(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

SEGREGATION AND RECOGNITION OF MICRO-ORGANISMS

Vanshika Goel and Sainyam Goel
Indraprastha Convent Senior Secondary School
Begumpura, Rohini, New Delhi

ABSTRACT

Every organism has its own habitat, which may or may not different from other micro organisms. For isolation of micro organism from different places or things can explain the flora of that place. Micro organisms are not present only on the things or places but they are very important part of human life. Human body can carry specific micro flora in their body. Different organ have different micro flora. Intestine carries ***E.coli*** *&* ***Clostridium spp****., vagina carries* ***Lactobacillus*** *such as* ***L.crispatus****, conjunctiva carries* ***Haemophillus spp.*** *& Streptococci (various spp.), ear carries* ***Diptheroids****,* ***Pseudomonas spp****. and throat carries* ***Staphylococcus aureus*** *etc as their normal flora. As well as skin is the most exposed part to the outer environment of the body so maximum number of micro organisms present on it. Most Moist and dry areas of body has large number of organisms because pH and temperature of the body are altered.* ***PASTEUR****,* ***KOCH****, and their contemporaries were among the first to identify a specific bacterium with a specific disease. Since that time, medical microbiology has made great advances in laboratory diagnosis of disease, and in understanding pathogenesis and the role of immunity in the infectious cycle. Yet, many of the diseases common in the late 1800s remain with us today.*

Like human body everything either it is living or non living on earth is covered with micro organisms. In today's life people are in touch with technology more than any other thing. So the chances of number of organism on mobile phones, laptops, tablets or other gadgets are very high. We can isolate the organisms by these gadgets easily. All organisms are not hazardous. Some are very important for mankind. For fermentation, industrial purposes, food purposes micro organisms are very helpful. When the number of micro organisms altered then problem begins. More or less micro organisms can cause diseases or many other complications. Isolation of organisms is not very easy. The requirements of every organism cannot fulfill by artificial conditions. Some organisms are hard to cultivate on laboratory levels. Organisms like bacteria, viruses, algae, fungi, protozoa and helminthes. Organisms like viruses are not culturable in normal institutes and laboratory. Bacteria are easily culture in laboratory but not all because some bacteria need more incubation or more favorable conditions that are hard to provide sometimes. Isolation of organism/bacteria from mobile phones is possible by taking their swab. And we get number of bacteria there. Mobile phones are the most common gadget which is used by every person. Wherever we are, mobile phone remains in our hand or pocket. They exposed to environment mostly. So micro-flora of phone is also very interesting.

(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

INTRODUCTION

Till today, only 1% of organisms are known and 99% of organisms are unknown. Organisms can smartly challenge the humans by their population and ability of resistance. Every organism has its own favorable condition to grow and multiply. Some organisms grows at 1 degree C and some grows at even 100 degree C or more than this temperature. lots of variations are present in the metabolism of organisms. **pH**, **salinity**, **respiration** etc are some other factors on which organisms rely. Traditional methods or modern techniques are present to isolation of organisms. For isolation we need freshly prepared media, **optimum temperature** for incubation, proper inoculation etc. Every single parameter is important for the growth, multiplication and isolation of organism. If any of this parameter has any problem or lacking of anything in media (liquid or solid media), temperature issue or contamination of the sample then the result will not be accurate or we get false positive or negative results.

Like all living organisms, microbes need nutrients, such as a carbon and nitrogen source as well as vitamins and other growth factors, to survive and grow. The source for these nutrients usually comes from the enzymatic degradation of other complex nutrients derived from plants and animal sources. The composition of the nutrients represents a culture medium. Such media may be in the form of a liquid or solid. A liquid solution in which microbes, especially bacteria and protozoa, will grow is called **nutrient broth**. A broth medium is one way to grow micro-organisms to a high cell density. However, if the broth contains more than one species of micro-organism, each individual species cannot be differentiated with the naked eyes. A broth supplemented with a solidifying agent, called **agar**, produces a relatively solid medium on which bacteria and fungi easily grow.agar is polymer of galactose that is extracted from the cell walls of seaweed(red algae); agar has no nutritional value in an agar culture. In solution, powdered agar liquefies at 100 degree C and then will solidify at 40 degree C as the solution cools. This means micro organisms isolated from humans can be cultivated at human body temperature(37 degree C) without fear of the medium liquefying.

AIM

Isolation of micro organism from mobile phones

REQUIREMENTS

Cotton swabs, alcohol, glass slides, Bunsen burner, nutrient broth, nutrient agar (liquid and solid media), hydrogen peroxide, bio-chemicals tubes, normal saline, incubator, inoculating loop, peptone water, microscope.

(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

THEORY

Mobile phones are used by everyone and everywhere. We have done all the work by hands and most of variable organisms are present there. Normal flora, hazardous organisms, organisms which present in faecal matter they also present on hand sometimes. So mobile phones carry same organism maximum time on their outer body. Due to the micro-flora of mobile phones, scientist tells to avoid mobile phones during having meals. They may be hazardous sometimes. So we isolated the organisms from mobile phones.evidence of lactose fermenting bacteria, which will appear as pink/red colonies. **Escherichia coli** will usually be brick red while **Enterobacter**, **Klebsiella** and other lactose fermenters will be pink to red with a mucoid texture.

PREPARATION

Need to prepare the cotton swabs for the swabing of organism present on the mobile phones. Prepare the media for the growth of organisms.

- Nutrient broth

Composition

1) Yeast extract 10gm
2) Peptone water 10gm
3) Sodium chloride 5gm
4) Distilled water

Preparation

1) Nutrient broth 1.3gm
2) Distilled water 100gm
3) Mix them well in flask and plug it tightly
4) Autoclave it properly for sterilization at 121 degree C for 15mins
5) Cool it down for pouring in plates.

- Nutrient agar

(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Composition

1) Peptone water 0.5%
2) Yeast extract 0.3%
3) Agar 1.5%
4) Sodium chloride 0.5%
5) Distilled water

Preparation

1) Nutrient agar 2.8gm
2) Distilled water 100ml
3) Mix them well and plug it properly
4) Sterilizing by autoclaving at 121 degree C for 15min
5) Cool the flask and pour it.

- MacConkey agar

Composition

1) Protease peptone (meat and casein) 3gm
2) Lactose monohydrate 10gm
3) Bile salt mixture 1.5gm
4) Sodium chloride 5gm
5) Peptone pr gelysate 17gm
6) Neutral red 0.03gm
7) Crystal violet 0.001gm
8) Agar 13.5gm
9) Distilled water 1liter

PREPARATION

1) MacConkey agar 4.95gm
2) Distilled water 100ml
3) Mix them well in flask
4) Autoclave it at 121 degree C for 15min
5) Cool it at 45-50 degree C
6) Mix well before pouring into sterile petri plates.

(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Immediately after sterilization media does not use. Because the organism get shock treatment by sudden rise of temperature and walls get disrupted. This is the main reason why organisms cannot detect in the sample.

After pouring media, incubate them overnight. If any contamination is there then next day growth appears on plates which shows plate cannot use for isolation because of contamination.

So sterilization of equipment & media and pouring in sterilize condition is very important.

PROCEDURE ON 1ST DAY

I. Took the swab from mobile phone. Inoculate the swab into the nutrient broth to grow or multiply more. A nonporous,clean vessel should be used for collection. Incubate it overnight.
II. Pour nutrient agar into petri plates and let them solidify, after the solidification invert the plates and incubate it overnight at 37 degree C.

PROCEDURE ON 2ND DAY

III. After overnight incubation of nutrient broth took the inoculum through sterilized inoculating loop and make a thin smear on the glass slide.
IV. Air dry it and heat fix it.
V. Stain the smear with gram stain. Put the drops of crystal violet dye (stains all the cells blue/purple) on the smear and let it dry for 1min, drops of iodine (iodine form a crystal violet iodine complex) is added for 1 min, put alcohol (decolorizing agent) for 30sec and then add drops of safranine dye (stain decolorizing cells) for 1min.
VI. After every stain rinse it with normal water. Air dry the smear.
VII. Examine the smear under 100X of light microscope with synthetic oil immersion.
VIII. If the bacteria are gram positive then cells appeared blue and gram negative bacteria appeared as pink in color. Cells appeared as coccus, bacillus, spiral shaped, cocco bacillus etc.
IX. On the basis of morphology cells can differentiate the type of cells. Whether it is gram positive coccus, gram positive bacillus & gram negative coccus, gram negative bacillus. And any kind of mixture present in the sample.
X. Then for pure culture streak the inoculum on solid media plate by inoculating loop and incubate the inverted plate at 37 degree C overnight.
XI. To check the motility, hanging drop method is done. This technique is for examining the living organisms which are unstained.
XII. Examine the solid plate media after 5/6 hours.

RESULTS

Gram negative, non-capsulated bacillus, motile organism, no growth/colony appeared on solid plate media on 2nd day.

PROCEDURE ON 3RD DAY

I. Examine the colony characteristics on solid plate media after the incubation of 16 hours. More the incubation more changes of colony can be seen.
II. Colony characteristics are different for every organism that helps in examination or identification.
III. Gram stain the colony and confirm the morphology of organism.
IV. One type of colony present if only one kind of bacteria are present in the culture or inoculum. And if two or more than two type of colony are present on the plate than it signifies that more than one type of bacteria are present in culture.
V. Done the catalase test by hydrogen peroxide and colony. In all living organism catalase enzyme are present which are exposed to oxygen. Catalase enzyme catalyzes the decomposition of hydrogen peroxide (H2O2) into water and oxygen.
VI. Inoculate the peptone water by colony and incubate it for 2-4 hours. In peptone water organisms can multiply and increase their number rapidly.
VII. Inoculate the bio-chemicals by peptone water to test the abilities of organism. Test are-
Indole, methyl red, voges-proskauer, phenylalanine deaminase (PPA), urease, citrate, HL, triple sugar iron (TSI), glucose, sucrose, manitol, xylose, lysine, ornithine, arginine.
Incubate the bio-chemicals over night.

RESULTS ON 3RD DAY-

One type of colonies are present on both the plates

CHARACTERS	COLONY	COLONY
Media	Nutrient agar	MacConkey agar
Shape	Circular	Circular
Color	White	Brick red
Size	2-3 mm	Small

Elevation	Low convex	Low convex
Lusture	Shiny	Shiny
Margin	Regular	Regular
Texture	Smooth	Smooth
Opacity	Opaque	Opaque
Odour	Yeast like	Yeast like
Motility	Motile	Motile
Temperature for incubation	37 degree C	37 degree C
Time for incubation	16 HRS	18 HRS
Catalase	POSITIVE	POSITIVE

Presence of pink color on MacConkey agar plate shows that the organism is lactose fermentor.

RESULTS ON 4TH DAY OF BIOCHEMICALS-

INDOLE	POSITIVE
METHYL RED	POSITIVE
VOGES PROSKAUER	NEGATIVE
SUGARS- GLUCOSE	POSITIVE

SUCROSE	POSITIVE
MANITOL	POSITIVE
XYLOSE	POSITIVE
AMINO ACIDS-	
LYSINE	POSITIVE
ARGININE	NEGATIVE
ORNITHINE	NEGATIVE
CITRATE	NEGATIVE
PPA	NEGATIVE
H2S IN TSI	NEGATIVE
UREASE	NEAGTIVE
HL O/F	POSITIVE/POSITIVE

Principle of different bio-chemicals-

Indole- This test is used to determine the capability of organism to split tryptophan and form a compound indole. Red ring shows the positive result. It is the Part of IMVIC test

Methyl red test- This test is used to detect the capability of organism to produce the stable acid end products. It is the part of IMVIC test. Presence of red color shows the positive result.

Voges-proskauer- VP test is used to detect the capability of organisms to produce non acidic or neutral end products, like acetyl methyl carbinol. Red color shows positive result.

Citrate- organism use citrate as their sole energy source. If citrate permease enzyme is present in organism then it converts sodium citrate into pyruvic acid, oxalo-acetic acid and carbon dioxide. Carbon dioxide combines with sodium and water to form sodium carbonate (an

alkaline product). This alkaline product raises the pH and turns the pH indicator into blue color which the test is positive. Absence of color change shows the test is negative.

Urease test- This test is used to determine the capability of organism to form the ammonia and carbon dioxide. Ammonia combines with water to produce ammonium hydroxide which increases the pH of medium. Because of increase in pH, phenol red turns into pink color. Presence of pink color shows the positive result of the test.

TSI test- three sugars are present in this; Sucrose 1%, lactose 1% and glucose 0.1%.

- Reduce sulfur into H2S
- Lactose or sucrose fermentation
- Glucose fermentation and gas production
- Phenol red as indicator (to detect acid production from carbohydrate and gas production).

PPA test- It denotes phenylalanine deaminase test. This test determines whether the microbe produces the enzyme phenylalanine deaminase, which is need for it to use the amino acid phenylalanine as a carbon source for growth. Presence of green color shows the positive result.

HL test- this test shows the organism is fermentative or oxidative. If the butt turns to yellow, it signifies that organism is fermentative. If only upper surface turns to yellow, it signifies that organism is oxidative. If both the portions are yellow then it means organism is fermentative as well as oxidative. It confirms the motility also.

IDENTIFIED ORGANISM IS- **ESCHERICHIA COLI**

CONCLUSION

E.COLI –

This organism is an aerobe and facultative anaerobe. E.coli is inhibited by sodium selenite, sodium tetrathionate and brilliant green in media. It is also inhibited by 7% sodium chloride in salt media used for isolation of staphylococci.

E.COLI is excreted in faeces of man and animals in very large number. It contaminates soil, water and food through crops. It can survive in water and soil for many days.

This organism can cause many severe diseases by its toxins. Toxins like-

- Enterotoxins
- Haemolysins
- Verocytotoxin

(IJIASE) 2016, Vol. No. 2, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Diseases like-

- Urinary tract infection
- Diarrhea and dysentery
- Pyogenic infection
- Septicaemia

This bacteria is harmful if ingested. And presence of this bacteria on mobile phone cause severe health issues. Mobile has its own advantage or disadvantage but people should avoid mobile phone during meal specially.