ADARSH COLLEGE

OMERGA, DIST. OSMANABAD

DEPARTMENT OF MICROBIOLOGY

CERTIFICATE

Practical paper – P- VI

Certified that Shri / Miss____________________________

has satisfactorily completed the course of practical work in B. Sc. I YEAR (SEMESTER I & II) prescribed by Dr. B. A. M. University, Aurangabad under my supervision in the Microbiology Laboratory during the academic year 20 – 20

Staff member incharge **Head of the department**

Date: Date:

Signature of the Examiner

Date:

Exam Seat No. ______________________

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Expt. No. 01 i) **Date: -**

Bacterial Cell wall staining by Chance's method

Aim: - To observe the cell wall of bacteria by Chance's method.

Theory:-

All true bacteria possess a rigid cell wall; it gives rigidity and shape to bacteria. The cell wall is the structure that immediately surrounds the cell membrane.

The chemical composition of the cell wall may vary in different species of bacteria. Peptidoglycan (also known as murein and mucopeptide) substance found only in prokaryotes cell wall. According to chemical nature of cell wall there are two classes of bacteria, Gram positive bacteria and Gram negative bacteria.

Principle:-

In this method two stains are used, one is basic fuchsin [basic stain] it stains cell wall and cytoplasm of the cell. The second stain is Congo red [acidic stain] it reacts with basic fuchsin from cytoplasm and neutralizes it therefore cytoplasm appears colorless but can't decolorize cell wall because it strongly stained with basic stain.

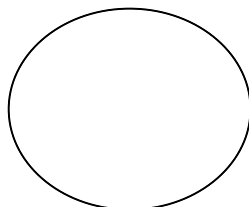
Requirement:-

1. 24 hrs, old culture of bacteria
2. 0.5 % Basic Fuchsin
3. 0.5 % Congo red

Procedure

1. Prepare a smear and heat fix.
2. Flood the smear with 0.5% Basic fuchsin for 1 minute.
3. Wash with water
4. Flood the smear with 0.5 % Congo red for 5 minute Wash with water
5. Air dry and observe under oil immersion lens.

Observation : - Cell wall appears pink, cytoplasm appears colorless.



Result: -

Pink coloured cell wall is observed / not observed

Conclusion: - By Chance's method we can observe cell wall of bacteria.

Expt. No. 1 ii) **Date: -**

Bacterial Spore Staining by Schaefer and Fulton's method

Aim: - To observe the bacterial spores by Schaefer and Fulton's method

Theory: -

The German botanist Ferdinand Cohn discovered the existence of endospores in bacteria; spore production is a very important characteristic of some bacteria such as members of anaerobic genera *clostridium* and aerobic genus *Bacillus*. They are highly resistant and metabolically inactive forms. They occur when environmental conditions become unfavorable for continuing vegetative cellular activities, particularly with the exhaustion of nutritional carbon sources. Because of chemical composition of spore layers, the spore is resistant to heat, freezing radiation, desiccation and chemical agents. The heat resistance of spores has been linked to their high content of calcium and dipicolinic acid.

A single bacterium forms a single spore by a process called sporulation.

Principle: -

The application of heat facilitates penetration of primary stain, malachite green. Water act as decolorizing agent it removes stain from vegetative cell but not from endospore and spore remains green. Red color safranin as counter stain is used as the second reagent to color the decolorized vegetative calls. The spores look green and vegetative cell red.

Requirements:-

1. Bunsen burner, beaker of boiling water, staining tray, glass slides, inoculating loop

2. Malachite green and safranin.

Preparation of malachite green: -The stain is prepared by dissolving 5gm of malachite green in 100ml of distilled water.

Preparation of safranin stain:-

The stain is prepared by dissolving 0.5 grams of safranin in 100ml of distilled water.

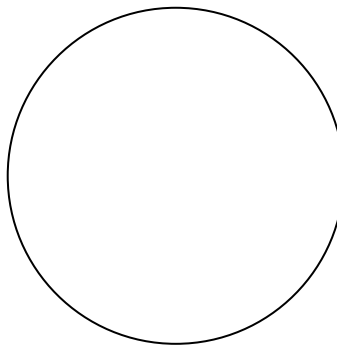
Procedure:-

1. Prepare a smear and air dry. Heat fix by passing the slide 2-3 time gently over the flame with the smear slides up.
2. Put the slide with the smear over a beaker of boiling water resting it on the run with the bacterial film upper most.
3. Flood the smear with 5% aqueous solution of malachite green and allow to act for 1 minute while water continues to boil.

4. Wash the smear with water.
5. Then cover the smear with 0.5% safranin or 0.05% basic fuchsine. Allow it to act for 30 seconds.
6. Rinse the smear again under tap water and air dry.
7. Observe the smear under oil immersion objective.

Obsrvation: -

Red colored vegetative cells with green colored spores are observed.



Result:

Green coloured spores are observed / not observed

Conclusion

We can observe bacterial spores by Schaefer and Fulton's method

Expt. No. 01 iii) **Date: -**

METACHROMATIC (VOLUTIN) GRANULE STAINING BY ALBERT AND NEUSSER'S METHOD

Aim: - To observe metachromatic (Volutin) granules by Albert and Neusser's method.

Theory: - The diphtheria bacillus, *Corynebacterium diptheriae* has well developed granules with the bacterial cytoplasm. These granules are known as volutin granules. These granules are made up of polymetaphosphate and are seen in unstained wet preparations as round, refractile bodies within the bacterial cytoplasm. With basic dyes, granules tend to stain more strongly than the rest of the bacterium, with toludine blue or methylene blue, they stain metachromatically, and appear reddish purple in color. These granules are demonstrated best by special stains such as Albert's, Neusser's stain. With Albert's staining, the bacilli appear green with bluish black metachromatic granules.

Principle:-

The granules present in the diphtheria bacilli exhibit metachromasia property and hence appear bluish=black colored when stained with the toludine blue present in Albert reagent and diphtheria bacillus appears green due to malachite green present in Albert reagent.

Metachromasis is a phenomenon when a substance is stained with particular coloured dye, and if a change in original color is observed, this is called Metachromasis.

Requirements

1. Albert's stain I and II

Preparation of Albert's Stain I

Composition of stain

Toluidine blue	- 1.5 grams.
Malachite green	- 2 grams
Glacial acetic acid	- 10 ml.
Alcohol (95% ethanol)	- 10 ml
Distilled water `	- 1000 ml

Toluidine blue and malachite green are dissolved in the alcohol and then added to the water and acetic acid. The stain is then allowed to stand for one day and then filtered.

Preparation of Albert's stain II: (Albert's iodine)

Iodine – 1 gram

Potassium iodide - 2 grams

Distilled water - 300 ml.

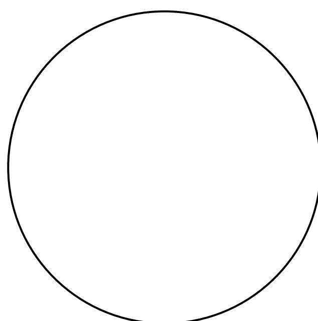
2. Specimen: - Culture of *Corynebacterium diphtheria*

Procedure:-

1. Prepare a smear and air dry. Heat fix the smears by passing the slide 2-3 times gently over the flame with the Smear side up.
2. Put the smear on a slide rack and cover the smears with Albert's stain I , Allow it Stain for 3-5 minutes.
3. Rinse the smear gently under tap water and dry.
4. Then cover the smears with Albert's stain II, Allow it to act for 1 minute.
5. Rinse the smears again under tap water and dry.
6. Observe the smear oil immersion (100*) objective.

Observation: -

Green coloured bacilli showing bluish-black metachromatic granules.



Result

Bluish-black metachromatic granules are observed / not observed.

Conclusion

We can observe metachromatic granules by Albert and Neusser's method

Expt. No. 02 i) **Date:**

Preparation of culture media

Nutrient Broth and Nutrient Agar

Aim: - To prepare Nutrient broth and Nutrient agar medium.

Theory: -

Nutrient broth and Nutrient agar are liquid and solid media respectively. These are one of the simplest and most commonly used laboratory media. These media contain Peptone, Yeast extract, NaCl, Meat extract.

Peptone serves as source of amino acids (nitrogen & carbon). Meat & yeast extract serve as vitamins and growth factors. NaCl maintains osmotic pressure balance. The pH of the medium is adjusted to 7 to 7.4 at which most of the bacteria have a maximum growth rate. Agar powder is for solidification of the medium, when we want solid medium. Agar is prepared form seaweeds, *Gelidium Eczema, pericardia* etc. The chief component of agar is a long-chain polysaccharide .Agar liquefies on heating 96^0 C and hardens into a jell us on cooling to $40\text{-}45^0$ C

This medium is also used for the study of some common pathogenic bacteria.

Composition of Nutrient broth:

1. Distilled Water 100 ml
2. Peptone 2 grams
3. Yeast extracts 1 gm
4. Meat extracts 0.3 gm
5. NaCl 0.5 gm
6. pH 7 to 7.4

When Nutrient agar is to be prepared, add 2.5 gm of agar powder in 100 ml of nutrient broth.

Procedure:

1. All ingredients are weighed accurately and dissolved in distilled water in conical flask.
2. The pH of the medium is adjusted by adding either NaOH (0.1 N) or dilute HCL (0.1 N). pH is tested by using pH paper.
3. The medium is distributed in suitable container and plugged properly with cotton.
4. The containers are rapped with paper and then sterilized in autoclave at 15 lbs / $inch^2$ pressure for 15 minutes.
5. Sterile nutrient agar is displaced in sterile petriplates.

Result: Thus, Nutrient broth and nutrient agar are prepared.

Expt. No. 02 ii) **Date:**

MacConkey's Broth and Agar

Aim:

To prepare MacConkey's broth and agar medium.

Theory:

MacConkey's agar is used for the cultivation of bacteria of enteric group (intestinal bacteria coliforms). It contains bile salt either Sodium Taurocholate or Sodium Deoxycholate, which are inhibiting non-intestinal bacteria i.e. inhibitory to non-coliforms. Thus this medium is selective medium. This medium also differentiates lactose fermenting and non-fermenting organisms. Lactose fermenting organisms produce lactic acid, which changes colour of the indicator in the medium. The pink colour is developed to the lactose-fermenting colony where as lactose non-fermenting organisms produce colourless colonies.

The pH 7.4 is favourable for the growth of intestinal bacteria. The peptone in the medium provides amino acids.

Coliforms: These are Gram negative, rod shaped, motile, non-spore forming, and lactose fermenting bacteria, present in colon region of intestine.

Composition of MacConkey's broth:

Distilled Water	100 ml
Peptone	2 gm
Lactose	1 gm
Sodium taurocholate	0.5 gm
pH	7.4
Neutral red solution	
(2 % in 50 % ethanol)	0.35 ml

To prepare MacConkey's agar add 2.5 gm of Agar- Agar powder to MacConkey's 100 ml broth.

Procedure:

All the ingredients are weighed accurately and are added in a definite amount of distilled water, mixed thoroughly and after the pH is adjusted to 7.4, Neutral red solution is added.

MacConkey's broth is distributed in test tubes and Durham's tubes are placed in inverted position. The air form the Durham's tube is removed. Tubes are plugged with cotton and sterilized.

MacConkey’s agar medium in a conical flask is plugged, wrapped with paper and sterilized in autoclave. After sterilization it is poured into sterile petri plates.

Sterilization temperature and time:

MacConkey’s broth and agar medium is sterilized in autoclave at 10 lbs / $inch^2$ pressure for 40 minutes of time.

Result:

Thus MacConkey’s broth and agar medium is prepared.

Expt. No. 02 iii) **Date:**

Sugar medium

Aim: To prepare sugar medium

Theory:

Sugar media used to determine the ability of microorganisms to degrade and ferment carbohydrate with the production of an acid or acid and gas.

Carbohydrates are organic molecules. There are three types, i.e. monosaccharide, disaccharides and polysacchrides. The power of break down of carbohydrate is possess by a large number of bacteria, organisms use carbohydrate differently depending upon their enzyme complement. The pattern of fermentation is characteristic of certain species, genera , or groups of organisms and for this reason this property has been extensively used as a method for biochemical differentiation of microbes using different types of sugar we can prepare glucose medium, sucrose medium lactose medium etc.

In sugar medium PH indicator (Bronothymol blue, 0phenol red, neutral red, phenol red,) present it indicates formation of acid by changing colure of the medium. The medium in the tube is also provided with a small tube called as Durham's tube in inverted position to collect the gas produced by the organism.

Composition:-

1. Peptone 1 gram
2. Sugar 1 gram
3. Nacl 0.5 gram
4. Distilled water 100 ml
5. Neutral red solution
   Or 1ml
   Phenol red solution
6. pH 7.4

Procedure:

All the ingredients except indicator are weighed accurately and added in definite volume of distilled water, mixed thoroughly. pH is adjusted at 7.4. The indicator is added. Medium is distributed in test tubes (about 5 ml in each tube). Durham's tube is placed in inverted position and air in the Durham's tube is removed completely. The tubes are plugged with cotton and are wrapped with paper and autoclaved by steaming for 30 to 40 minutes.

Note:

1. Sugar media are not sterilized at 15 lbs / $inch^2$ pressure.
2. Indicator is added after adjustment of pH of the medium.

Expt. 02 iv) **Date: -**

Potato dextrose agar

Theory :-

Potato dextrose agar (PDA) is routinely used for the isolation and maintenance of common fungi, *Penicillium Aspergillus*, *Rhizopus*.

Media used for the isolation of fungi are designed to be inhibitory to bacteria. .The PH of media should be adjusted towards acidic range 5.4 to avoid bacterial growth.

Requirement:-

Composition

I. Potato (Peeled) - 200 .0g

Dextrose - 20.0 g

Agar - 20.0 g

Distilled water - 1000 ml

PH is self adjusted – 1000 ml

II. Muslin cloth / Cheese cloth.

III. 1 liter corning beakers (2)

IV. 250 ml corning Erlenmeyer flasks.

IV. Potato peeler

Procedure:-

1. Take 500 ml of water is one liter beaker
2. Add 200g washed peeled and sliced potatoes to the beaker.
3. Boil potatoes gently for 30 minutes till they are easily penetrated by a glass rod.
4. Filtered through chase cloth, squiring out all liquid.
5. Add 20 g of dextrose to the potato extract.
6. Take 500 ml of water in another beaker and heat it.
7. Add 20 g agar, bit by bit to the hot water to dissolve it
8. Mix agar with the potato extract.
9. Bring volume up to 100 ml
10. Dispense 200 ml each five conical flasks
11. Dispense 5 ml each in screw-capped or (cotton plugged) tubes for slant preparation.

12. Plug the flaks and test tubes contain medium.
13. Sterilize at 121^0 C, 15 lb pressure for 15 minutes in an autoclave
14. Allow the tubes to cool in slanting position for PDA slants.
15. Pour the medium (15-20ml) into Petri dishes quickly under aseptic condition.
16. Allow to solidify to produce PDA plates and slants.

Expt. No. 02 v) **Date: -**

Blood agar

Theory:-

Blood agar is enriched culture media it can be prepared by adding blood in nutrient agar. Blood agar is widely used in medical bacteriology. It is especially suitable for the Gonococcus, the hemophilic group of bacteria. In addition to being an enriched medium, it is an indicator medium showing the hemolytic properties of bacteria such as ***Streptococcus pyogense.***

It is differential medium for hemolytic organisms. Three patters of hemolysis can occur on blood agar plate.

A] Beta hemolysis (complete hemolysis) where formation of clear Zone with a clear edge around colony take places.

B] Alpha hemolysis (Incomplete hemolysis), where production of methenoglobin and green cloudy zone around the colony is formed.

C] Gamma hemohyis. (No hemolysis) , where no change in cooler surrounding the colony on blood agar takes place.

For preparation of blood agar 10% blood from human or animal may be used horse blood is commonest.

Requirement: -

Nutrient agar – 100 ml

(PH 7.2)

Defibrinated blood – 10 ml

Procedure:-

1. Autoclave the nutrient agar of 121^0 C for 15 min
2. Cool to $45\text{-}50^0$ C and add 10 ml of sterile blood aseptically.
3. Rotate to mix thoroughly avoiding accumulation of air bubbles and pour immediately into sterile tube or plate i.e. before solidification
4. Allow to solidify plates.

Expt. No. 02 vi) **Date: -**

Photosynthetic bacterial growth medium

Composition I

$(NH)_4 Cl$	-	1.0 g
$KH_2 PO_4$	-	1.0 g
$MgCl_2$	-	0.5 g
NaCl	-	0.3 g
Na H Co_3	-	2.0 g
Na_2 S. 9H20	-	1.0 g
Fe	-	500 micro grams
Tap water	-	1000 ml
PH	-	7.3

Steam sterilize the medium at 121^0 C for 30 ml vex used for cultivation of photosynthetic bacteria from chlorobeacae family.

Composition II

(NH4)2 So4	-	1.0 g
K2H Po4	-	0.5 g
Mg So 4	-	0.2 g
Nacl	-	2.0 g
Na Hco3	-	5.0 g
Yeast extract	-	0.1 g
(Malate) organic substrate	-	1.5 g
Tap water	-	1000 ml
PH	-	7.0

Steam sterilize the medium at 121^0 C for 30 min used for cultivation of photosynthetic bacteria form "Athiorhodaceae' Family

Expt. No. 3 **Date:**

Isolation of microorganisms from soil, water, air and milk

Aim:

To isolate different types of microorganisms from soil, water and air.

Theory:

All regions of the earth that contain living organisms are known collectively as the "BIOSPHERE". This includes Soil, Water and Air.

Soil is the upper layer of most of the earth's surface and varies in depth from inches to over 20 feet. Microorganisms are present in soil because there is a food for them. Soils are excellent cultural media for the growth of many types of organisms. These include Bacteria, Fungi, Algae, Protozoa and Viruses. A spoonful of soil contains billions of microorganisms. The number and kinds of microorganisms found in the soil depend up on the nature of soil, depth, season of the year, state of cultivation, reaction, organic matter, temperature, moisture, aeration etc.

All metabolic reaction of living organisms depends on the presence of water. Microorganisms get into waters from air, soil, sewage, organic wastes, dead plants & animals etc. Thus any type of microorganisms may be found in water. The numbers and kinds of microorganisms in water depend upon food material, Climatic, geographical and biological conditions.

Air is not a natural environment for the growth of microorganisms. It does not contain necessary amount of moisture and nutrients. The microorganisms found in air come from soil, organic wastes of man and animal, the oral, nasal and rectal passages of man and animals and from the lungs through coughs and sneezes. The kinds and numbers of microorganisms in the air vary, depending upon the sources of contamination in the environment and locality and also speed of air current, humidity, sunlight, temperature and the size of particles on which they are attached.

Milk is good culture medium for the growth of microorganisms as it contains protein, carbohydrate, fat, vitamins and minerals. Microorganisms enter in milk from milking animals, milking man, utensils, and during handling and transporting of it.

Requirements:

1. Sterile nutrient agar plates
2. Sterile saline tube

Procedure:

A) Isolation of bacteria from soil:

One gram of soil is added in 10 ml sterile saline. A loopful from this is streaked on sterile nutrient agar plate aseptically. The plate is incubated at 37 ^{o} C for 24 hours. After incubation colony characters and Gram nature are studied of well-isolated colonies.

B) Isolation of bacteria from water:

A loopful of water sample is streaked on sterile nutrient agar plate aseptically. The plate is incubated at 37 ^{o} C for 24 hours. After incubation colony characters and Gram nature are studied of well-isolated colonies.

C) Isolation of bacteria from air:

Sterile nutrient agar plate is exposed to air for 5 minutes. The plate is incubated at 37 ^{o} C for 24 hours. After incubation colony characters and Gram nature are studied of well-isolated colonies.

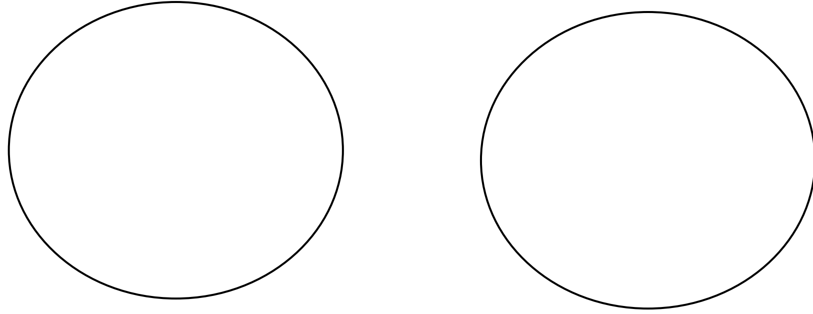
D) Isolation of bacteria from MILK:

A loopful of milk sample is streaked on sterile nutrient agar plate aseptically. The plate is incubated at 37 ^{o} C for 24 hours. After incubation colony characters and Gram nature are studied of well-isolated colonies.

Observations: Different types of colonies are observed on nutrient agar plates. Following are the colony characters and Gram nature of selected colony from each plate.

Colony Characters:

Colony characters	**Soil**	**Water**	**Air**	**Milk**
Size				
Shape				
Colour				
Margin				
Elevation				
Opacity				
Consistency				
Gram nature				



Result:

Bacteria are isolated from air / water / soil / milk

Conclusion

Bacteria are present in air / water / soil / milk and by this method we can isolate them.

Expt. No. 4 **Date: -**

Isolation of bacteria from mixed cultures by streak plate method.

Aim: - To isolate the bacteria from mixed cultures by streak plate method.

Introduction:-

A pure culture, one containing single kinds of microbe, is required in order to study its various characteristics like growth, physiology, metabolism, pathogencity etc.

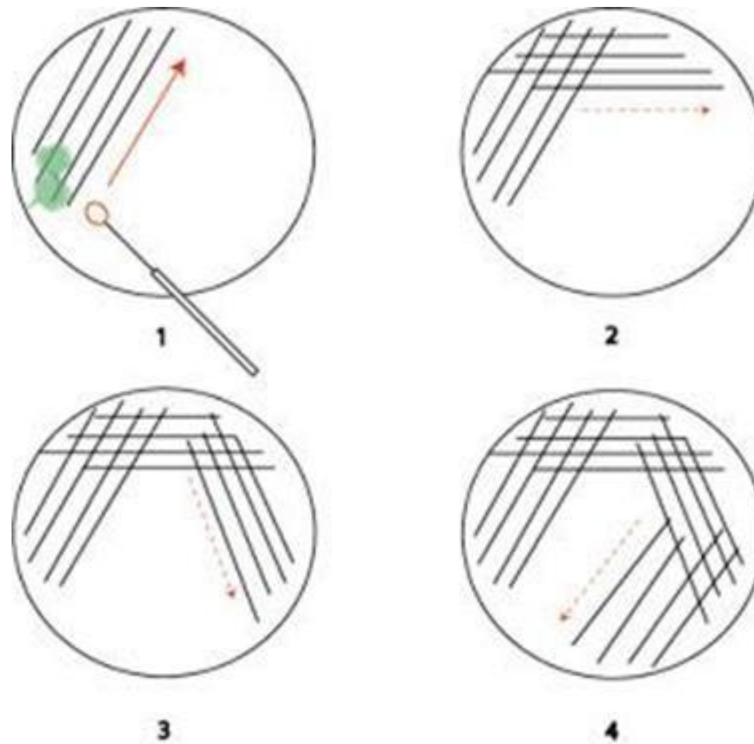
In mixed culture, any particular microorganism being sought must be recognized among, and isolated away form other organisms. Colonies of the desired organism must picked out of the mixed culture and grown in isolated pure culture.

Then proceed for identify the isolated organism by enjoining its biological properties pure culture technique is a critical to successful , accurate identification of microorganism .When cells are separated by dilution in a solid medium and allowed to form colonies, they can be separated form each other and use for further studies,

Colonies differ in size, shape, texture and colure with different microorganisms, Therefore colony appeared is a valuable clone in identification. By a two methods organisms can be separated form a mixed culture on solid medium.

Streak plate method:-

Microbial cultures may be applied to the surface of agar and spread them with a loop. This is called streaking and a plate so prepared is a streak plate. Streaking is generally done by four quadrant method. During streaking bacterial cells are separated from each other and after incubation each cell develops into colonies.



Requirements:-

1. Sterile nutrient agar plate.
2. Inoculating 100 p / Nichrome wire loop
3. Mixed bacterial culture.

Procedure

1. Following aseptic conditions open the Petri plate containing the nutrient agar near burners.
2. Sterilize the loop by red hot heat method cool 2 sec. and take one loop full of mixed culture.
3. Streaking can carried by quadrant method.
4. In this method Petri dish at its base marked by four lines. Means make four parts as shown in fig A,B,C,D
5. Takes a loop full of mixed culture and make few parallel line of streak to 'A' line
6. Flame the loop and without taking mixed culture make few parallel streaks to B line.
7. Flame the loop and streak few parallel lines to C line and lastly to D line without touching A side.
8. After streaking incubate the plates at 37 ^{0}C in inverted position for 24 hrs.
9. After 24 hours, note the colonies developing one the plate with regard to their size, shape and appearance.
10. Mark the isolated colonies, transfer one isolated colony on nutrient agar slant that will give you pure culture of that type.

Observation

Well isolated colonies are observed

Result:

From observation it can be said that there are different types of bacteria in the given sample which can be isolated from each other by streak plate method.

Conclusion

During streaking bacterial cells are separated from each other and after incubation each cell develops into colonies. Thus streak plate method is useful technique to obtain pure culture.

Expt. No. 5 i) **Date: -**

Effect of physical and chemical agent on bacterial growth

Effect of Temperature

Aim: - To study effect of temperature on bacteria growth by turbidometry method.

Introduction:-

Microbial growth is directly dependent on how temperature affects cellular enzymes. With increasing temperature, enzyme activity increases, but at high temperature enzyme molecules denatured and enzyme activity completely stops. On the other hand, as the temperature is lowered towards the freezing point, enzyme inactivation occurs and cellular metabolism gradually diminishes. On the basis of temperature, microorganisms are classified as Psychrophilic, Mesophilic and Thermophilic. Psychrophilic grow in the temperature range 0 to 20^0 C, mesophilic grow in the temperature range 20 to 50^0 C, thermophilic grow in the temperature range 50 to 80^0 C.

Requirement:-

1. Actively growing culture of *E. Coli* , / *S. aureus*
2. Test tubes with 10 ml of nutrient broth
3. Freeze, Incubator set to differed temperature [10^0 C, 37 ^{0} C, 50^0 C]
4. Colorimeter.

Procedure:-

1. Prepare the nutrient broth and dispense about 10 ml of it into each tube and autoclave.
2. Inoculate 0.1 ml of actively growing culture cell suspension into each tube. Mix well and take OD at 540 nm.
3. Incubate tube at 10^0 C, 37^0 C, 50^0 C according to their label for 24 hrs.
4. After incubation read optical density of each tube held at different temperatures against a blank at 540 nm in colorimeter.
5. Record the results.
6. The temperature at which O.D. is maximum is considered as optimum temperature of the given organism.

Observation table: -

	OD at 10 ^{0}	OD at 37 ^{0}	OD at 50 ^{0}
Control			
Test			

Result: -

As OD of given test sample is more at ______ ^{o}C , optimum temperature for growth of given test organism is ______ ^{o}C.

Conclusion: -

Microbial growth is directly dependent on how temperature affects cellular enzymes.

Expt. No. 5 ii) **Date: -**

Effect of UV rays on growth of bacteria

Aim: - To study effect of UV rays on bacteria growth.

Theory: -

Certain form of electromagnetic radiation is capable of producing a lethal effect on cells and therefore can be used for microbial control. Ultraviolet rays produce lethal effect in the range of 210 nm to 300 nm. Most of the ultraviolet are absorbed by DNA and damage it by forming thymine dimmers, which is the covalent bonding of two adjacent thymine molecules on the nucleic acid strand in the DNA molecule. This dimmer formation distorts the configuration of the DNA molecule and the distortion interferes with DNA replication and transcription during protein synthesis. UV rays are used for the sterilization of air in operation theater and water in water purifiers.

Requirements:-

1. 24 hrs bacterial culture.
2. Sterile nutrient agar plates.
3. Ultraviolet chamber.

Procedure:-

1. Label 5 nutrient agar plates with exposure time period, 30, 60, 90, 120, 150 Seconds.
2. Inoculate all plates with given bacterial culture by spread plate technique
3. Open the plates in the uv chamber. The plates are exposed to UV rays as per respective time by covering half portion of the plate. After exposure put the lid.
4. Incubate all plates in an inverted position at 37^0 C for 24 hrs.
5. Observe all nutrient agar plate for number of colonies on exposed and unexposed area on each plate.

Observation table: -

UV exposure time	**No. of colonies on exposed area**	**No. of colonies on unexposed area**
30 Seconds		
60 Seconds		
90 Seconds		
120 Seconds		
150 Seconds		

Result: -

Number of colonies on UV exposed area of each plate is less than unexposed area.

Conclusion: - As the number of colonies on UV exposed area of each plate is less than unexposed area, there is lethal effect of UV rays on the growth of bacteria in a given sample.

Expt. 05 iii) **Date: -**

Effect of chemical agents on bacterial growth

Effect of pH

Aim: - To study the effect of pH on bacterial growth by turbidometry method.

Introduction: -

The H^+ ion concentration of the medium provides stimulation or inhibition of growth of microorganisms. The metabolic activity of microorganisms is carried by enzymes that catalyze specific reactions with narrow pH range. The presence of buffers tends to stabilize the pH of growth medium. More change in pH of growth medium affects bacterial growth. This can be observed by allowing the culture growth at different pH media by keeping all other factors constant.

Requirements:-

1. Actively growing bacterial culture
2. Nutrient broth media tubes with different pH (3, 7 & 11)
3. Colorimeter
4. Incubator

Procedure:-

1. Prepare Nutrient broth with different pH and 10 ml dispensed into each test tube (3 pH, 7 pH, 11 pH)
2. pH of nutrient broth adjusted by 1 N HCl and 1 N NaOH, before autoclaving.
3. Inoculate 0.1 ml of culture in each tube. One test tube of each pH is kept as control (blank) without inoculation of culture.
4. Incubate the tubes for 24hrs at 37 ^{0} C temperature.
5. Read optical density each tube against a blank at 540 nm.

Observation: -

pH	OD
3	
7	
11	

Result: -

OD of given bacterial culture is more at pH ___.

Conclusion: - As OD of given bacterial culture is more at pH ___, the optimum pH for growth of given bacterial culture is __. The pH other than optimum pH affects the growth.

Expt. No. 5 iv) **Date: -**

Effect of Antibiotics on growth of bacteria

Aim: - To study the effect of antibiotics on bacterial growth.

Introduction:-

Antibiotics are chemical substances which are synthesized and secreted by some microorganisms (Bacteria, fungi, actinomycetes) that destroy or inhibit the growth of other microorganisms e.g. Penicillin is produced by *Penicillium notatum,* Streptomycin and tetracycline is produced by *Streptomyces* species.

Today, some antibiotics are laboratory synthesized or modified, however their origins are living cells.

Different antibiotics have different mode of action on bacteria for example penicillin kills bacteria by inhibiting cell wall synthesis, streptomycin inhibit protein synthesis, polymyxin carry destruction of cell membrane.

Antibiotic sensitivity test by paper disc method determine the effect of antibiotic on growth of bacteria. This method allows the rapid determination of the efficiency of a drug by measuring the diameter of the zone of inhibition that result from diffusion of the agent into the medium surrounding the disc.

Requirements:-

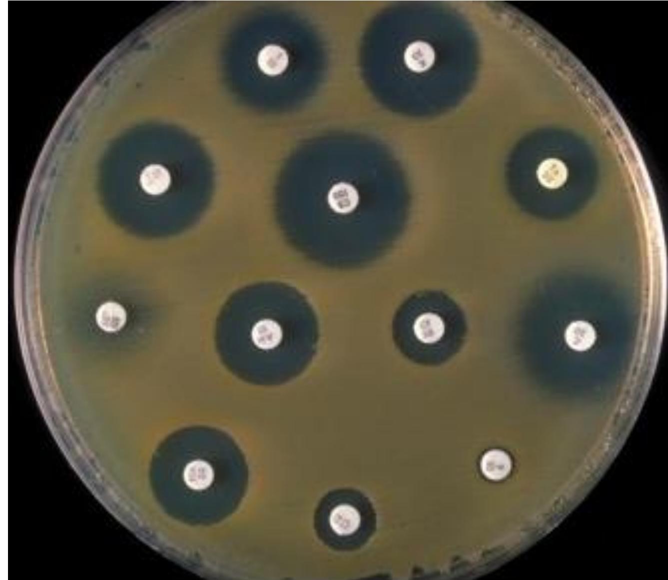
1. Saline suspension of *Staphylococcus aureus*
2. Nutrient agar plates
3. Antibiotic discs of Penicillin
4. Forceps

Procedure:-

1. Using sterile technique, inoculate agar plate with 0.1 ml given bacterial culture by spread plate method.
2. Using sterile forceps keep the antibiotic disc on agar surface at centre and pressing it gently.
3. Keep the plate in freeze for 15 minutes for the diffusion of antibiotic in the medium.
4. Incubate the plate in upright position (not inverted) for 24 hrs at 37 ^{0} c.
5. Observe the zone of inhibition production around the disc.

Observation: -

Zone of inhibition is observed / not observed.



Result: -

There is a lethal effect / no effect of given antibiotic on the given bacterial culture.

Conclusion: -

As zone of inhibition is observed / not observed, there is a lethal effect / no effect of given antibiotic on the given bacterial culture.

Expt. No. 5 vi) **Date: -**

Effect of Heavy metal ions (oligodynamic action) on bacteria

Aim: -

To study effect of Heavy metal ions (oligodynamic action) on bacteria.

Introduction:-

Most of the heavy metals either alone or in certain chemical compound are germicidal .The most widely used heavy metals are mercury, silver and copper. These metals are so toxic that even in small concentration, they are lethal to bacteria, this inhibition of bacteria, in low concentration is called oligodynamic (Oligos = small, Dynamic = power).

Sliver nitrate 1 % solution added in eyes of infants to prevent gonococcus infection of eyes, Copper sulphate is effective algicide in swimming pools, it is also used as a fungicide to prevent certain plant diseases.

Heavy metal ions combine with enzymes of microorganisms and affect with sulfahydryl groups and inactivate them.

Requirements:-

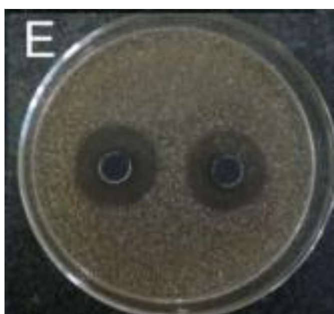
1. 24 hrs old culture of bacteria.
2. Silver or copper coin
3. Sterile nutrient Agar plate.

Procedure:-

1. Inoculate sterile nutrient agar plate with bacterial culture by spread plate method.
2. Using sterile forceps keep copper / silver coin at the center of plate.
3. Incubate plate at 3 7 $^{0\,C}$ for 24 hr.
4. After incubation observe the zone of Inhibition (No growth) of bacterial growth.

Observation: -

Zone of inhibition around the coin is observed / not observed.



Result: - There is a lethal effect of heavy metal on the growth of given bacterial culture.

Conclusion: -

As zone of inhibition around the coin is observed / not observed, there is a lethal effect / no effect of heavy metal on the growth of given bacterial culture.