MEDIA

GROWTH PERIODS of MICROORGANISMS

MOVEMENT EXAMINATION

Microorganisms ;

- synthesize the necessary constituents by using environmental conditions

  and

- maintain their lives by performing their chemical and physical processes under the influence of extracellular chemical and physical factors

  to sustain their lives.

- They provide the continuation of their strain through reproduction.

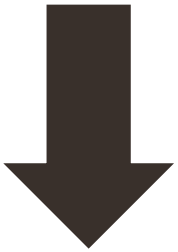
Reproduction = Increase in cell count

Why Microbial Production?

- In clinical microbiology laboratories, the isolation and identification of pathogenic microorganisms for the diagnosis of infectious diseases is very important.

- Microorganisms must be able to be produced under suitable conditions for conducting researchs in medical microbiology, pharmaceutical microbiology, food technology, industrial microbiology, environmental microbiology and many other fields.

Because of differentiation of enzyme systems



the physical and chemical conditions that microorganisms need for nutrition and reproduction differ.

Microorganisms can be classified in various groups according to the resources they need:

- In terms of energy source;
  - Phototroph (using light energy)
  - Chemotroph (using chemical substance)

- In terms of carbon source;
  - Autotroph (organic matter synthesis from inorganic substances)
  - Heterotroph (using ready organic material)
- In terms of hydrogen source;
  - Lithotroph (using inorganic substances) and
  - Organotroph (using organic substances)

The factors effective in the production of microorganisms

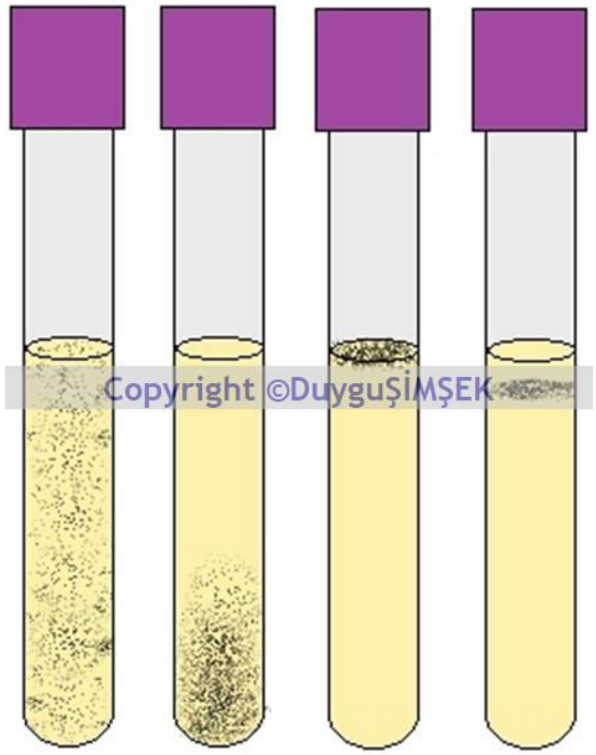
- Heat
  - Psychrophilic bacteria → -8 / +15
  - Mesophilic bacteria → +20 / +45
  - Thermophilic bacteria → +50 / +70
- Osmotic pressure
  - Optimal osmotic pressure
  - High osmotic pressure
  - Low osmotic pressure

The factors effective in the production of microorganisms

- Carbon source
- Hydrogen donors and receivers
- Nitrogen source
- Oxidation / reduction potential
- pH
  - Most microorganisms are in the range of pH 6-8 (neutral pH)
  - Some microorganisms such as yeasts and molds are in acidic conditions
  - Some microorganisms such as *V. chlorea* are in alkaline conditions…

The factors effective in the production of microorganisms

- Oxygen
  - Aerobes →
  - Anaerobes →
  - Facultative anaerobes →
  - Microaerophiles →



- CO_2

The factors effective in the production of microorganisms

- Minerals
- Growth factors and vitamins
- Water
  - **holophytic nutrition**

Growth environments of microorganisms

- Microorganisms can be produced *in-vitro* or *in-vivo* under suitable environmental conditions in laboratory.
- Microorganisms such as ***viruses, ricketsia, chlamydia*** that can survive only in living cells.
- *Most of the* ***bacteria*** *and* ***fungi*** can be growth in non-living environments.

Growth environments of microorganisms

Living environments

- Experimental animals
- Embryonated eggs
- Cell cultures
- Tissue cultures

Non-living environments

- Media

Media

- A nutrient blend that microorganism can be produced *in vitro* and contains materials necessary for the production are called as **medium**.

There is no common optimum medium formula for all microorganisms.

Classification of media

According to physical characteristics

- Liquid
- Semi-solid
- Solid

According to usage purposes

- Simple (basal)
- Complex
  - Spesific
  - Differential
  - Selective
  - Both selective and differential
  - Enrichment
  - Preservation
  - Transfer

According to chemical structures

- Natural
- Semi-synthetic
- Synthetic

Agar agar;

- It is derived from some red seaweed species.
- It is thickener.
  - The ratio of agarose / agaropectin in it determines the consistency.
- It is not a food source for microorganism.
- Dissolves above 90 °C, solidifies below 45 °C.
- It does not affect the pH, but at low pH it hardens the solidification of the medium.
- It has a water holding capacity of 300-500 times.

Media by physical characteristics

Liquid

Agar free

Nutrient Broth
Mueller Hinton Broth

Semi Solid

Agar ratio is between 0.3 - 0.5 %

Stuart transport media
Kirschner semi-solid media

Solid

Agar ratio is between 1.5 – 3 %

Nutrient Agar
Mueller Hinton Agar

Media by physical characteristics

- There are also biphasic media that carry both solid and liquid phases in the same medium.
  - Castenada medium

Media by chemical characteristics

- **Natural media**
  - Contains natural substances such as bouillon, peptone, milk, egg, blood, serum, potatoe.
  - Their chemical content is undefined.
- **Synthetic media**
  - Medium containing pure chemical substances.
  - It is a well-known medium containing defined pure chemical substances at certain ratios.
- **Semi-synthetic media**
  - It contains both chemical substance and various organic substances.
  - The content is not exactly known.

Media by usage purposes

- **Simple (basal) media**
  - Routinely used in laboratories
  - Contain sufficient amounts of nutrients for the development of many microorganisms
  - Do not contain inhibitory substances

Media by usage purposes

- **Simple (basal) media**
  - Basic media containing essential nutrients such as peptone water, bouillon
    - peptone water → peptone + salt + water
    - Bouillon → peptone + salt + bouillon
    - gelose → bouillon + agar
  - Usage areas; first isolation, counting, production.

Media by usage purposes

- **Simple (basal) media**
  - Growth inducing **enriched media** can be obtained by adding more nutrients such as blood, serum, egg to the basic medium.
    - blood agar, chocolate agar, tomato juice agar

Media by usage purposes

- **Complex media**
  - It is used for purposes such as production and identification of microorganisms, obtaining pure culture, examination of physico-chemical activities.
  - Indicators → may contain some special substances that induce or inhibit the germination.

Media by usage purposes

- **Complex media**

a) Spesific media: Used for the production of only one species. Spesific to species.

- Löwenstein Jensen media → *Mycobacterium tuberculosis*

Media by usage purposes

- **Complex media**

b) Selective media: The medium prepared with the substances that allow the growth of the desired microorganism while inhibiting the growth of unwanted groups of microorganisms from a mixed culture.

- This feature is provided by dyes, antibiotics, etc.
- SS Agar → *Salmonella* and *Shigella spp*.
- Selenite F → *Salmonella spp*

Media by usage purposes

- **Complex media**

c) Differential media: It is the medium which shows its metabolism end products and whether the microorganism uses a specific nutrient.

- It contains various indicator.
- Endo Agar → Lactose (+) and Lactose (-)
  Enterobacteriaceae differentiation

Media by usage purposes

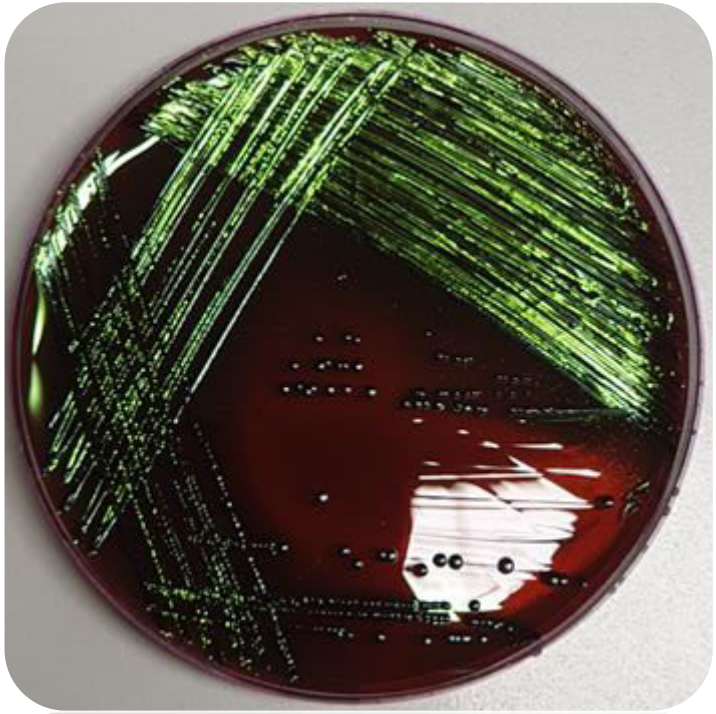
- **Complex media**

  d) Both selective and differential nutrients: Selectivity by allowing the growth of a group of microorganisms and inhibiting the growth of the other group; as well as by differentiating the biochemical characterization of the microorganisms.

Media by usage purposes

- **Complex media**

  d) Both selective and differential nutrients: Eosin Methylen Blue Agar → Allows the generation of Gram (-) bacteria. Different *E. coli* and Klebsiella spp.



Media by usage purposes

- **Complex media**

  e) Reagent media: It is the medium which enables the formation of various reactions according to the biochemical character of the microorganism.

  - Results interpreted by the colour change

    - Simmons Citrate Agar media →the use of citrate as a carbon source
    - Clark-Lubs media → the presence of (+) or (-) glucose fermentation

Media by usage purposes

- **Complex media**

  f) Enrichment media: If the number of desired microorganisms in the mixed culture is low, it is the medium which multiplies it by the addition of various substances and causes the others to reproduce in a lesser amount.

  Selenite F media → *Salmonella spp*.

  Alkaline-peptonic water → *Vibrio chlorea*

Media by usage purposes

- **Complex media**

  g) Storage medium: It is the medium used to keep the isolated microorganisms in the refrigerator at -20 ºC or -80 ºC for a long time. For example;

  - Certain proportions of glycerol or fat-free milk.

Media by usage purposes

- **Complex media**

  h) Transfer media: The medium from which the samples are carried until they are transferred to the laboratory and cultured.

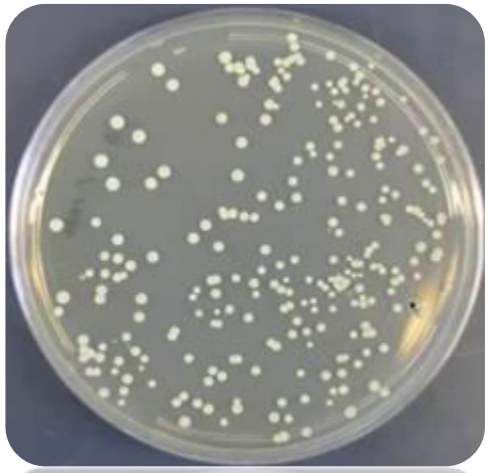
Apperance of culture;

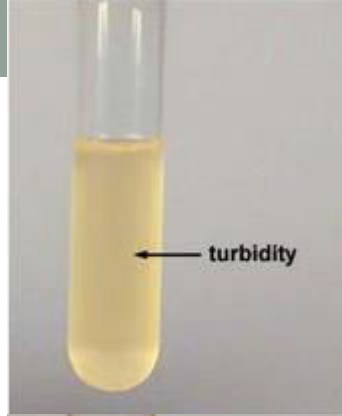
- Cultural appearance may be
  - homogeneous turbidity,
  - granular reproduction,
  - bottom depression
  - membrane form on the surface

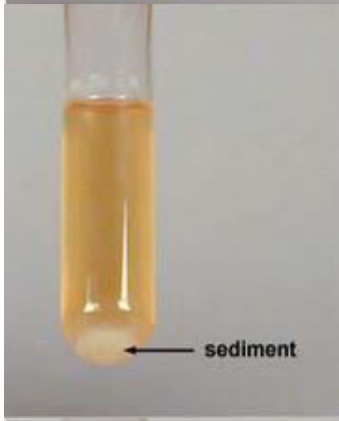
  for the liquid medium and

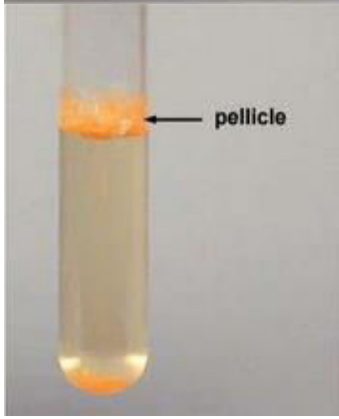
  - colony shape

  for solid medium.









Colony types

- The size, shape, colour, flavour, structure, appearance of the colony varies depending on the type of bacteria.

Colony types

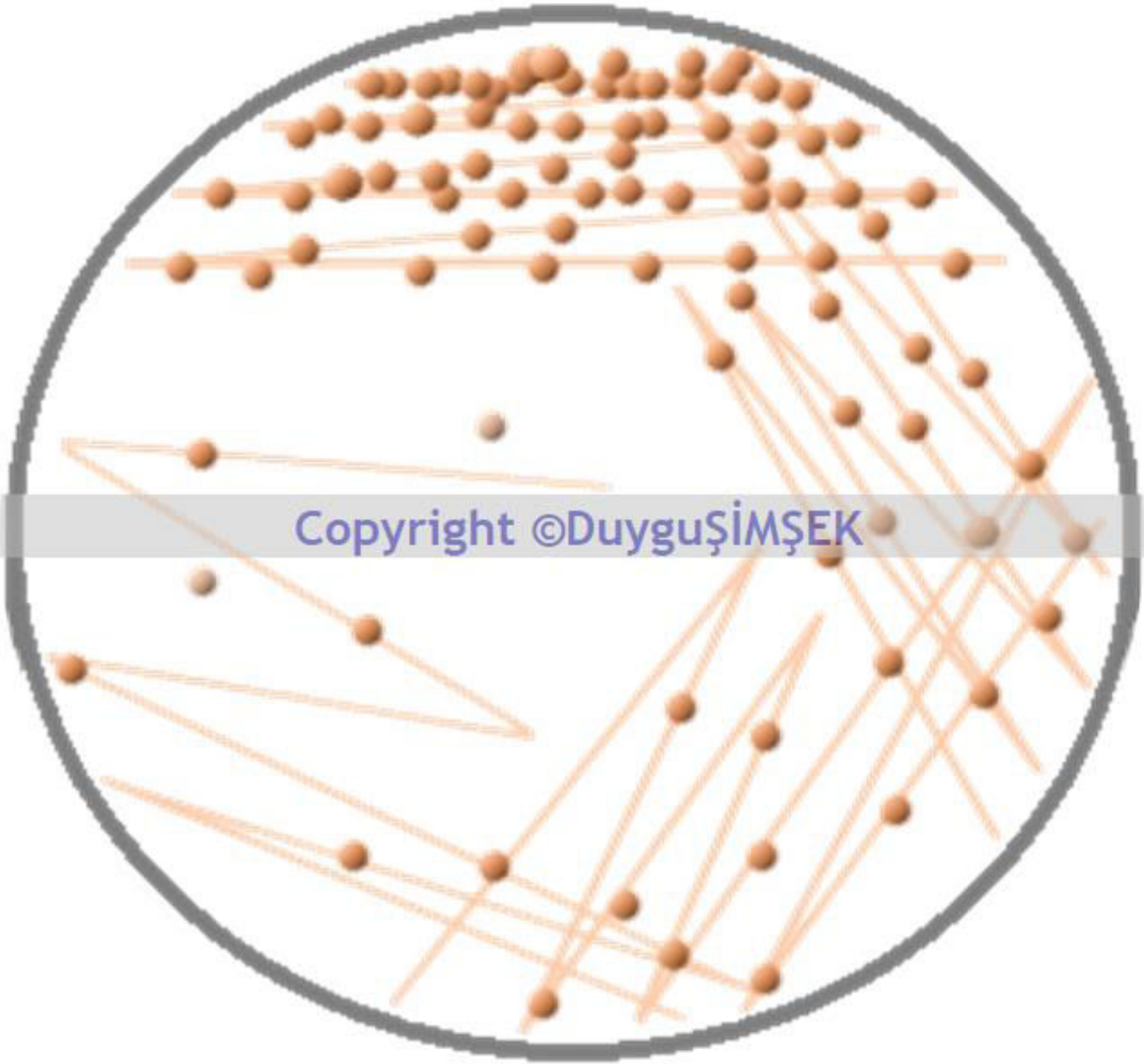
- **S** *(smooth)* type colony:
- **R** *(rough)* type colony:
- **M** *(mucoid)* type colony:
- **L** type colony:

Some terms in microorganism production

- Production of microorganisms → **culturing**
- Produced microorganisms → **culture**
- One type of microorganism culture → **pure culture**
- Sample inoculated to the medium → **inoculum**
- Culturing to medium → **inoculation**

Some terms in microorganism production

- Apart from the microorganisms present in the culture medium or in the medium, other microorganisms called as **contaminant**s, this state is called as **contamination**.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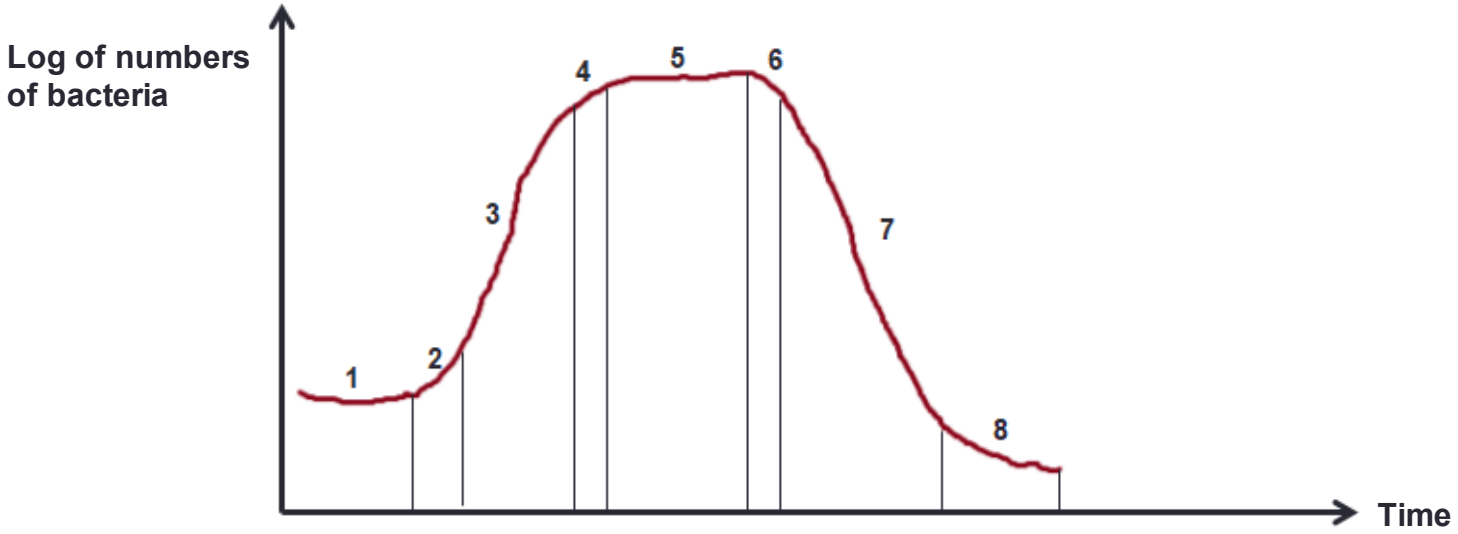
Growth periods of microorganisms

- Bacterial growth rate during **reproduction (*generation time*)** depends on the;
  - *species of bacteria and*
  - *ambient conditions*

Growth periods of microorganisms

- When a certain number of microorganisms are added to a liquid medium and incubated;
  - with sampling at regular intervals, it is observed that there are different periods of the generation depending on the time.



Growth periods of microorganisms

1. Latent period:

- *Adjustment period*
- *No multiplication, even some of them die*
- *Enlarge the volume, synthesize enzymes and intermediate metabolites*
- *The duration depends on the type, number, age of the microorganism*

2. The period when the generation accelerated:

- *Period of the beginning of the generation*
- *Cell size is at its maximum size*

3. Logarithmic reproduction period:

- *Exponential reproduction period as soon as possible*

Growth periods of microorganisms

4. The period of decrease in reproductive speed:

- *Food is reduced, toxic substances start to form*
- *Reduced amount of oxygen in aerobic conditions*
- *Multiplication speed is reduced*

5. The period of proliferation:

- *Proliferation continues*
- *Bacterial death keeps the number of living ones in balance*

6. Reduction period of bacteria:

- *The number of dead cells is greater than the number of cells divided*

Growth periods of microorganisms

7. Logarithmic reduction period:

- *Conditions become very inappropriate*
- *Rapid death starts*
- *Logarithmic reduction in count*

8. Re-arrangement period:

- *Number is miserable*
- *Low number of deaths and generation*
- *Survivors can stay alive for weeks or months, depending on their genus*
- *Sporadic species form spores*

Motions in microorganisms

1. Amoeboid Motion: It's seen in amoeba. The microorganism moves with the help of pseudopods.

2. Slip Motion: Some blue-green algae and algae-like bacteria, make a sliding motion by curling ,with their twisting bodies in moist solid media.

3. Spiral Motion: Microorganisms whose bodies are spirally twisted act as auger with the help of axial filaments on moist media.

4. Colony Motion: The colonies of some microorganisms are displaced by a very slow sliding movement in the moist solid medium.

Motions in microorganisms

5. Flagellar Motion: Microorganisms with flagella are replaced by this movement. It is an **active motion**. For this movement there is a need for energy that is provided from ATP.

(***taxis***)

6. Brownian Motion: Bacteria that do not have movement organelles can cause vibration, rotation, bending, swing, etc. in the environment without changing their place. It is called a **passive motion**, which is caused by the collision of microorganisms with other molecules in the liquid medium.

Motility tests of microorganisms

- Hanging Drop Slide inspection
- Wet Mount Slide inspection
  (Inspection between slide-cover glass)
- Inspection by Soft Agar Stabbing
  (Inspection in Semi-Solid Media)
- Inspection on Solid Medium

Motility test of microorganisms

Topic: Motility test of microorganisms

Method: Wet mount slide

Brownian motion →

Flagellar motion →

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