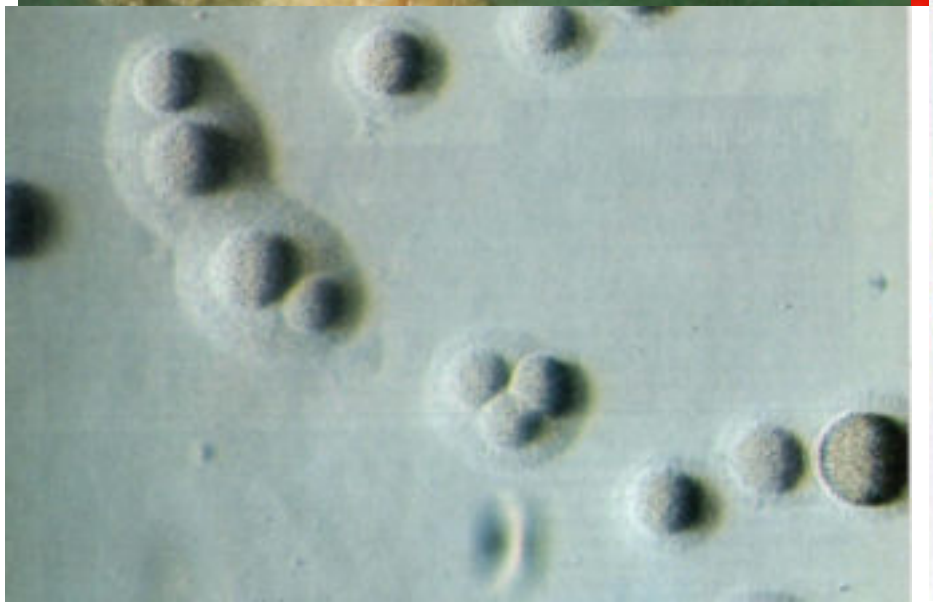
(b)

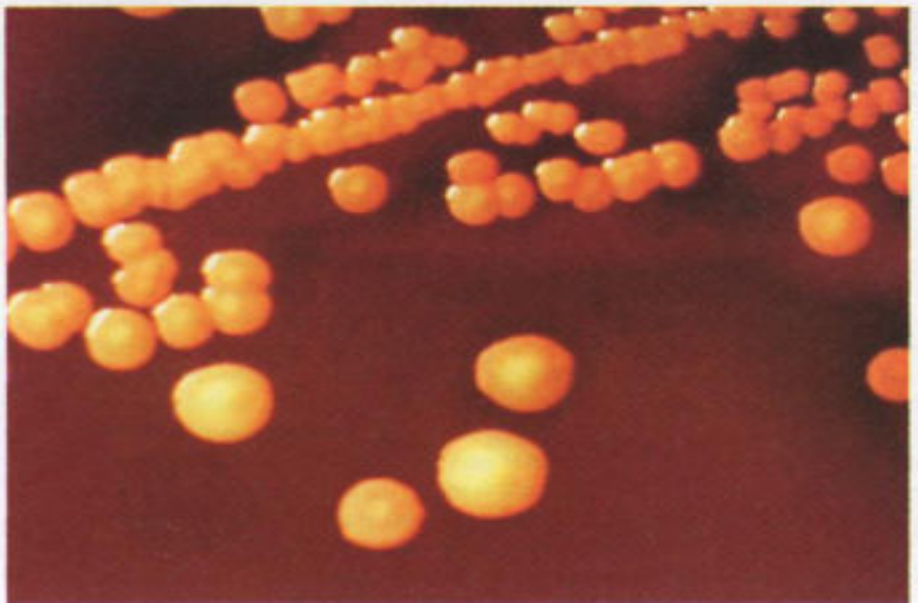
Figure 5.10 **Bacterial Colony Morphology.** (*a*) Variations in bacterial colony morphology seen with the naked eye. The general form of the colony and the shape of the edge or margin can be determined by looking down at the top of the colony. The nature of colony elevation is apparent when viewed from the side as the plate is held at eye level. (*b*) Colony morphology can vary dramatically with the medium on which the bacteria are growing. These beautiful snowflakelike colonies were formed by *Bacillus subtilis* growing on nutrient-poor agar. The bacteria apparently behave cooperatively when confronted with poor growth conditions, and often the result is an intricate structure that resembles the fractal patterns seen in nonliving systems.

Different types of bacterial colonies









Cultural method of laboratory diagnostics

- **Routine procedure of the pure culture isolation takes at least 2 days:**

First day:

1. **Microscopy of a smear from sample stained with Gram`s technique**
2. **Inoculation of a collected specimen on the appropriate plate medium by suitable way: streak culture, pour culture, carpet culture (exception is anaerobic bacteria isolation: sample is inoculated into Robertson cooked meat broth) with successive overnight incubation at 37^0C**

Cultural method of laboratory diagnostics

Second day:

1. Appeared colonies are studied and described (cultural characteristics of a pathogen)
2. Inoculation of the separated suspect colony with stroke technique onto the slope agar to isolate a pure culture with successive overnight incubation at 37^0C

Third day:

Tests for bacteria identification, antibiotic susceptibility tests


1st day


2nd day


Identification

Identification of a germ

- Pure culture of the pathogen has to be identified
- Methods of the identification:

1. Microscopic observation (detection of morphological features)
2. Examination of the cultural characteristics
3. Determination of the bacterial enzymes or metabolic products with biochemical tests
4. Detection of the antigens with serologic reactions
5. Determination of the susceptibility to bacteriophages
6. Determination of the resistance to antimicrobial preparations (antibiotics, chemotherapeutic agents, and disinfectants)