
بسم الله الرحمن الرحيم

MICROBIOLOGY LAB.

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OBJECT NO. 1:
CONTROL OF BACTERIAL GROWTH BY MOIST HEAT

Purpose :

- Effect of moist heat on spore forming and non spore forming bacteria
- Thermal resistance of bacterial spore

Introduction:

Microorganisms can grow over a wide range of temperature from low to higher temperature every type has an optimum minimum maximum growth temperatures

High temperature combined with high moisture is one of the most effective method of killing microorganisms it is important to differentiate between dry heat and moist heat in any procedure for microbial control

Sterilization:

- A process that completely removes or destroys all living organisms and their products in or on the object called as Sterlization
- For example :
- Heating (different organisms require different time and temperature for their destruction or complete removal)

Thermal death point (TDP):

- The lowest temperature at which all the organisms of the given species killed in 10 minutes is called as thermal death point

Thermal Death Time (TDT):

- The length of time required to kill all microorganisms of a certain type at a particular temperature is known as TDT

Types of Heat :

Moist Heat

- Effectively kills microbial cells by coagulating their proteins
- for example :
- Boiling, Pasteurization ,Autoclaving

Dry Heat

- It kills the microorganisms by the oxidation of cell components
- for example :
- Flaming ,incineration and baking (hot air oven ,mainly used for is sterilizing metallic instrument, powder, and oil)

Requirements :

- Cultures : *Escherichia coli* , *Bacillus subtilis*
- 10 Nutrient broth tubes (Each tube containing 5ML Nutrient Broth
- Water bath

Procedure:

- Set a water bath at 100C
- Transfer 1-2 loops of E.coli to a tube of Nutrient Broth label it (C 1) control +ve and take another tube without adding culture into the tube and label it (C 2) control –ve
- Repeat 2nd step for 2nd culture B. subtilis
- Labelled 2 sets (1 for E.coli and 2 for B.subtilis) of 04 nutrient broth tubes
- labeled 04 tubes with 5mins,15mins , 25mins ,35mins repeat same for other 04 tubes
- Than inoculate one set with one loop of E.coli and other set of B.subtilis
- Place both the sets in the water bath take out the respectively labeled tubes from the water bath after 5,15,25,and 35mins of heat exposure and incubate all sets of tubes at 37C for 24 hours.
- Observe the result by visual determination of turbidity due to bacterial growth

Inoculation of bacteria in to Culture media

- Plate

Broth

Slant

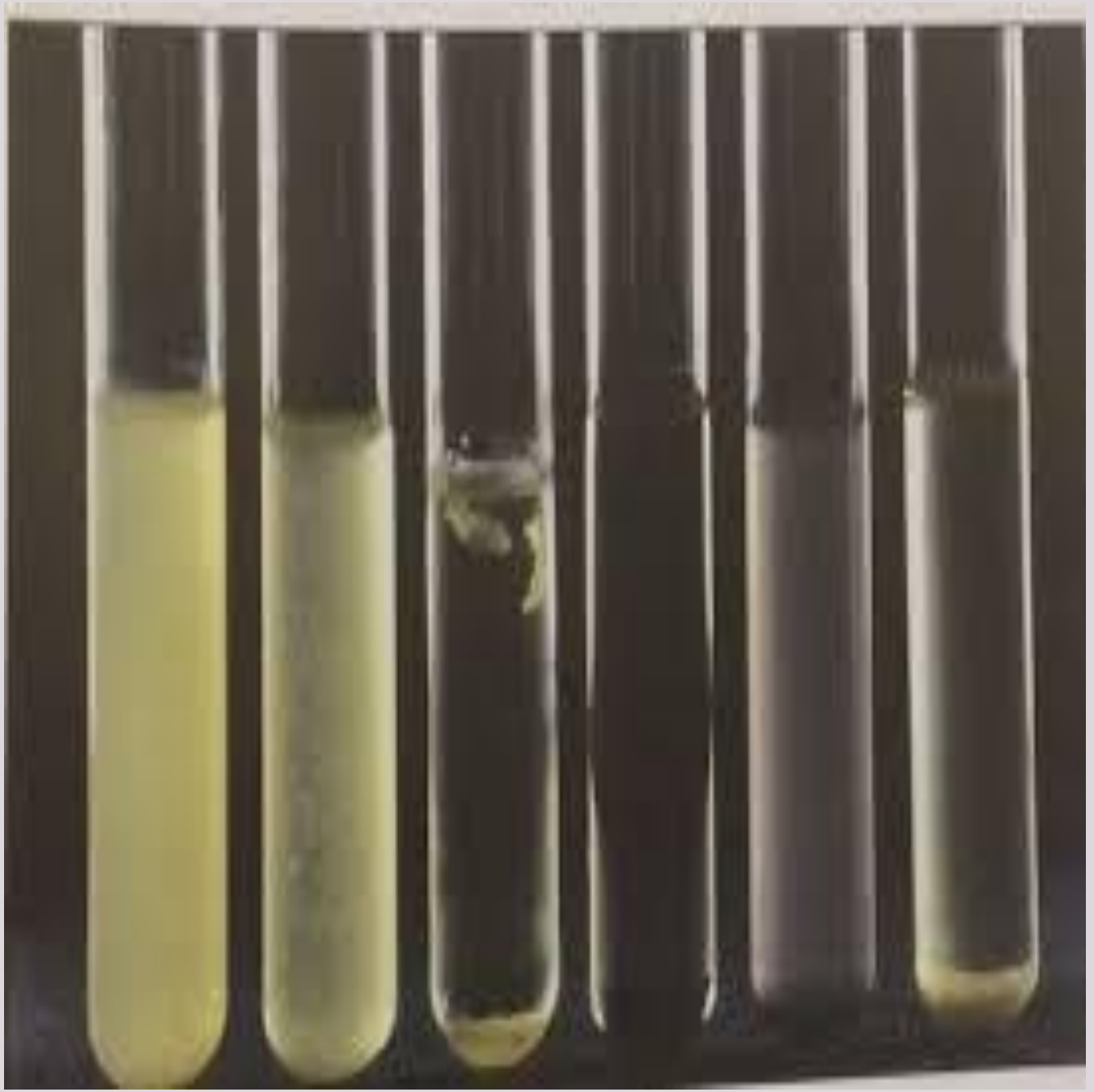
Deep

NAME OF ORGANISM	5 MINS	15 MINS	25 MINS	35 MINS	CONTROL
1. E.coli	+	-	-	-	C 1= + C 2= -
2. B. subtilis	+++	+++	+++	+++	C 1 = + C 2 = -

KEY :

ZERO = NO GROWTH
+ = LOW
++ = MODERATE
+++ = ABUNDANT

C1= +VE CONTRO
C2= -VE CONTROL



OBJECT NO. 2:

EFFECTS OF DISINFECTANT ON BACTERIAL GROWTH

14

Effects of Disinfectants On Bacterial Growth

PURPOSE

Once you have completed this experiment, you should understand the following.

- Determination of bacterial susceptibility, of varying degree, to different disinfectants.
- Comparison of the efficiency of different disinfectants with phenol.
- Modes of action of disinfectants on microbial growth.

GENERAL CONSIDERATIONS

A process which eliminates the vegetative forms of most pathogenic organisms, but does not assure the removal of all microbes and their spores, is known as **disinfection**. A chemical agent that is used to achieve disinfection is called **disinfectant**. Disinfectants are efficiently applied only on inanimate objects. The chemical agents which can be used on the surface of living body in order to destroy pathogens are called **antiseptics**. They are less toxic chemical agents and therefore commonly used in the medical field for cleansing the skin or wound of a patient. The disinfecting agents are widely applied for the preparation of many articles intended for the use in patient care. These include bedpans, urinals, clinical thermometers, and many other utensils. Different types of disinfectants are also used for disinfecting hospital floors, walls, tables, trolleys and work benches. They are also sprayed on sites which are contaminated with blood, pus, exudates or microbial cultures. Disinfection is the only method applicable to the skin of hands, operation sites and injection sites for killing transient contaminants or reducing the microbes to a low level.

It should be emphasized that not all chemicals are disinfectants. There are certain qualities that a chemical agent should have in order to be an ideal disinfectant for general use. These qualities include broad-spectrum activity, rapidity of action, nontoxic for tissues, high penetrating power, high degree of solubility, stability, low cost, nonstaining and noncorrosive in nature. The efficiency of disinfectants and antiseptics is influenced by several factors, such as concentration, exposure time, types of microorganisms to be destroyed, temperature, pH and type of material to be disinfected.

Phenol [another name fo carbolic acid] was the original disinfectant first used by Joseph Lister in 1867 in order to prevent surgical sepsis. It is now also used as a standard of comparison for determining the antimicrobial activity of a chemical compound. Table 14-1 summarizes the mode of action and uses of some selected disinfectants.

TABLE 14-1: Some common disinfectants and antiseptics.

AGENT	MODE OF ACTION	USES
Phenol	Denatures proteins. Disrupts membrane by lowering surface tension.	Used as antiseptic in soaps, lotions, body deodorants, cosmetics. Disinfectant for lab surfaces.
Alcohols	Lipid solvent and protein denaturation.	Antiseptic for skin. Disinfectant and sterilant for medical instruments and lab surfaces.
Chlorine compounds	Oxidizing agent	Dairy and food industry equipment. Domestic water supplies.
Chlorine gas	Oxidizing agent	Disinfectant used for purification of water supplies.
Iodine compounds	Oxidizing agent and protein denaturation.	Antiseptic for skin. Disinfection for medical instruments and lab surfaces.
Mercuric chloride	Combines with sulfhydral groups of proteins.	Disinfectant for lab surfaces.
Mercurochrome	Combines with sulfhydral groups of proteins.	Antiseptic for skin.
Silver nitrate	Protein precipitant	Drops in eyes of newborn to prevent gonococcal infection.
Formaldehyde	Alkylating agent	3-8% solution used as surface disinfectant. 37% solution (formalin) or vapors used as sterilant.

NEEDS

Cultures. 24-hour broth cultures of *E. coli* and *B. subtilis*.

Medium. Two sets of nutrient broth tubes. Each set should have 6 tubes, each containing 5 ml broth.

Disinfectants. 2% phenol, 2% silver nitrate, 70% ethyl alcohol, tincture of iodine, 3% formaldehyde.

Equipment. Sterile 12 x 100mm test tubes, Bunsen burner and sterile 1-ml and 5-ml pipettes.

PROCEDURE

Each group of 2 students should be provided any one of the disinfectants and antiseptics available in the laboratory. At the end of the experiment, the antimicrobial activities of different agents should collectively be tabulated and a comparative study should be made.

First Day Session

1. Transfer 5.0 ml disinfectant solution into a sterile 12 x 100 mm test tube.
2. Using sterile technique, transfer 0.5 ml of *E. coli* culture to disinfectant tube.
3. Mix the contents in order to distribute the cells uniformly. Note the time.
4. At intervals of 5, 10, 15, 20 and 25 minutes, transfer two loopfuls of the disinfectant-culture mixture to a tube of fresh nutrient broth. Mark the organism code, name of the agent and the time of exposure on each tube.
5. Repeat the step 1 to 4 with the *B. subtilis* culture.
6. Prepare two CONTROLS by directly inoculating *E. coli* into one nutrient broth tube and *B. subtilis* into another broth tube.
7. Incubate the tubes at 37°C for 24 hours.

Second Day Session

1. Clean the outside of all tubes with a tissue and place them in a test tube rack organized into groups by organisms.
2. Gently shake each broth until uniform turbidity is achieved.
3. Compare all tubes in a group to each other and to the CONTROL tube. Rate each one as: 0, +, ++ or +++ according to its turbidity [0 = no growth; + = scanty growth; 2+ = moderate growth; 3+ = abundant growth].
4. Record your results in the table.
5. Comment on the results you obtained.

NAME OF ORGANISM	DISINFECTANT USED	5 MINS	10 MINS	15 MINS
1. E.coli	Alcohol	++	+	-
2. B. subtilis	Alcohol	+++	++	+

KEY :

ZERO = NO GROWTH

\+ = LOW

++ = MODERATE

+++ = ABUNDANT

Object no. 3:

Effects of Osmotic pressure on Bacterial Growth:

Effects of Osmotic Pressure On Bacterial Growth

PURPOSE

Once you have completed this experiment, you should understand the following.

- The possible effect of osmotic pressure environments on different bacteria.
- Differentiation between hypotonic, isotonic and hypotonic solutions.

GENERAL CONSIDERATIONS

Like all other living forms, the bacteria and other microorganisms require water as an essential source of electrons and hydrogen ions and also to maintain cellular turgor pressure. A continuous availability of water in the environment is necessary for the excellent growth and reproduction of microorganisms. Whereas eukaryotic animal cells burst with a constant influx of water, prokaryotes require it to prevent shrinkage of the cell resulting in the separation of the membrane from the cell wall [a harmful condition known as **plasmolysis**].

How do bacteria regulate turgor pressure? The bacteria have the capacity to allow transportation of potassium or sodium ions from their environments to the interior of cells. They maintain high concentration of these ions in the cytoplasm, thus creating a concentration gradient that promotes inward diffusion of water. Not only this, but bacterial cells also allow transportation of **compatible solutes**, composed primarily of amino acids, into the cell to help maintain turgor pressure and provide essential building blocks for cellular components.

Beside the bacterial cell's own efforts to maintain its internal pressure, there are certain natural forces as well that cause movement of water molecules through the semipermeable membrane from an area of low **solute** concentration to an area of high solute concentration. In a solution where solute concentration is low, water concentration is high and *vice versa*. It is, therefore, understandable that water molecules move from where their concentration is high to water where their concentration is low. The process of net movement of water molecules [solvent] across a semipermeable membrane from a solution of their higher concentration to a solution of their lower concentration is called **osmosis**. The pressure exerted by water molecules on the membrane is called **osmotic pressure**.

One point should be made clear right at this stage. The determining factor in the relative water concentrations of the two solutions is their respective solute concentrations. A solution that possesses higher solute concentration, and therefore it has lower water concentration, is called **hyperosmotic** or **hypertonic solution**. If a bacterium is placed in such a solution, there will be a net diffusion of water out of the cell, a condition known as plasmolysis. The plasmolysis is harmful for cells as it inhibits cell growth and reproduction. A solution that possesses a lower solute concentration, and therefore it has a higher water concentration, is called **hypotonic** or **hyposmotic solution**. If bacterial cell is placed in this solution, there will be a net movement of water down its gradient and into the cell. This may lead to the bursting of the cell, a condition known as **plasmoptysis**. Microorganisms which have rigid cell walls are not usually susceptible to lysis in hypotonic environments. Instead, they usually prefer a slightly hypotonic environment to maintain themselves in a turgid state. For bacteria living in an environment where the concentrations of solutes are equal on either side of semipermeable membrane, and therefore equal water concentrations, water will tend to move in both directions equally. It means there is no osmosis and thus no net movement of water molecules. Such solution is called **isotonic** or **isosmotic solution**. This is the most ideal environment for most of the cell types so that they are not susceptible to damage from osmotic pressure.

The natural environments present highly variable osmotic conditions. Microorganisms have a tremendous ability to adapt these all types of osmotic pressure environments, such as soil, fresh water, sea water, and air. Different groups of microorganisms adjust themselves to different levels of salinity for growth. As a rule, they can adjust to salt concentrations of 0.5% to 3%. Concentrations above this range are highly deleterious for the growth. There are, however, some organisms which grow only in high osmotic environments. They are called **osmophiles**. Some of them are adapted to life in waters of high salinity and require high concentrations for growth — in some cases 20% or above. These organisms are known as **halophiles**. Such bacteria are normally found in slat lakes, seas, and oceans, for examples, *Halobacterium salinarium, Pediococcus halophiles,* and *Sarcina morrhuae*. Some of them grow well in the presence of 36% sodium chloride. They are sometimes called **extreme halophiles.** They do not grow if the salinity is lower. Some bacteria can tolerate high slat concentration but do not require it for growth. Such bacteria are called **osmotolerant**, for example, *Staphylococcus aureus* can tolerate 10% slat.

There are certain osmophiles which grow well in the presence of sugar. They are called **saccharophiles**. They may be naturally found in honey. Most of such organisms are fungi, such as *Candida* and *Saccharomyces*, and a few bacteria such as *Leuconostoc mesenteroids.*

The following exercise is designed to determine the maximum tolerance of two common human commensals when they are grown in the presence of a variety of sodium chloride concentrations. One of these commensals is *Staphylococcus aureus* which is a common inhabitant of human skin and nasal passage. The other is *E. coli* which is a common bacterial species living in human intestine.

NEEDS

Cultures. 24-hour broth cultures of *Staphylococcus aureus* and *E. coli*.

Medium. Two sets of nutrient broths containing 1%, 3%, 5%, 7%, 9% and 11% NaCl respectively. Two tubes of normal nutrient broth to be used as control.

Equipment. Sterile 1-ml pipettes, Bunsen burner and glass marker

PROCEDURE

First Day Session

1. Label each set of NaCl broth tubes with the name of bacterium to be inoculated.
2. Mark CONTROL on tubes containing normal nutrient broth.
3. Mix the cultures thoroughly and using sterile technique, transfer 0.1 ml of the experimental culture in each of the appropriately labeled tubes. Use the same pipette for all transfers with a single organism.
4. Incubate all the tubes at 37°C for 24 hours.

Second Day Session

1. Clean the outside of all tubes with a tissue and place them in a test tube rack organized into groups by organisms.
2. Gently shake each broth until uniform turbidity is achieved.
3. Compare all tubes in a group to each other and to the CONTROL tube. Rate each one as: 0, +, ++ or +++ according to its turbidity [0 = no growth; + = scanty growth; 2+ = moderate growth; 3+ = abundant growth].
4. Record your results in the observation table.

Name of Organisms	0% conc.	0.9% conc.	10% conc.	20% conc.
1. B.subtilis	+++	++	+	-
2. S.aureus	+++	+++	+++	++

KEY :

ZERO = NO GROWTH
\+ = LOW
++ = MODERATE
+++ = ABUNDANT

C1= +VE CONTROL
C2= -VE CONTROL


Thank
you!!