PHYSIOLOGY OF MICROORGANISMS

By

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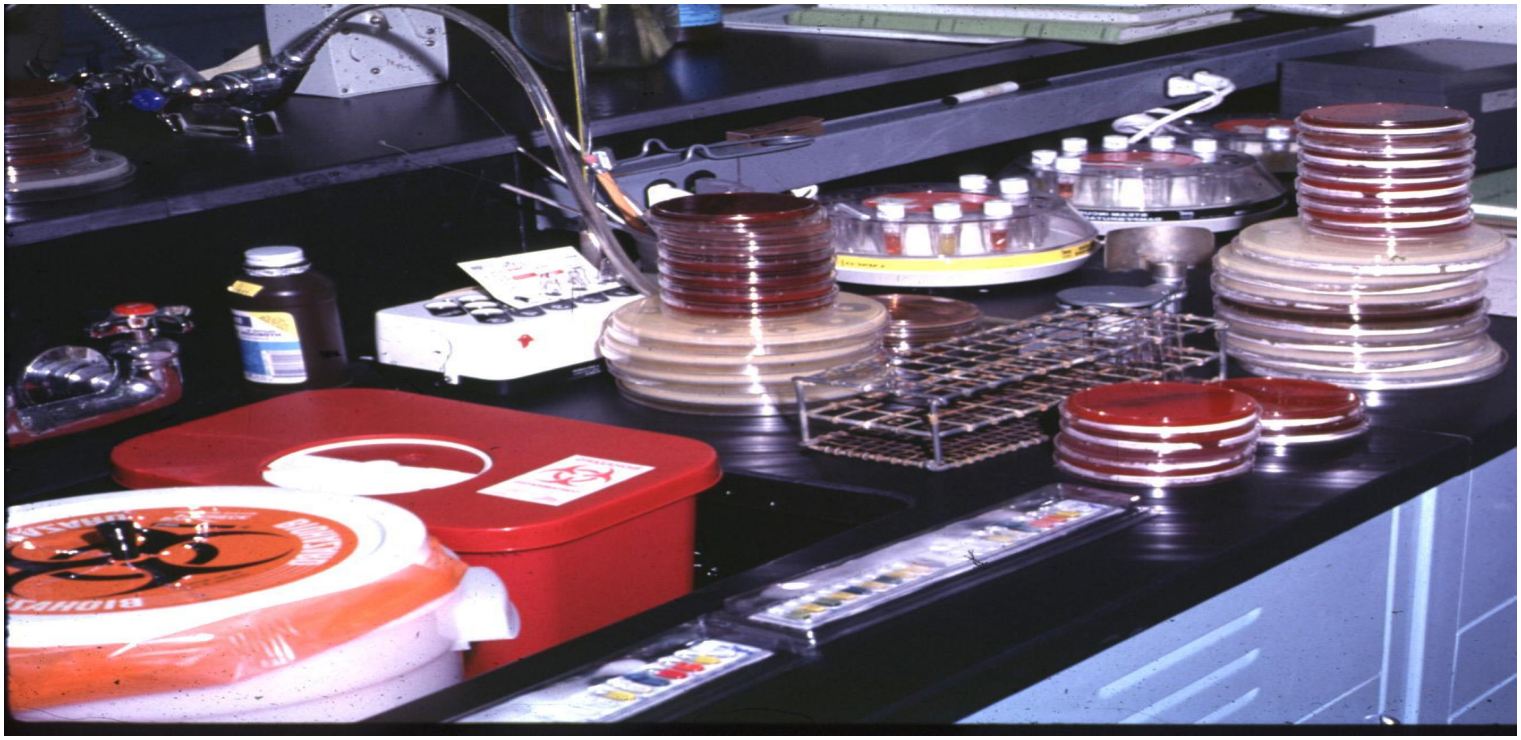
1. Methods of laboratory diagnosis
2. Methods of the bacterial cultivation
3. Identification of bacteria
4. Bacterial metabolism
5. Media for bacterial growth
6. Sterilization

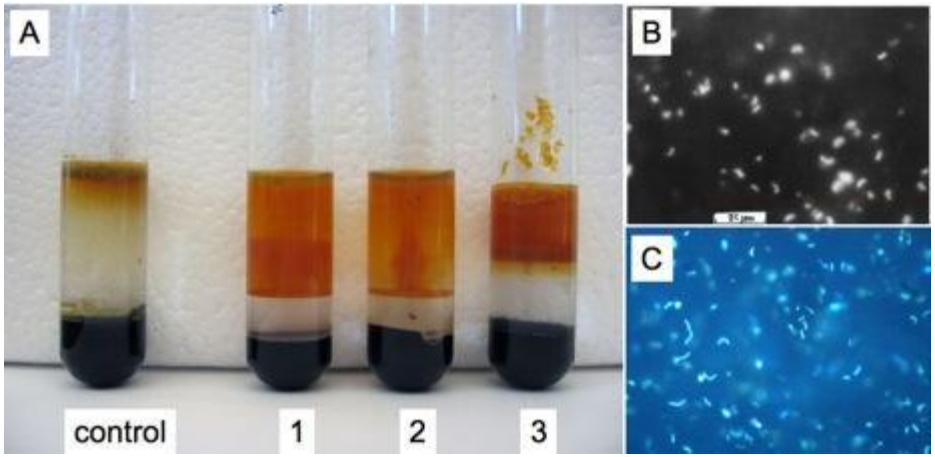
Methods of laboratory diagnosis

1. *Bacterioscopical (Microscopic examination)*
2. *Bacteriological (Culture method)*
3. *Detection sensitivity of bacteria to antibiotics*
4. *Serological*
5. *Biological*
6. *DNA-technology test (PCR)*



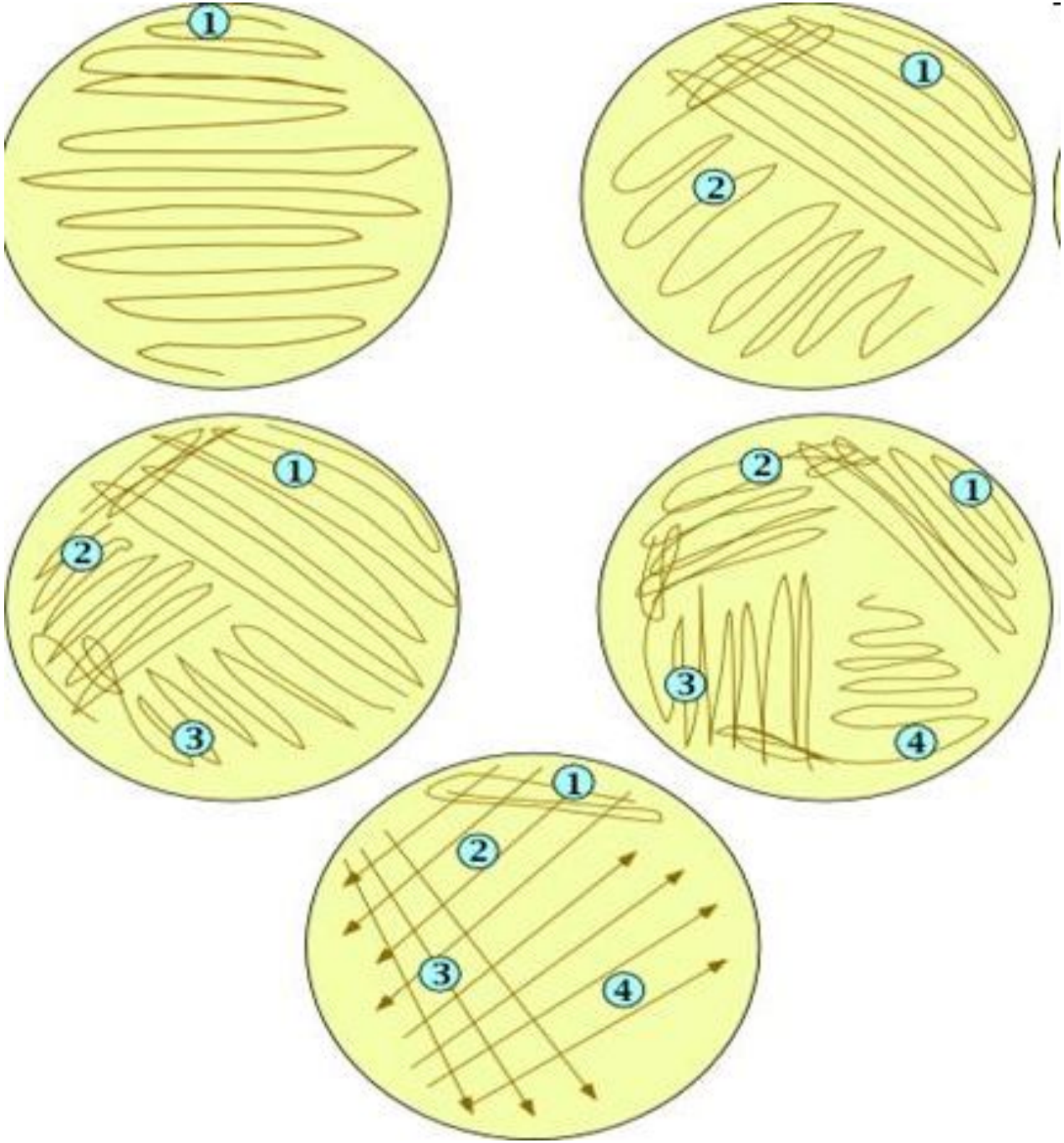
In the clinical laboratory it is necessary:

- **isolate bacteria in pure culture;**
- **obtain sufficient growth of bacteria for demonstration their properties such as study of morphological, cultural, biochemical, antigenic and pathogenic properties, bacteriophage and bacteriocin susceptibility;**
- **determine a sensitivity to antibiotics.**



Methods of the cultivation

- ***Streak culture (surface plating).*** The inoculum is spreaded thinly over the plate of a culture media in series of parallel lines in different segment of the plate. On inoculation well separated colonies are obtained over the final series of streaks.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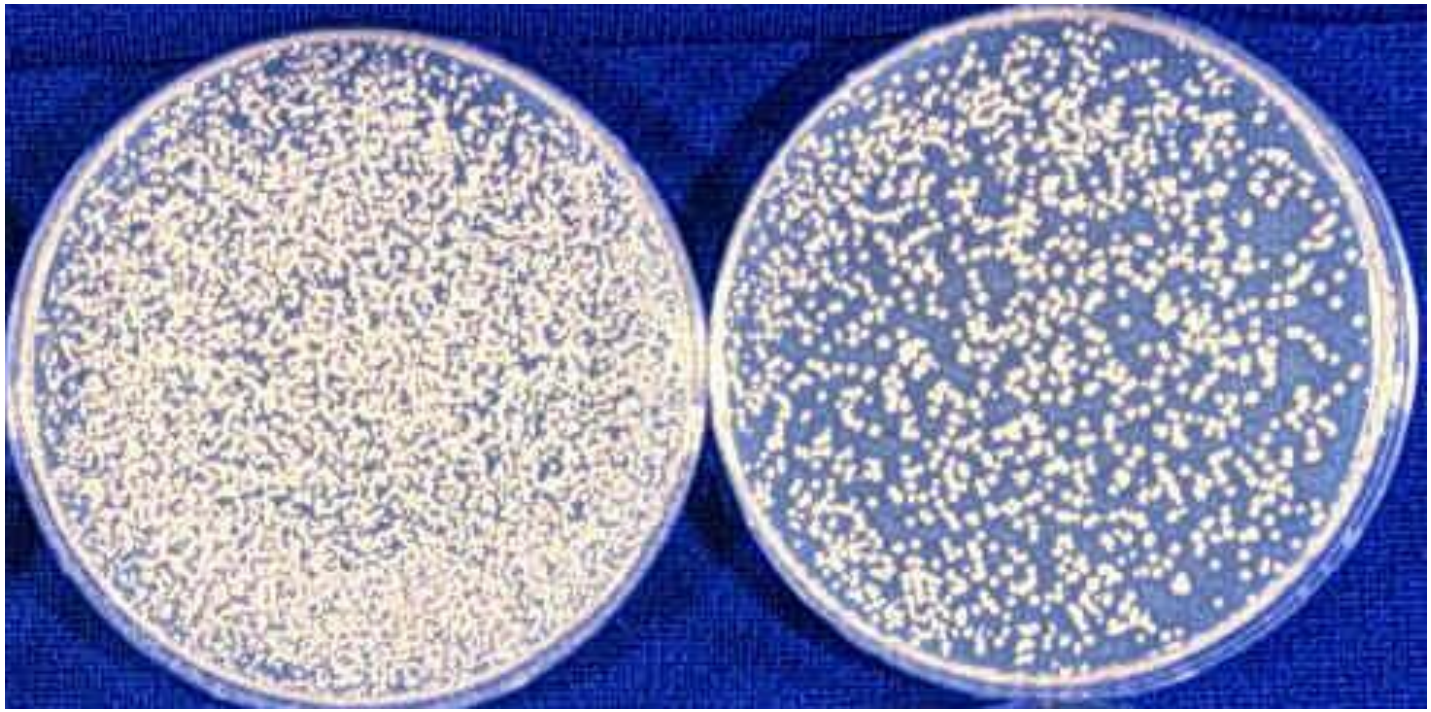
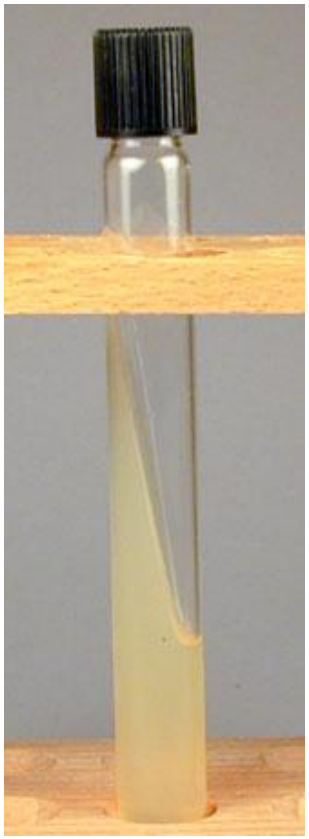
Methods of the cultivation

- ***Lawn or carpet culture***. Lawn cultures are prepared by flooding the surface or plate with suspension of bacteria. It provides uniform surface growth of bacteria. It is useful for bacteriophage typing and antibiotic sensitivity test.



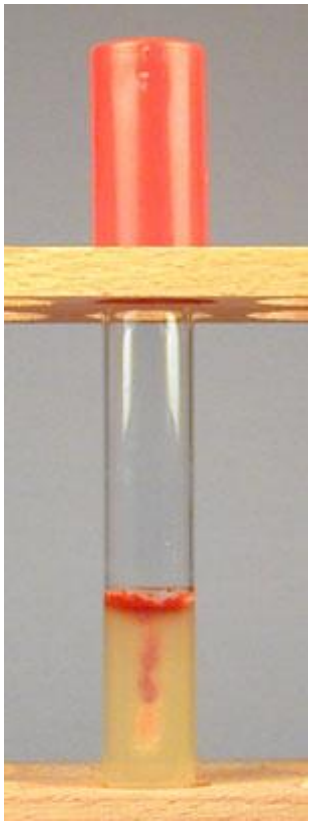
Methods of the cultivation

- ***Stroke culture.*** It is made in tubes containing agar slopes. It is used for providing a pure growth of bacterium (for slide agglutination).

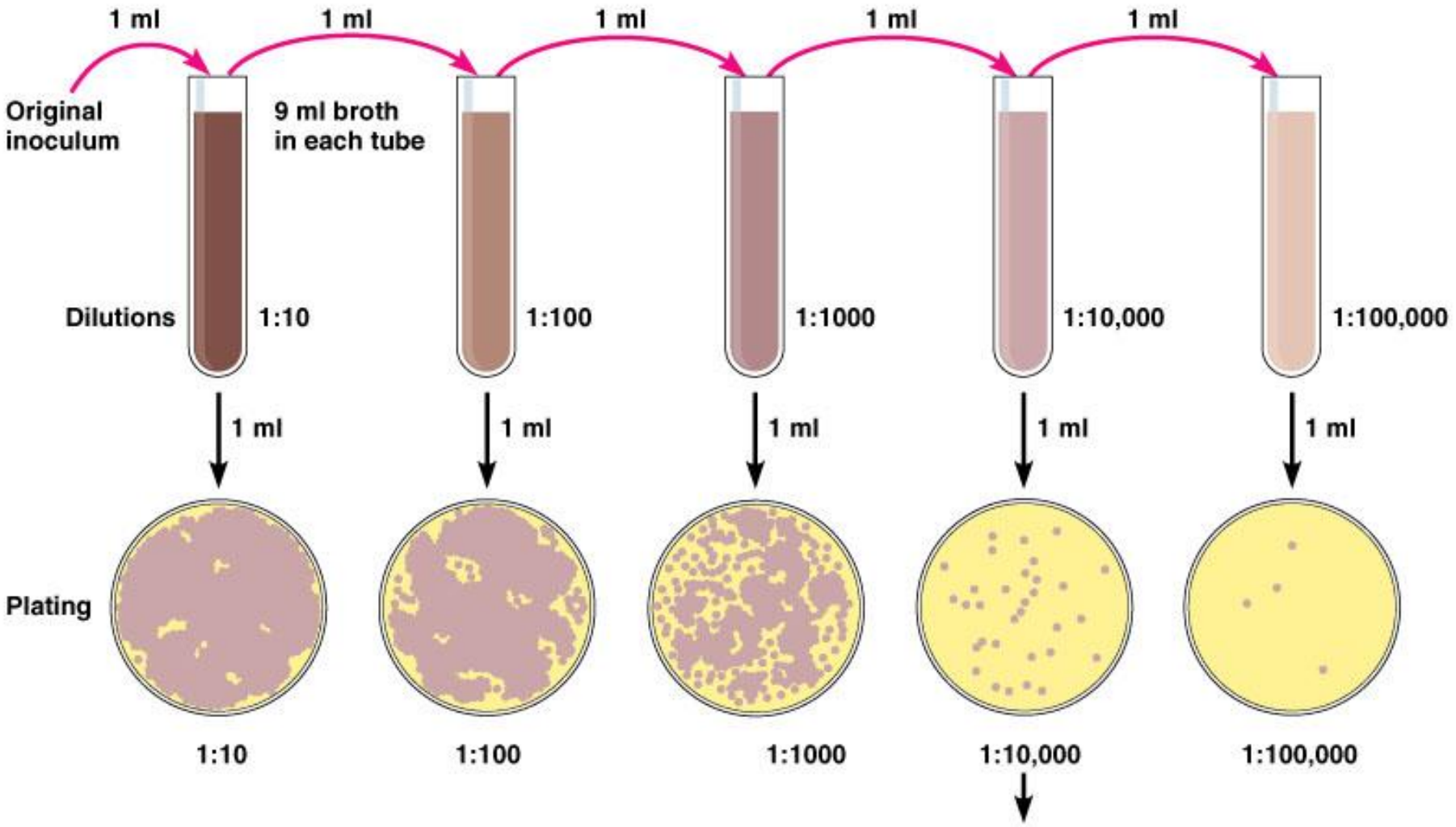


Methods of the cultivation

- ***Stab culture.*** It is prepared by puncturing with charged long straight wire (loop). Stab culture is employed mainly for cultivation of anaerobes.



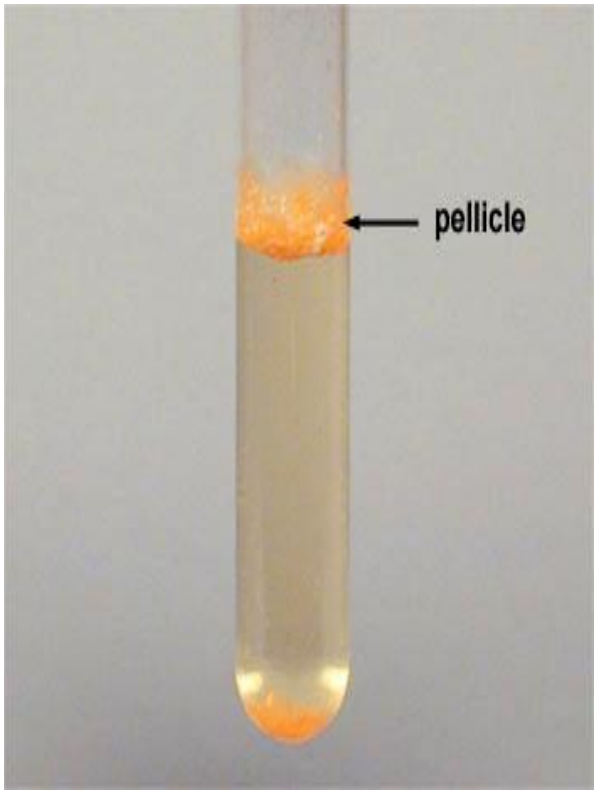
Pure plate culture

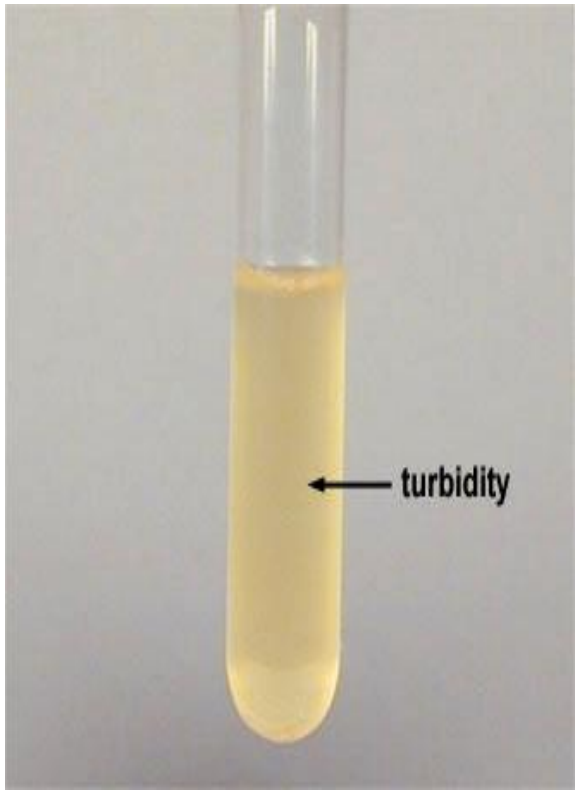


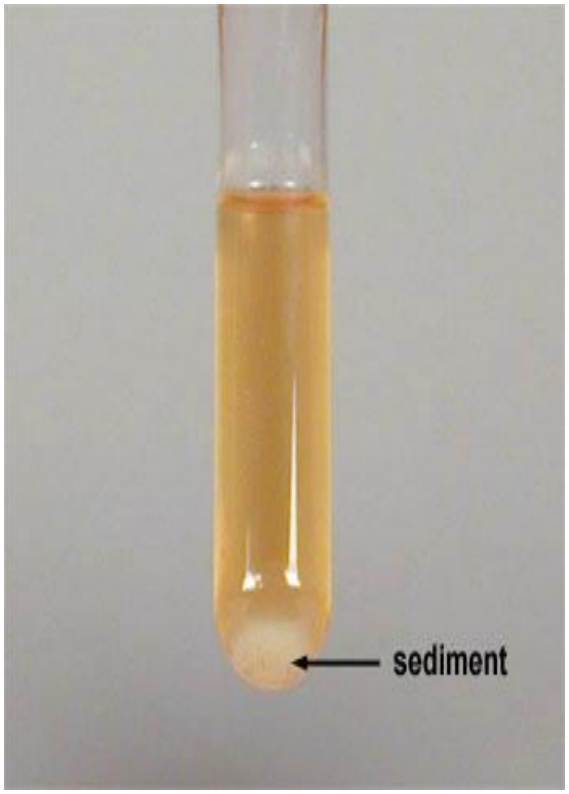
Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)

Methods of the cultivation

- ***Liquid culture*** in a tube, bottle or flask may be inoculated by touching with a charged loop

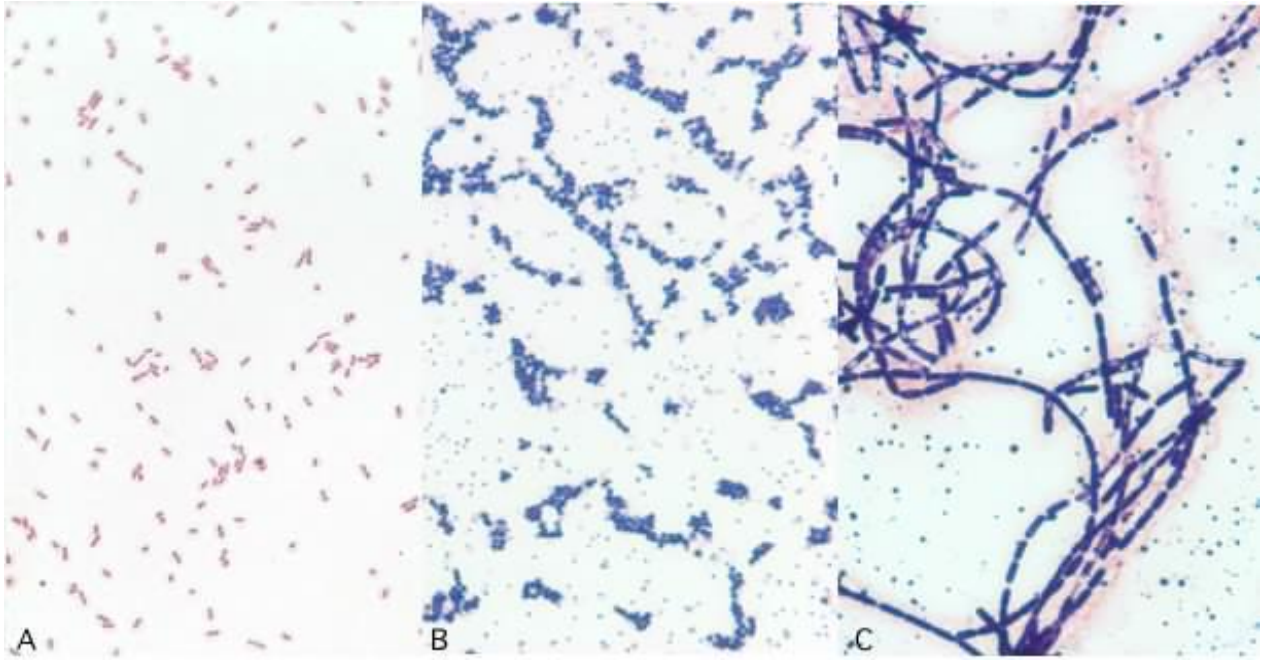






Identification of bacteria

- ***Microscopic examination:*** It helps to detect a shape, a size and an arrangement of microorganisms
- ***Staining reaction:*** On gram staining we can have two groups of microorganisms: Gram positive and Gram negative.

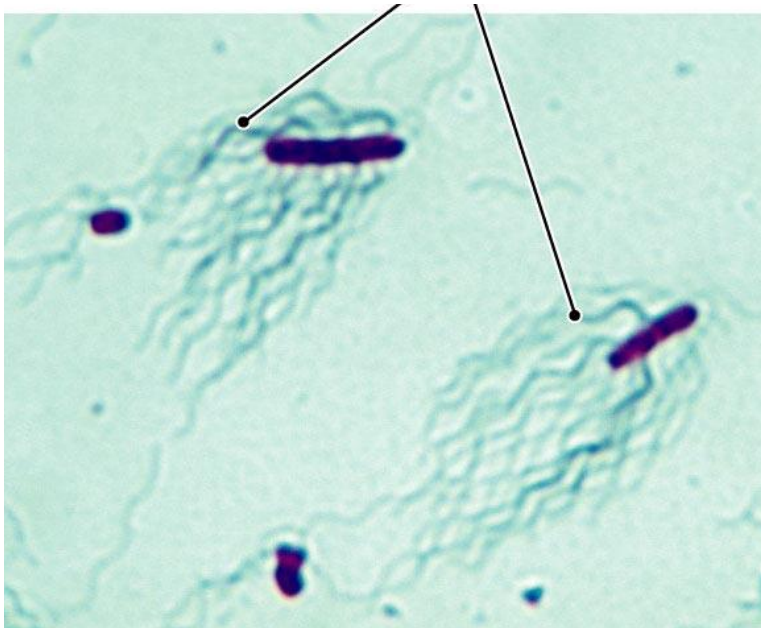


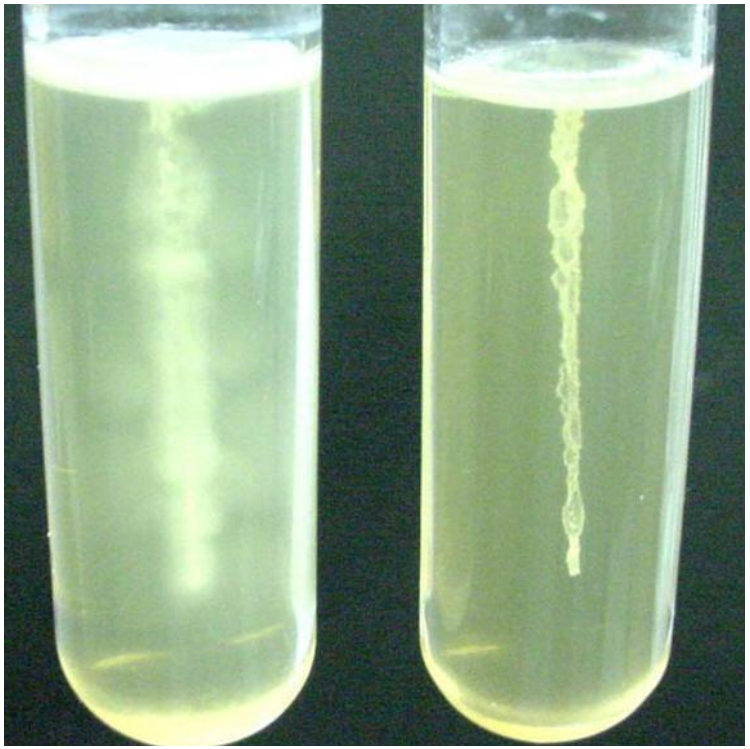
E. coli, Gram negative (A), Staphylococcus epidermidis, Gram positive (B) and Bacillus cereus, Gram positive

Identification of bacteria

- ***Motility:*** Some bacteria can move (Salmonella, E. coli, Proteus, Pseudomonas, Vibrions, Clostridia). **Dark ground microscopy and Phase contrast microscopy, special culture media use for studying motility of bacteria**

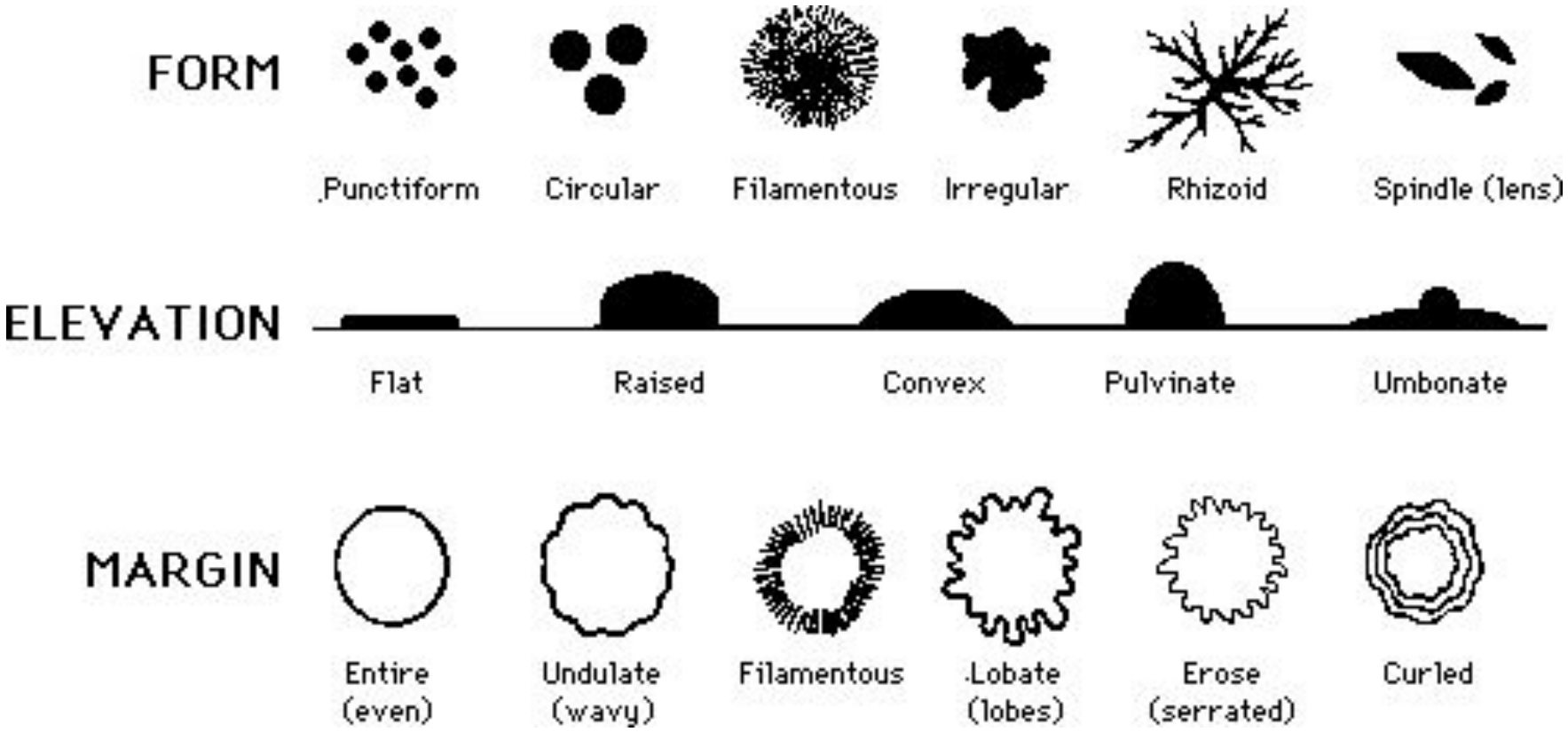
<u>Special stain for flagella</u>





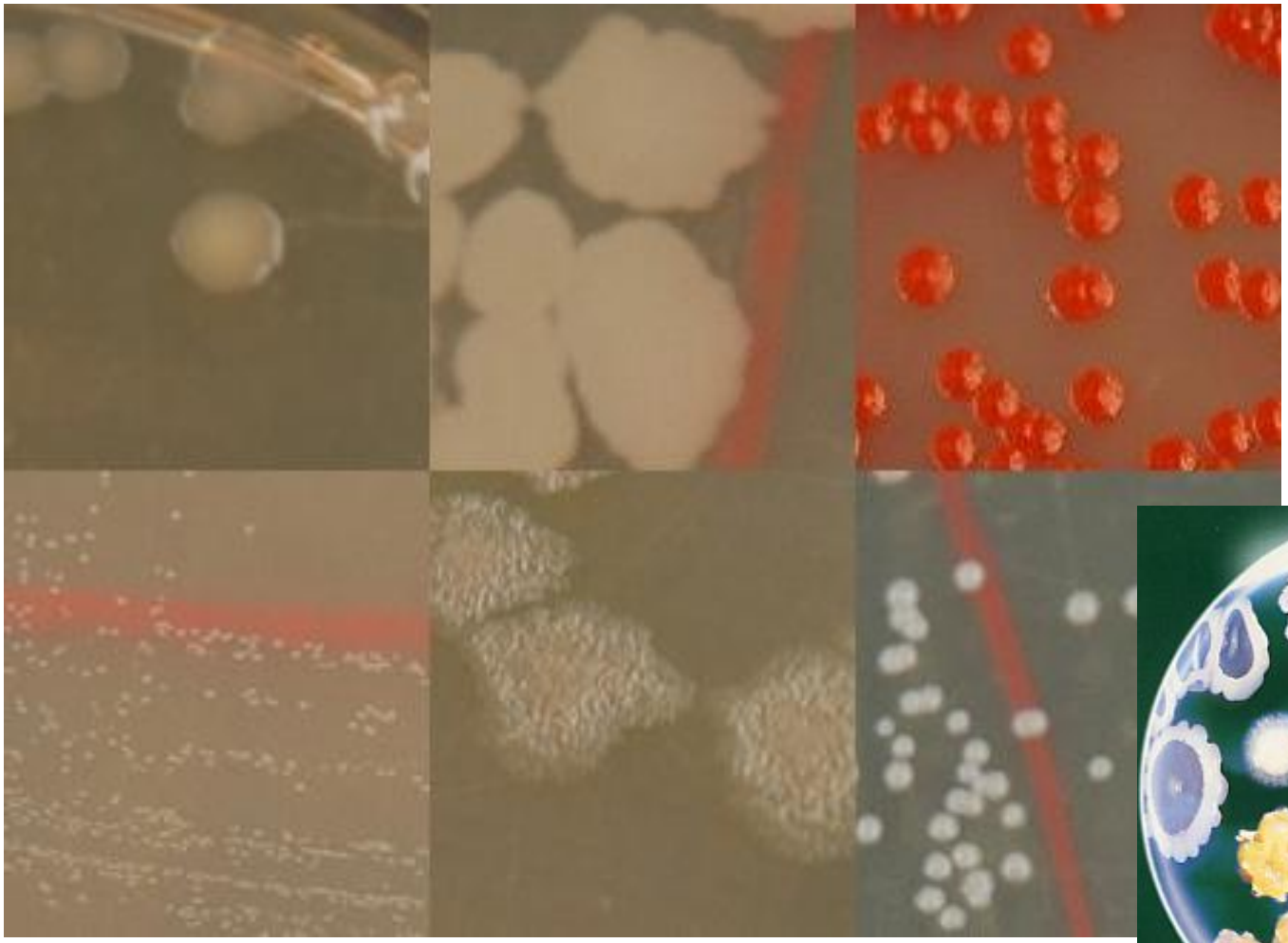
Identification of bacteria

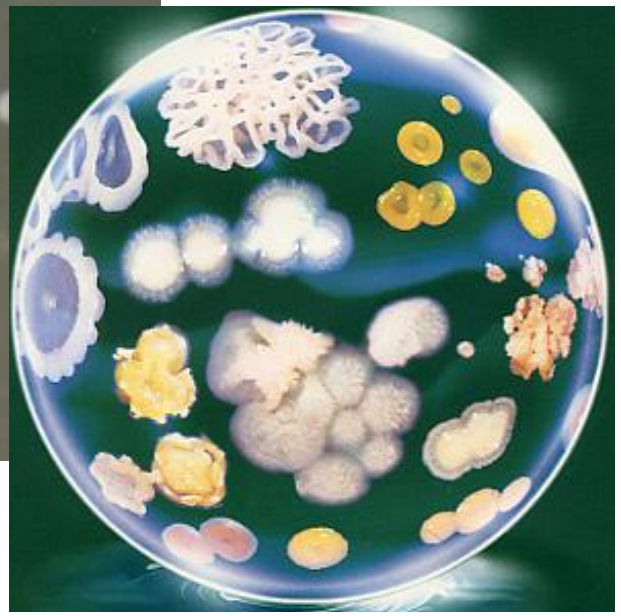
- ***Culture character:*** Growth requirement, colonial characteristics in culture



Colony morphology descriptions

Colony morphology

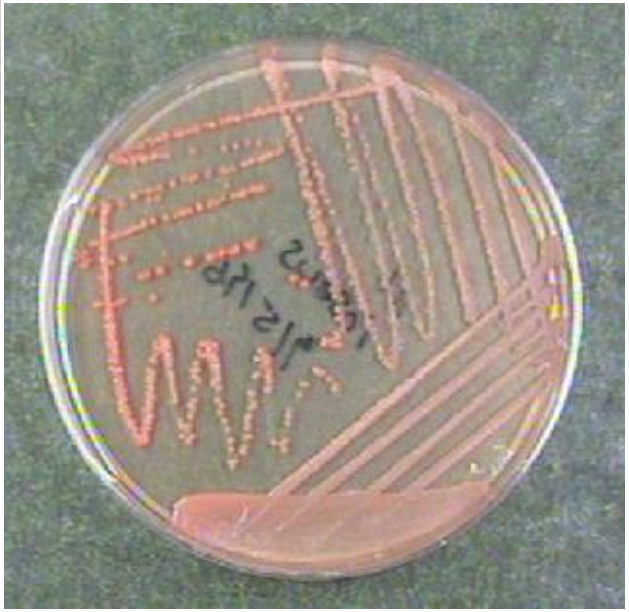




Identification of bacteria

- ***Metabolism:*** Capacity to form pigment and power of haemolysis is help for classification of bacteria

Staphylococcus aureus



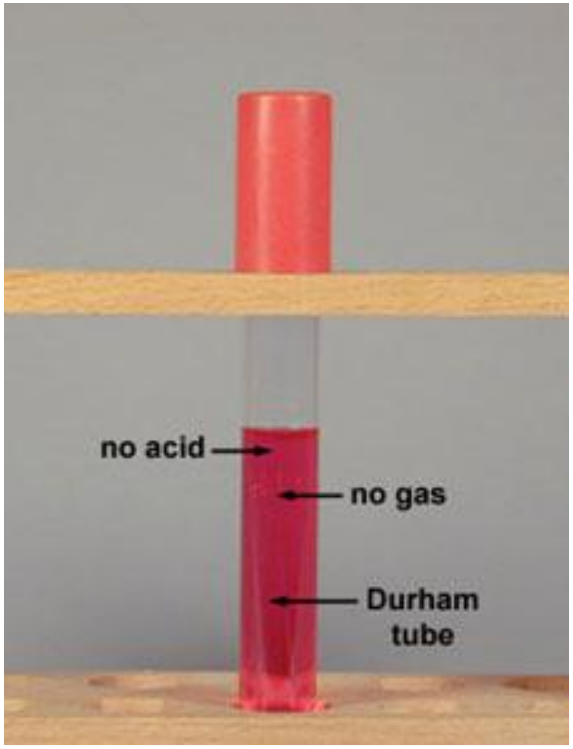
Micrococcus roseus

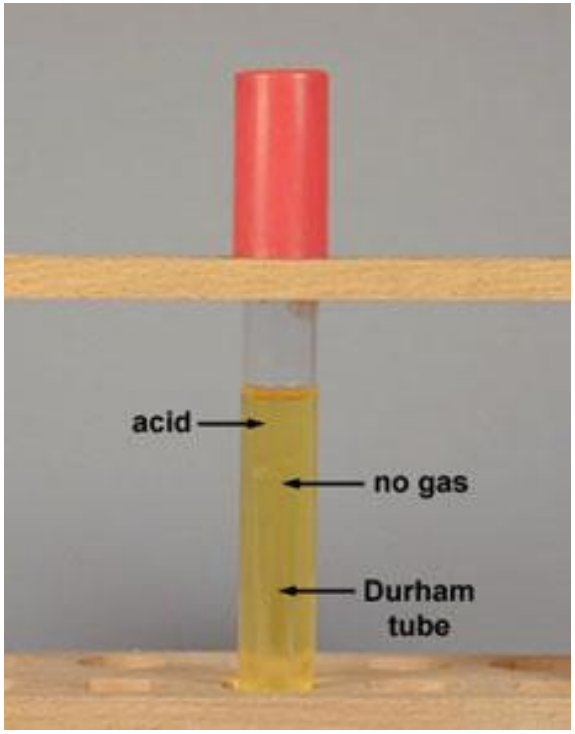
Studying of haemolysis

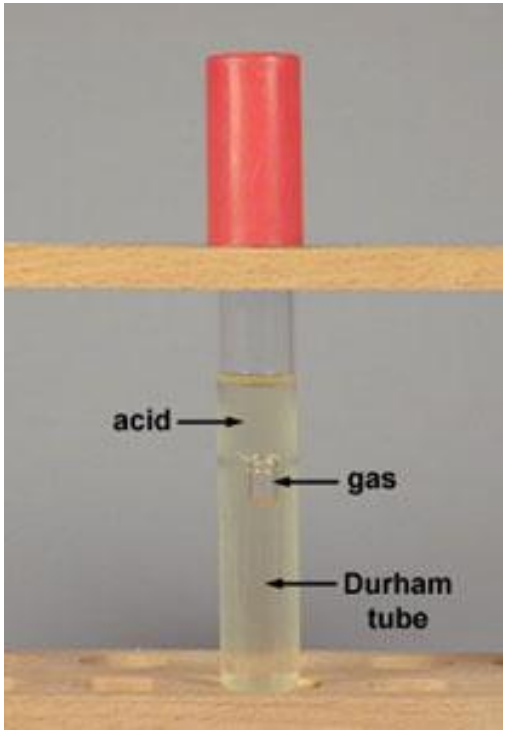
Colonies and pigments of bacteria

Identification of bacteria

- ***Biochemical reactions:*** The more important and widely used tests are as under:
- ***a) Sugar fermentation***

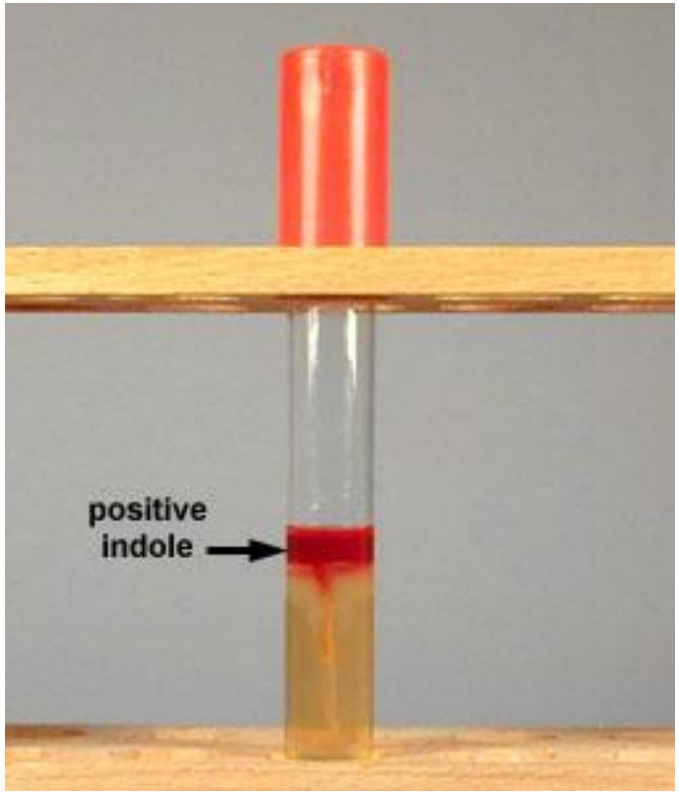


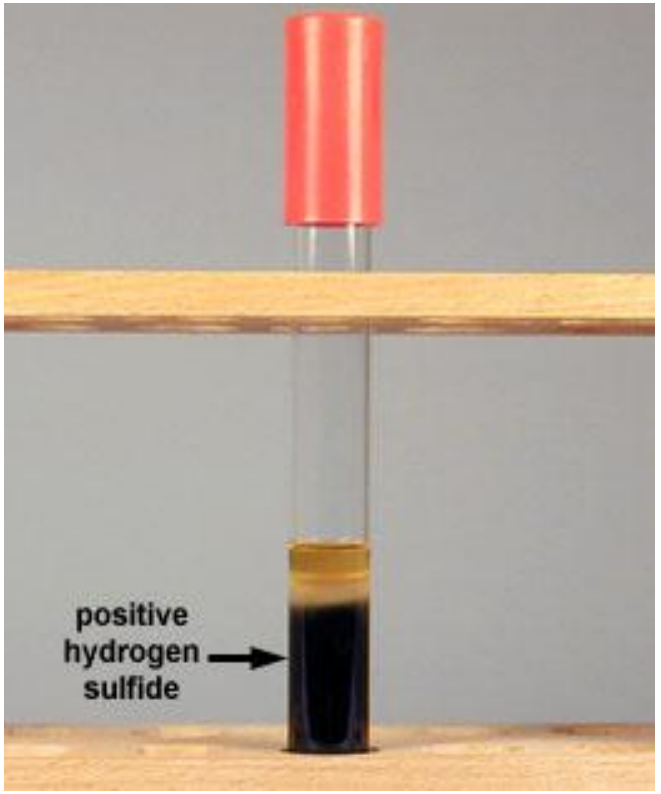




Identification of bacteria

- ***b) Indole production***
- ***c) Hydrogen sulfide production***





Identification of bacteria

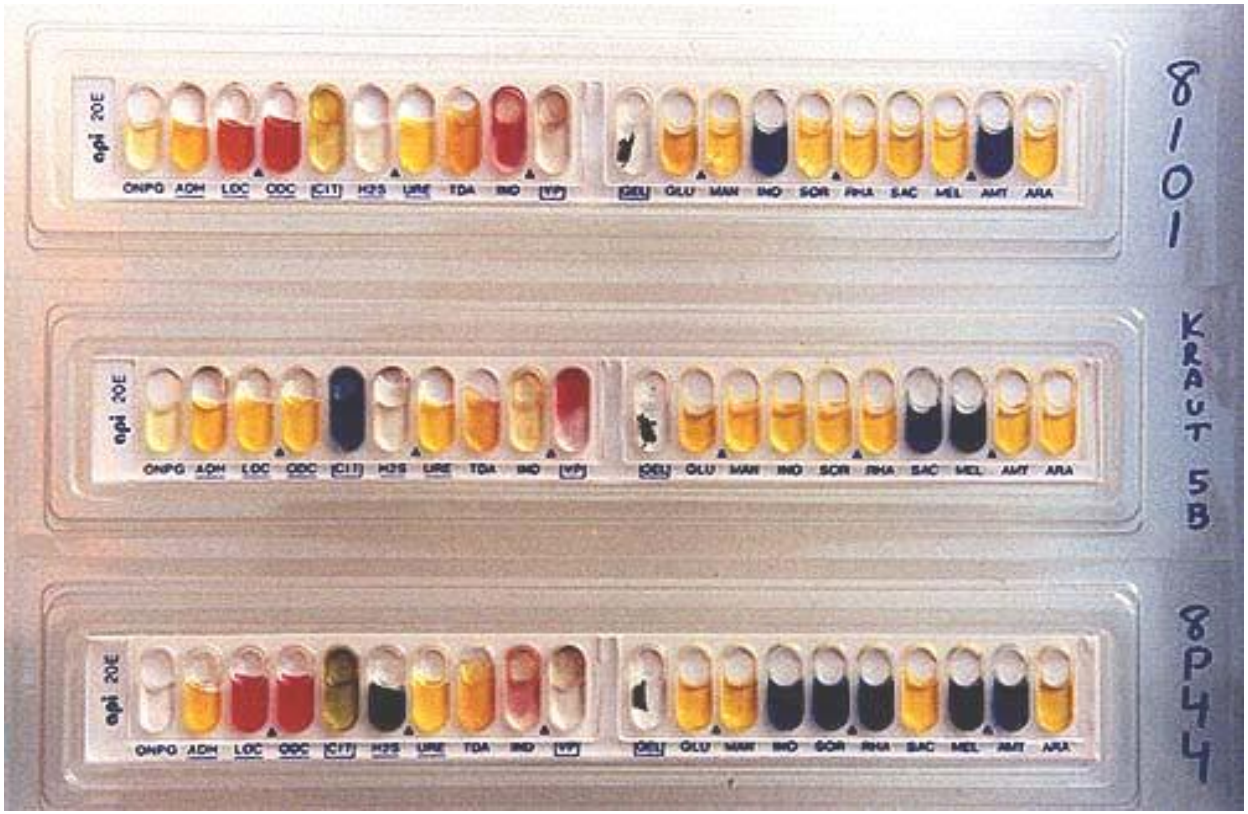
- ***d) Other tests:*** Citrate utilization; Nitrate reduction; Methyl red test; Urease test; Catalase test; Oxidase reactions.

Positive Catalase Test on *Staphylococcus aureus*

Negative Catalase Test on *Streptococcus lactis*

API-20 "Bio Merieux" (France) strip test

- ***Twenty tests are performed on this strip by a simple procedure, saving time and money.***



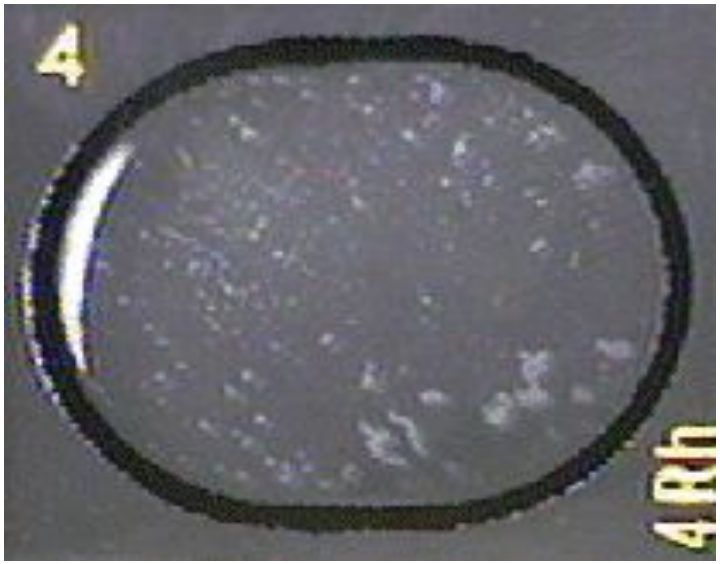
Escherichia coli

Enterobacter agglomerans

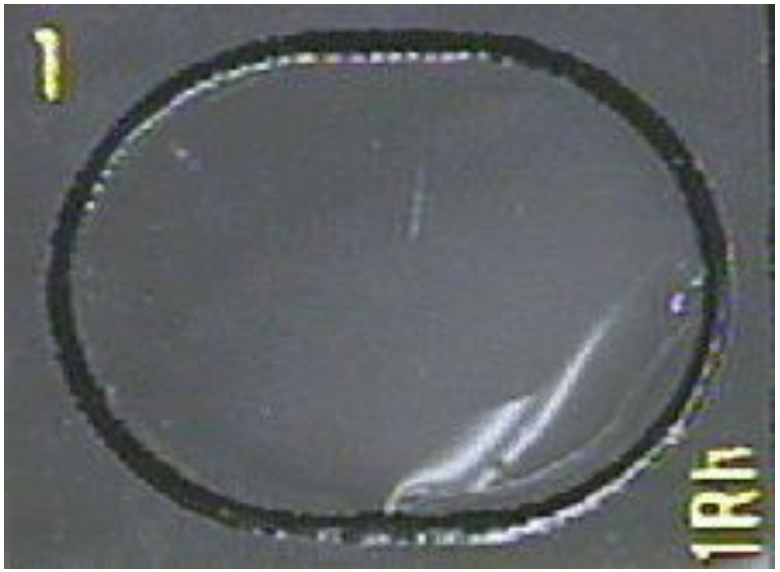
Edwardsiella hoshinae

Identification of bacteria

- ***Antigenic analysis:*** by using specific sera we can identify microorganism by agglutination reaction (Serologic Typing of *Shigella*).



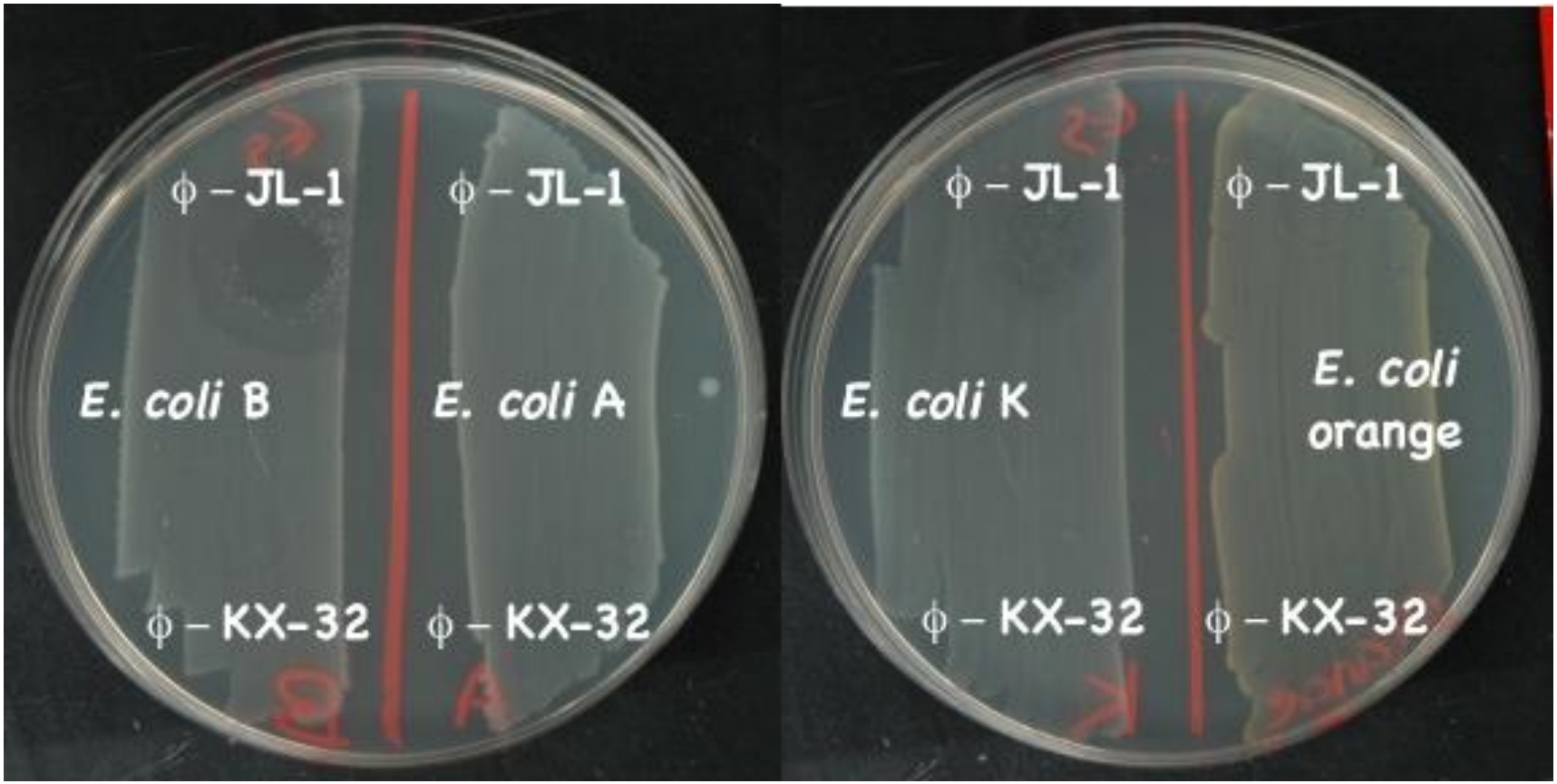
The clumping of the bacteria is seen in this circle



No clumping of the bacteria is seen in this circle

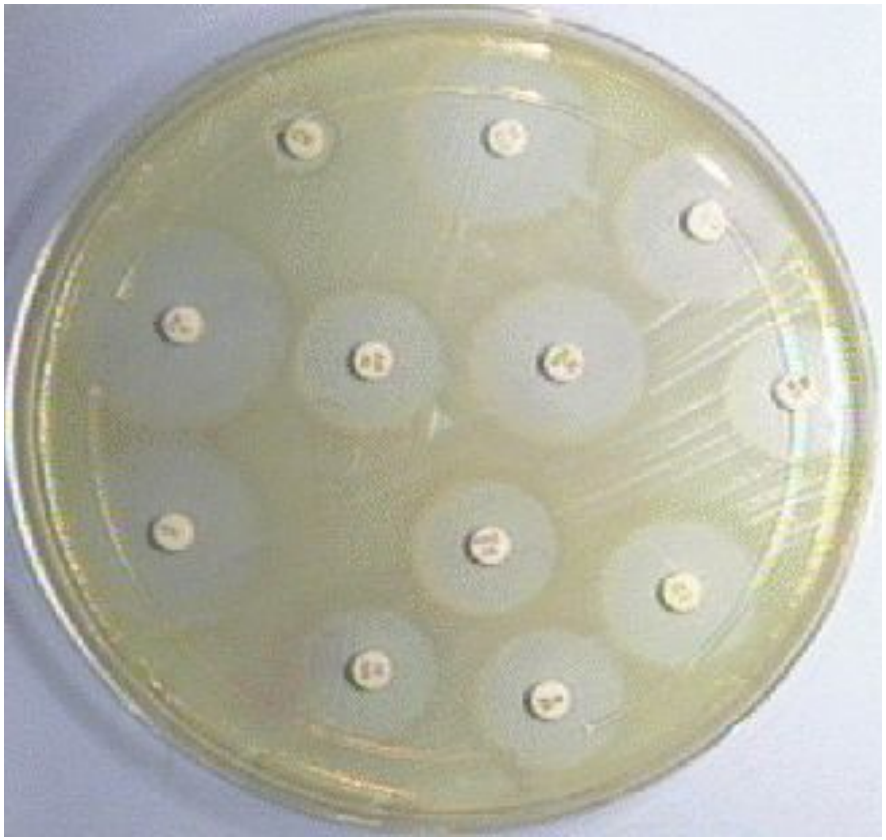
Identification of bacteria

- ***Bacteriophage typing:*** Phage brings about lysis of susceptible bacterial cells.



Identification of bacteria

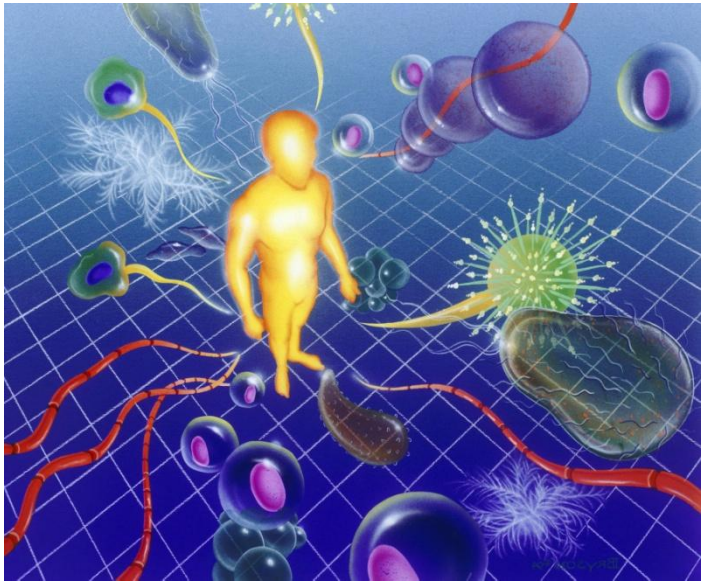
- ***Pathogenicity:*** For pathogenicity test commonly used laboratory animal models are guinea pig, rabbit, rat and mouse.
- ***Resistance to antibiotics and other agents***



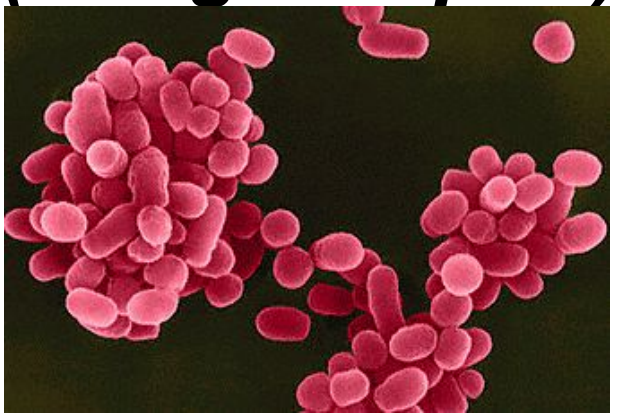
- **Metabolism** is the process of building up chemical compounds in the cell and their breaking down during activity to receive the required energy and the building elements.
- **Metabolism** comprises of ***anabolism*** (assimilation) and ***catabolism*** (dissimilation)

Chemically, bacteria consist of:

- ***Water (75-85%) – bound water and free water***



- ***Dry matter (15-25%) – organic part and mineral substances (inorganic part)***



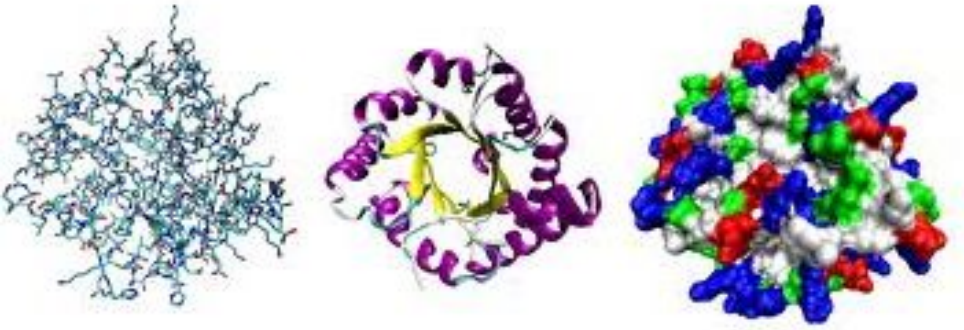
Dry matter

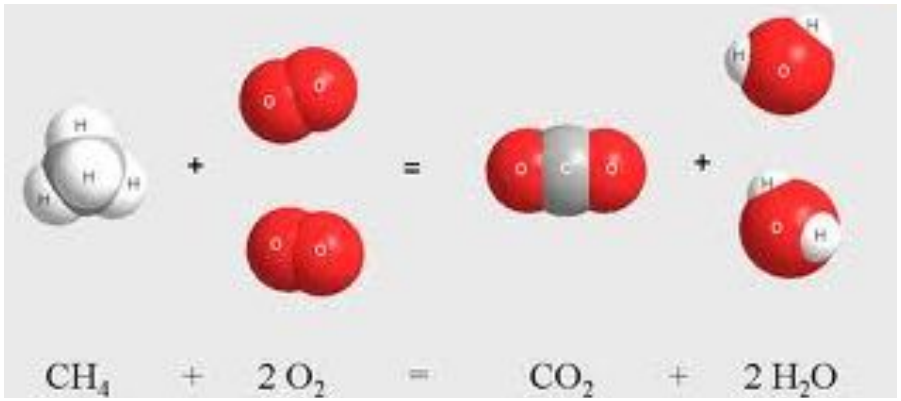
- ***Organic part***

- *proteins – 50-80%*
- *nucleic acid – 10-30%*
- *carbohydrates – 12-18%*
- *polysaccharides – 3-5%*
- *lipids – 5-10%.*

- ***Inorganic part***

- *nitrogen (N), carbon (C), oxygen (O), hydrogen (H), phosphorus (P), sulfur (S), sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), iron (Fe) and other*



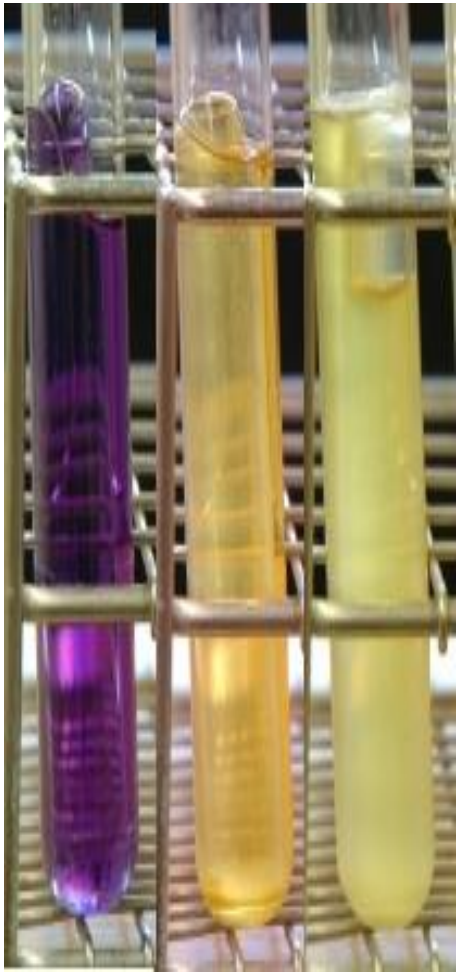


Classification of bacteria based on nutritional requirements

- ***Autotrophs*** are free-living, most of which can use carbon dioxide as their carbon source. The energy can be obtained from:
- sunlight – **protoautotrophs** (get energy from photochemical reactions)
- inorganic compounds, by oxidation – **chemoautotrophs** (get energy from chemical reactions)
- ***Heterotrophs*** are generally parasitic bacteria, requiring more complex organic compounds than carbon dioxide, e.g. sugars, as their source of carbon and energy.

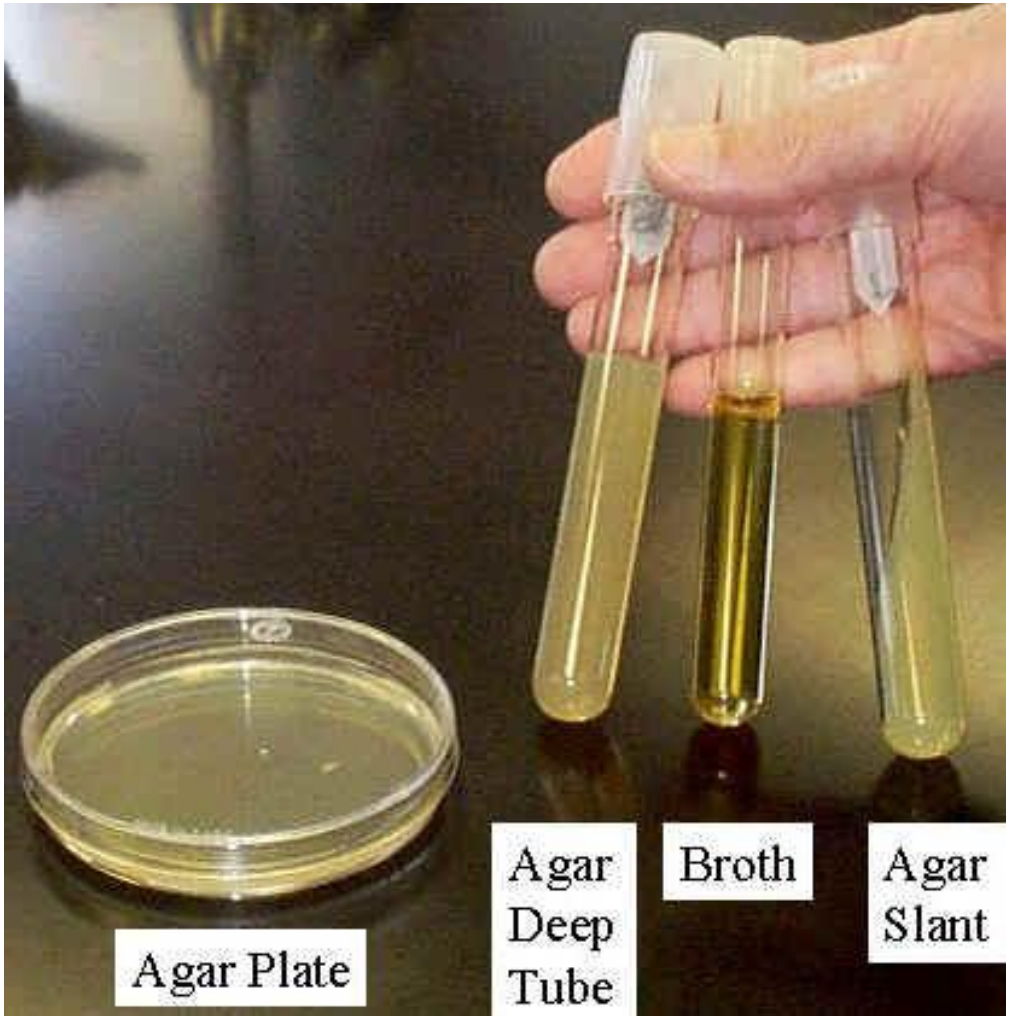
The basic requirements of culture media

- energy source;
- carbon source;
- nitrogen source;
- salts like sulphates, phosphates, chlorides and carbonates of sodium, potassium, magnesium, ferric, calcium and trace elements, like copper, etc.;
- satisfactory pH 7.2-7.6;
- growth factor like vitamins.



Classification of Media

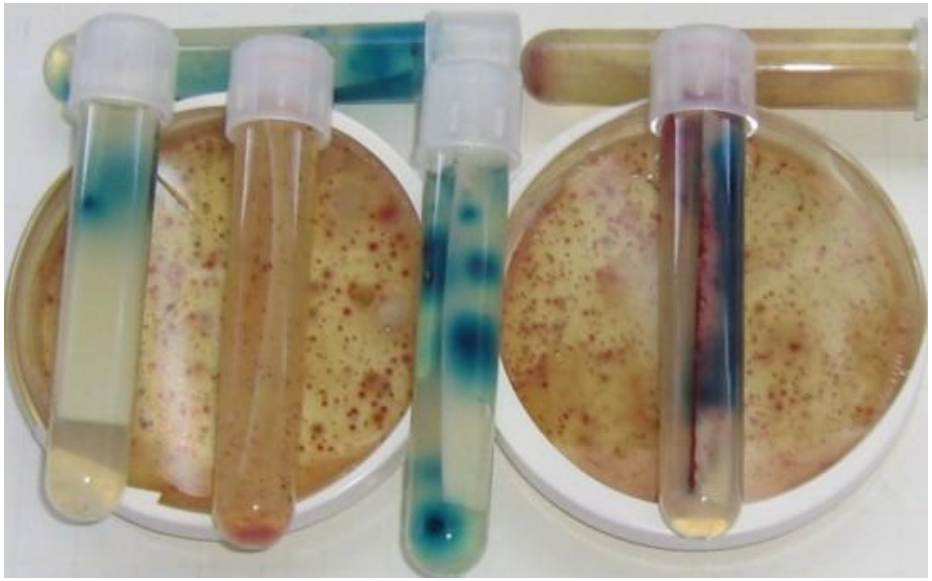
- **A. *On the basic of consistency*:**
- Solid media
- Liquid media
- Semisolid media

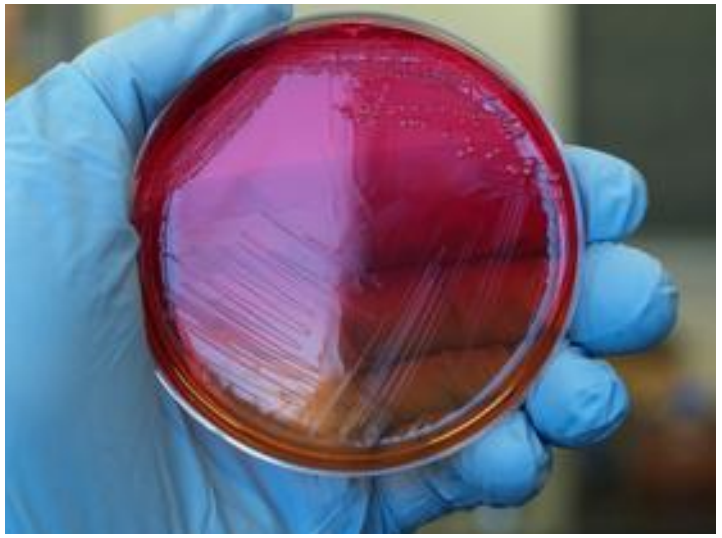


Classification of Media

- **Nutrient media can be subdivided:**
- 1. Simple media - meat-peptone broth (MPB), meat-peptone agar (MPA)
- 2. Synthetic media
- 3. Complex media
- 4. Special media: a) Enriched media; b) Enrichment media; c) Selective media; d) Indicator and differential media; e) Sugar media; f) Transport media.

- 5. <u>Aerobic and anaerobic media</u> **-** according to type of respiration bacteria subdivided into 4 groups:
- ***Obligate aerobes*** ***(Brucella)***
- ***Microaerophils*** ***(H.pylori)***
- ***Obligate Anaerobes*** ***(C.tetani)***
- ***Facultative Anaerobes*** ***(E.coli)***





Sterilization

TREAT-MENT	TEMPE-RATURE	EFFECTIVENESS
Incineration	>500 C	Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process.
Boiling	100 C	Thirty minutes of boiling kills vegetative forms of bacteria but may not kill bacterial endospores. There are also toxins that are not inactivated at 100C.
Intermittent boiling	100 C	Three 30-minute intervals of boiling, followed by periods of cooling kills bacterial endospores.
Autoclave (steam under pressure)	121 C for 15 minutes at 15 p.s.i.	Kills all forms of life including bacterial endospores. The substance being sterilized must be maintained at the effective temperature for the entire time.

Sterilization

Dry heat (hot air oven)	160 C for 2 hours	Used for materials that must remain dry. Good for glassware, metal, but not most plastic or rubber items.
Dry heat (hot air oven)	170 C for 1 hour	Same as above. Note that increasing the temperature by 10 C shortens the sterilizing time by 50 %.
Pasteurization (batch method)	63-66 C for 30 minutes	Kills most vegetative bacterial cells, including pathogens such as streptococci, staphylococci and *Mycobacterium tuberculosis.*
Pasteurization (flash method)	72 C for 15 seconds	Effect on bacterial cells is similar to batch method. For milk, this method has fewer undesirable effects on quality or taste.

AUTOCLAVES (1) AND HOT AIR OVEN (2)

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