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

Enumeration of microbes from indoor and outdoor

Aim:

To isolate different types of bacteria from air.

Theory:

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

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.

e.g. Bacterial species --- Species of genera *Alcaligens, Bacillus, Chromobacterium, Coliforms, Micrococcus, Serratia, Staphylococci* etc.

Fungal species --- Spores of Penicillium, Aspergillus, Alternaria, Cladosporium etc.

Requirements:

1. Sterile nutrient agar plates
2. Sterile saline tube

Procedure:

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.

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	**Colony - 1**	**Colony - 2**	**Colony - 3**
Size			
Shape			
Colour			
Margin			
Elevation			
Opacity			
Consistency			
Gram nature			

Result:

From observation it can be said that there are different types of bacteria in the air.

Conclusion: -

The number and kind of bacteria depends up on the air and its contents.

Expt. No.02a **Date: -**

Bacteriological examination of drinking water: Quantitative analysis of water (MPN)

Aim:

To determine the Most Probable Number (MPN) of coliforms in the given water sample

Theory:

Drinking water may be contaminated with sewage, human excreta. It may cause outbreak of intestinal infections like Typhoid, Dysentery, Cholera, Gastro etc. Coliform bacteria are commonly found in human intestine and considered as indicators of fecal pollution.

The coliform bacteria are able to ferment lactose with production of acid and gas within 24 hours at 37^0 C. Estimation of number of coliforms in water sample is made by adding various quantities of water from 0.1 ml to 10 ml in MacConkey's broth. MacConkey's broth contains pH indicator, which indicates the change in pH in the medium. It also contains Durham's tube for collection of gas.

In this way it is possible to test quality of water samples containing coliform and then to express the degree of contamination from number of tubes showing acid and gas. By referring MacCardy's or Swaroop's table, it is possible to find out number of coliforms present in 100 ml of water sample. After getting the results i.e. coliform count for 100 ml, we can determine the quality of water and say about the potability of water.

Sr. No.	Quality of Water	Coliform Count / 100ml
1	Excellent	0
2	Satisfactory	1 to 3
3	Intermediate	4 to 9
4	Unsatisfactory or Non potable	More than 10

Requirements:

1. Water sample to be tested
2. Sterile MacConkey's single strength broth tubes (10) each containing 5 ml of medium.
3. Sterile MacConkey's double strength broth tubes (5) each containing 10 ml of medium.
4. Sterile pipettes 10 ml and 1 ml capacity.

Note: In double strength medium the ingredients are added in double amount except water. When 10 ml water sample is added in this tube the medium becomes single strength. Therefore double strength medium is used.

Procedure:

1. In the first set of five test tubes of double strength medium, 10 ml of water sample is added in each tube.
2. In second set of five test tubes of single strength medium, 1 ml of water sample is added in each tube.
3. In third set of five test tubes of single strength medium, 0.1 ml of water sample is added in each tube.
4. All these additions are done in sterile condition and after shaking the tubes, they are incubated at 37^{0} C for 24 hours.
5. The number of tubes showing acid and gas production from each set are recorded and statistical estimation of number of coliforms per 100 ml of water is done from the MacCardy's or Swaroop's table.

Observation table:

Set of tubes	1	2	3	4	5
1) 10 ml double strength MacConkey's broth + 10 ml water sample					
2) 5 ml single strength MacConkey's broth + 1 ml water sample					
3) 5 ml single strength MacConkey's broth + 0.1 ml water sample					

Observations:

1) Number of positive tubes (Acid & Gas) in which 10 ml water sample inoculated i.e. Set First _____.

2) Number of positive tubes (Acid & Gas) in which 1 ml water sample inoculated i.e. Set Second_____.

3) Number of positive tubes (Acid & Gas) in which 0.1 ml water sample inoculated i.e. Set Third _____.

Result:

MPN of coliforms per 100 ml water sample is _____.

Conclusion:

1. As the MPN is above 10 / 100 ml, the given water sample is not potable.

2. As the MPN is between 4 to 9 / 100 ml, the given water sample is of intermediate quality.

3. As the MPN is between 1 to 3 / 100 ml, the given water sample is of satisfactory quality.

4. As the MPN is above 0 / 100 ml, the given water sample is of excellent quality.

Remark:

If the MPN is above 10 / 100 ml of water, it should be treated to remove pathogenic microorganisms by different methods like Chlorination, Ozonization, Ultraviolet radiation or boiling.

Expt. No. 2b **Date:**

Bacteriological analysis of water by Standard Plate Count (SPC)

Aim: - To find out the total viable microorganisms in a given water sample by Standard Plate Count (SPC).

Theory / Approach: -

Water provides the nutrients for the growth of variety of microorganisms. These microorganisms can be pathogenic, non-pathogenic and saprophytic. They remain viable and even multiply in the water. The pathogenic microorganisms may cause many serious epidemics if consumed by the human beings and the animals. Therefore it is very essential to analyze the water microbiologically.

SPC is one of the microbiological examination methods. By this method we can count the viable number of microorganisms in the given water sample. The series of dilutions of given water sample is prepared to get the countable colonies.

Advantages of SPC: -

1. It counts viable organisms only.
2. We can identify the organisms present in the food.

Disadvantages of SPC: -

1. For each cell to produce colony, the bacteria would have to be well separated otherwise many cells form single colony and count will be less.
2. All kinds of microorganisms cannot be grown on the supplied medium.
3. Some rapidly growing species inhibit the growth of other species.
4. Method is time consuming, expensive and requires skilled persons.

Requirements: -

1. Sterile nutrient agar
2. Sterile 9 ml saline tubes
3. Sterile empty petri plates
4. Water sample

Procedure: -

1. Different dilutions of given water sample are prepared in saline.
2. 0.1 ml of each dilution is added in sterile empty petri plates aseptically.
3. Sterile molten and cooled at 45 ^{0} C nutrient agar is poured over the sample.
4. Plates are shaken well to mix the sample, allowed to solidify and incubated at 37 ^{0} C for 24 hours.
5. Colonies are counted form each plate and calculations are done.

Observations: -

Sr. No.	**Dilutions (X)**	**No. of colonies (Y)**	**No. of organisms / ml of water sample** **X x Y x 10**
1	10^{-1}		
2	10^{-2}		
3	10^{-3}		
4	10^{-4}		
5	10^{-5}		

Average no. of organisms =

Result: -

Number of organisms in a given water sample is _____ per ml.

Conclusion: -

By this method we can find out number of viable microorganisms from water sample.

Expt. No.3 **Date: -**

Qualitative analysis of water: Presumptive, Confirmed and Completed test

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Aim:

To perform the qualitative analysis of water by Presumptive, Confirmed and Completed test.

Theory:

Bacteriological analysis of water includes the qualitative and quantitative analysis. By qualitative analysis, we can conclude about the presence of coliform bacteria in water. If they are abundant in water sample, this a strong evidence that the intestinal waste have polluted the water. *Escherichia coli* is an indicator bacterium because its survival period is more than other bacteria and the detection of it is easier and less expensive.

Qualitative analysis consists of following three types of tests.

1. Presumptive test
2. Confirmed test
3. Completed test

1. Presumptive test:

In this test selective medium is used. It contains lactose, bile salt (Sodium taurocholate which allow the growth of coliform bacteria). Coliforms ferment lactose into acid and gas. The gas is collected in Durham's tube but sometimes *Staphylococcus aureaus* and *Proteus vulgaris* synergistically ferment lactose to acid and gas. Hence sometimes we get false positive test. Therefore, further confirmation is to be done by confirmed test.

For this test LBB (Lactose Bile Broth), LTB (Lauryl Tryptose Broth) or MacConkey's broth media are used.

Composition of Lactose Bile Broth (LBB)

Distilled water	100 ml
Peptone	1 gm
Lactose	1 gm
Sodium taurocholate	0.5 gm
pH	7

Medium is distributed in the test tubes. Durham's tubes are 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.

2. **Confirmed test**:

If presumptive test is positive, then sample from this is streaked on selective medium like EMB (Eosin Methylene Blue) or Endo agar or sample is added in BGLBB (Brilliant Green Lactose Bile Broth).

EMB agar contains eosin and methylene blue, which give typical characteristics to the colony of E. coli.

Typical colony	**Atypical colony**
1. Small, nucleated	1. Large, nucleated
2. Dark red in colour	2. Pink
3. Well isolated	3. Mucoid
4. With or without metallic sheen	4. Dull
5. Ex. *Escherichia coli*	5. Ex. *Enterobactor aerogenes*

Composition of EMB agar

Distilled water	100 ml
Peptone	1 gm
Lactose	1 gm
Dipotassium Hydrogen Phosphate	0.5 gm
pH	7
Eosin	40 mg
Methylene blue	6 mg
Agar- Agar	2.5 gm

Sterilization at 10 lbs / inch^2 pressure for 40 minutes.

Endo agar contains Basic fuchsin dye, which binds with acetaldehyde which is produced during fermentation of lactose by *E. coli*. Thus the *E. coli* colonies become red coloured while others are colourless.

Composition of Endo agar

Distilled water	100 ml
Peptone	1 gm
Lactose	1 gm
Dipotassium Hydrogen Phosphate	340 mg
Sodium sulphite	250 mg
pH	7 – 7.4
Basic fuchsin	40 mg

Sterilization at 10 lbs / inch^2 pressure for 40 minutes.

Brilliant green in BGLBB inhibits the growth of bacteria other than E. coli.

Composition of BGLBB (Brilliant Green Lactose Bile Broth).

Distilled water	100 ml
Peptone	1 gm
Lactose	1 gm
Sodium taurocholate	0.5 gm
NaCl	0.5 gm
pH	7
Brillinat green	13 mg

Medium is distributed in the test tubes. Durham's tubes are 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.

3. Completed test:

This test is done to test whether *E. coli* in confirmed test can again give a positive presumptive test. Sample from typical colony from EMB agar or red colony from Endo agar or from BGLBB tube is inoculated in LBB or LTB or MacConkey's broth tube. Also one loopful suspension is streaked on nutrient agar slant and incubated at 37 ^{o} C for 24 hours.

If acid and gas is produced, the test is positive and if suspension from slant showing Gram negative, rod shaped, and motile bacteria then test is positive.

A. Presumptive test

Requirements:

1.Sterile LBB or LTB or MacConkey's broth tube

2.Water sample

Procedure:

One ml of given water sample is added with sterile pipette into LBB or LTB or MacConkey's broth tube and incubated at 37 ^{o} C for 24 hours. If gas is observed in Durham's tube, test is positive. If gas is not observed test is negative and further testing is not done.

B) Confirmed test

Requirements:

Sterile Endo agar plate or EMB agar plate or BGLBB tube
Suspension from positive presumptive tube

Procedure:

Loopful suspension from the positive presumptive test is streaked either on Endo or EMB agar plate or one loopful is inoculated in BGLBB tube. Incubation is done at 37^{o} C for 24 hours. If red colour colonies on Endo agar or typical colonies on EMB agar or gas production in BGLBB tube are observed, confirmed test is positive.

C) Completed test

Requirements:

1.Sterile LBB or LTB or MacConkey's broth tube

2.Sterile Nutrient agar slant

Procedure:

The suspension of red colour colony on Endo agar or typical colony on EMB agar or loopful from BGLBB positive tube is inoculated in Sterile LBB or LTB or MacConkey's broth tube and also streaked on Nutrient agar slant. Incubation is done at 37^{o} C for 24 hours.

If gas is produced and suspension from slant is showing Gram negative, rod shaped and motile bacteria then completed test is positive.

Observations:

A) Presumptive test: Gas is observed / not observed in Durham's tube in LBB tube.

B) Confirmed test: On endo agar plate red coloured colonies are observed / not observed and in BGLBB tube gas is observed / not observedin Durham's tube.

C) Completed test: Gas is observed / not observed in Durham's tube of LBB tube and suspension from Nutrient agar slant showed / not showed Gram negative, rod shaped motile bacteria.

Result:

A) Presumptive test: Test is positive / Negative
B) Confirmed test: Test is positive / Negative
C) Completed test: Test is positive / Negative

Conclusion:

A) **Presumptive test:** *E. coli* may be present / absent in the given water sample.

B) **Confirmed test:** Red coloured colonies on Endo agar and gas production in BGLBB tube indicate confirmation of *E. coli* in given water sample.

C) **Completed test:** Here *E. coli* are again giving presumptive test positive and Gram negative, motile, rod shaped bacteria are observed which indicates given water sample is contaminated by fecal matter.

Expt. No. 04 a) **Date: -**

Testing of water & domestic sewage for physicochemical parameters: Measurement of chloride

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Aim:

To measure amount of chlorides in a given water sample.

Theory:

Chloride in drinking water is relatively harmless if present in amounts below 250 ppm. In wastewater chloride content is higher than in raw water. However high chloride content in water bodies harms metallic pipes and structures as well as agricultural crops. Chloride is determined by titration with $AgNO_3$ solution using K_2CrO_4 (Potassium chromate) as an indicator. The end point is indicated by the appearance of a permanent reddish tinge. The method is valid for 0.15 to 10 mg chloride.

Chlorides are precipitated as AgCl (silver chloride) by titrating with $AgNO_3$, K2 CrO_4 (Potassium chromate) being used as an indicator. The addition of single drop of $AgNO_3$ in excess after all the chloride is used up, results in the formation of red Silver Chromate. The solution at this point changes suddenly from pale yellow to orange.

$$NaCl + AgNO_3 \longrightarrow AgCl + NaNO_3$$

$$2AgNO_3 + K_2CrO_4 \longrightarrow Ag_2CrO_4 + 2KNO_3$$

Requirements:

1. **Silver Nitrate ($AgNO_3$) solution 0.02 N**

Dissolve 3.397 gm of $AgNO_3$ in distilled water and dilute to one liter. Store the solution in dark glass bottle.

2. **K_2 CrO_4 (Potassium chromate) indicator**

Dissolve 10 gm of K_2 CrO_4 in about 20 ml of distilled water. Add few drops of $AgNO_3$ solution to produce red precipitate. Let stand it for 12 hours. Filter and dilute the filtrate to one liter with distilled water.

Procedure:

1. Take 10 ml of water sample in a flask and add 5 to 6 drops K_2 CrO_4 (Potassium chromate) indicator. The colour of the sample becomes yellow.

2. Titrate against $AgNO_3$ solution until persistent brick red colour appears.

Calculation:

$$\text{Chloride (mg / L)} = \frac{\text{Volume of titrate X Normality of } AgNO_3 \text{ X } 35.457 \text{ X } 1000}{\text{Volume of sample}}$$

$$= \frac{\text{Volume of titrate X } 0.02 \text{ X } 35.457 \text{ X } 1000}{10}$$

$$= \text{\_\_\_\_\_}$$

Result:

The amount of chlorides in a given water sample is _____ mg / L.

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

Testing (water/ sewage) for physicochemical parameters

BOD (Biological Oxygen Demand)

Aim: - To test biological oxygen demand of sewage.

Theory: - The amount of oxygen required by aerobic microorganisms to decompose the organic matter in a sample of water, such as that polluted by sewage. It is used as a measure of the degree of water pollution. Biological Oxygen Demand (BOD) is one of the most common measures of pollutant organic material in water. BOD indicates the amount of putrescible organic matter present in water. Therefore, a low BOD is an indicator of good quality water, while a high BOD indicates polluted water. Dissolved oxygen (DO) is consumed by bacteria when large amounts of organic matter from sewage or other discharges are present in the water. DO is the actual amount of oxygen available in dissolved form in the water. When the DO drops below a certain level, the life forms in that water are unable to continue at a normal rate. The decrease in the oxygen supply in the water has a negative effect on the fish and other aquatic life. Fish kills and an invasion and growth of certain types of weeds can cause dramatic changes in a stream or other body of water.

For results of the BOD test to be accurate, much care must be taken in the actual process. For example, additional air cannot be introduced. Temperature must be 20°C, which is the usual temperature of bodies of water in nature. A five-day BOD test is used in environmental monitoring. This test is utilized as a means of stating what level of contamination from pollutants is entering a body of water. In other words, this test measures the oxygen requirements of the bacteria and other organisms as they feed upon and bring about the decomposition of organic matter.

The **Winkler test** is used to determine the concentration of dissolved oxygen in water samples. Dissolved Oxygen, abbreviated D.O., is widely used in water quality studies and routine operation of water reclamation facilities.

In the first step, Manganese(II) sulfate (at 48% of the total volume) is added to an environmental water sample. Next, Potassium iodide (15% in potassium hydroxide 70%) is added to create a pinkish-brown precipitate. In the alkaline solution, dissolved oxygen will oxidize manganese(II) ions to the tetravalent state.

$$2\ Mn(OH)_2(s) + O_2(aq) \rightarrow 2\ MnO(OH)_2(s)$$

$MnO(OH)_2$ appears as a brown precipitate. There is some confusion about whether the oxidised manganese is tetravalent or trivalent. Some sources claim that $Mn(OH)_3$ is the brown precipitate, but hydrated MnO_2 may also give the brown colour.

$$4\ Mn(OH)_2(s) + O_2(aq) + 2\ H_2O \rightarrow 4\ Mn(OH)_3(s)$$

The second part of the Winkler test reduces acidifies the solution. The precipitate will dissolve back into solution. The acid facilitates the conversion by the brown, Manganese-containing precipitate of the Iodide ion into elemental Iodine.

The $Mn(SO_4)_2$ formed by the acid converts the iodide ions into iodine, itself being reduced back to manganese(II) ions in an acidic medium.

$$Mn(SO_4)_2 + 2\ I^-(aq) \rightarrow Mn^{2+}(aq) + I_2(aq) + 2\ SO_4^{2-}(aq)$$

Thiosulfate solution is used, with a starch indicator, to titrate the iodine.

$$2\ S_2O_3^{2-}(aq) + I_2 \rightarrow S_4O_6^{2-}(aq) + 2\ I^-(aq)$$

BOD can be calculated by:

- Undiluted: Initial DO - Final DO = BOD
- Diluted: ((Initial DO - Final DO)- BOD of Seed) x Dilution Factor

Requirements

BOD-free water (deionised distilled water passed through activated carbon and re-distilled)

BOD bottles, Erlenmeyer flak

Pipette

BOD incubator

Manganese(II) sulfate

Potassium iodide

Thiosulfate solution

Starch as indicator,

Allylthiourea solution (0.5%)

Procedure

(i) Add 1N acid/1N alkali in the water sample to adjust the pH to 7.0

(ii) Gently transfer this water into BOD bottles so as bubbles should not come out.

(iii) Add 1 ml of allylthiourea to each bottle to avoid nitrification

(iv) Measure dissolved oxygen following the steps as described for dissolved oxygen

(v) Incubate the other BOD bottle at 20°C for 5 days in a BOD incubator.

(vi) Measure the amount of oxygen as done earlier.

Results

Calculate the BOD of water by using the following formula:

BOD (mg/l) = D_1 - D_2

Where, D_1 = Initial dissolved oxygen (mg/l) in the first sample (mg/l)

D_2 = Dissolved oxygen (mg/l) in the second sample after 5 days of incubation

Expt. 05 **Date: -**

Isolation of *E. coli* and identification by IMViC tests

Aim: -

To isolate *E. coli* bacteria form sewage

Approach / Theory: -

E. coli are coliform bacteria of the family *Enterbacteriacae and Genus Escherichia.* These are rod shaped, Gram negative, motile, non-spore forming, noncapsulated, lactose fermenting into acid and gas, and facultative anaerobes. These are normal flora and are more in colon region of small intestine hence are called as coliform.

If they are abundant in water sample, this is strong evidence that the intestinal waste have polluted the water. *Escherichia coli* is an indicator bacterium because its survival period is more than other bacteria and the detection of it is easier and less expensive.

Drinking water may be contaminated with sewage, human excreta. It may cause outbreak of intestinal infections like Typhoid, Dysentery, Cholera, Gastro etc. Coliform bacteria are commonly found in human intestine and considered as indicators of faecal pollution.

For their isolation selective media such as MacConkey's agar, Eosin-Methylene Blue Agar, Endo agar, Brilliant Green Lactose Bile Broth (BGLBB) etc are used.

Endo agar contains Basic fuchsin dye, which binds with acetaldehyde, which is produced during fermentation of lactose by *E. coli.* Thus the *E. coli* colonies become red coloured while other are colourless.

E. coli are Indole positive, Methyl Red positive, Voges Proskauer negative, Citrate negative, Catalse positive, Oxidase negative.

Requirements on first day: -

1. Sewage sample
2. Sterile Endo agar plate

Requirements on second day: -

1. Cavity slide for motility
2. For Gram staining Crystal violet solution, Gram's Iodine, Absolute Ethanol, Saffranin solution.
3. Peptone water for Indol test,
4. Glucose phosphate broth for Methyl red test & Voges Proskauer test.
5. Simmon's citrate medium for citrate test

Requirements on third day: -

1. Kovac's reagent for Indol test
2. Methyl red solution for MR test
3. Alpha –Napthol & KOH solution for VP test
4. H_2O_2 solution (3 %) for catalase test
5. Dimetyl-p-phenylenediaminehydrochloride 1% solution for oxidase test

Composition of various media and reagents

1. Endo agar

Distilled water	100 ml
Peptone	1 gm
Lactose	1 gm
Sodium sulphite	250 mg
pH	7 – 7.4
Basic fuchsin	40 mg

Sterilization at 10 lbs / inch^2 pressure for 40 minutes.

2. Peptone water

Water	100 ml
Peptone	4 gm
pH	7

3. Glucose phosphate broth (GPB)

Distilled Water	100 ml
Peptone	1 gm
Glucose	0.5 gm
Potassium phosphate	0.5 gm
pH	7

4. Citrate medium

Water	100 ml
Sodium citrate	0.2 gm
$MgSO_4$	20 mg
$(NH_4)H_2PO_4$	100 mg
K_2HPO_4	100 mg
NaCl	50 mg
Bromothymol blue	8 mg
pH	7

5. Kovac's reagent

p- dimethylaminobenzaldehyde	5 gm
Isoamyl alcohol	50 ml
HCl	25 ml

6. Methyl red solution

95% Ethanol	100 ml
Methyl red	35 mg

7. Alpha napthol

Absolute ethanol	100 ml
Alpha napthol	5 gm

8. KOH solution

Water	100ml
KOH	40 gm

Procedure: -

1. Sewage sample is streaked on sterile Endo agar plate and plate is incubated at 37 ^{0} C for 24 hours.

2. Colony characters of red coloured colonies are studied and suspension of one colony is prepared in saline.

3. Motility and Gram staining is done.

4. Loopful of suspension is inoculated in peptone water tube, Glucose phosphate broth tube and Simmon's Citrate medium and all tubes are incubated at 37 ^{0} C for 24 hours.

5. **Indole test: -** 0.5 ml of Kovac's reagent is added to incubated peptone water tube.

6. **MR test: -** Incubated GPB is distributed into two tubes. One tube is used for MR test and other for VP test. For MR test, 5 drops of methyl red indicator is added.

7. **VP test: -** 0.6 ml of Alpha napthol is added in another GPB tube and 0.2 ml of KOH reagent is added to it.

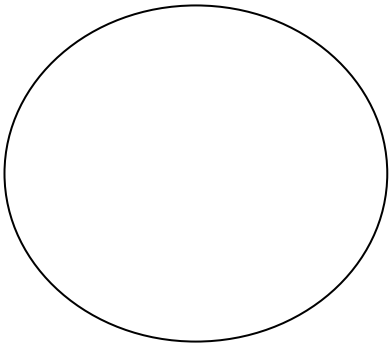
8. **Catalase test: -** Part of colony is dipped into 3% H_2O_2 solution with the help of nichrome wire loop and observed for effervescences of oxygen bubbles.

9. **Oxidase test: -** Part of colony is rubbed on a filter paper dipped in Dimetyl -p- phenylene diamine hydrochloride and observed for formation of purple colour.

Observations: -

1. Colony characters: -

Colony characters	*Colony - 1*
Size	
Shape	
Colour	
Margin	
Elevation	
Opacity	
Consistency	
Gram nature	
Motility	



2. Indole test: - Red coloured band is appeared/not appeared at the junction of medium and reagent.

3. MR test: - Red colour is observed/ not observed.

4. VP test: - Pink red colour is observed / faint brown or no colour is observed.

5. Citrate test: - Blue colour is observed / not observed.

6. Catalase test: - Effervescences of O_2 gas observed / not observed.

7. Oxidase test: - Purple colour is observed / not observed.

Result: -

1. Gram negative, rod shaped, motile bacteria are observed / not observed.
2. Indole test is positive / negative.
3. MR test is positive / negative.
4. VP test is positive / negative.
5. Citrate test is positive / negative.
6. Catalase test is positive / negative.
7. Oxidase test is positive / negative.

Conclusion: -

From the colony, morphological, biochemical characteristics, the isolated bacterium is *E. coli.*

Expt. No. 06 **Date: -**

Isolation of coliphages from sewage sample

Aim: -

To isolate coliphages from sewage sample.

Theory: -

Phages are the viruses that attack microorganisms. They are noncellular, obligate intracellular parasites, always growing in association with its respective host. The phages have host specificity and are named on the basis of their host type. The phages that attack actinomycetes are called actinophages, that attack on algae are called cyanophages, that attack on bacteria are called bacteriophages. The viruses that attack on coliform bacteria are called coliphages. The phages are of great industrial importance because of their ability to destroy desired culture used for product formation. The destruction of the culture or its mutation by transduction results into either decrease or loss of product or decrease the quality of product.

Coliphages can be detected and counted by using phage plaque technique. The sewage sample is inoculated along with a fresh active culture of host (E. coli) and the plate showing inhibition of host are detected. Palques are round clear zones lacking the visible growth.

Requirements: -

1. Bacteria free sewage sample
2. Actively growing E. coli culture in MacConkey's broth
3. MacConkey's agar paltes
4. MacConkey's butts

Procedure: -

The sewage sample is made bacteria free either by filtering through bacteriological filter or by treatment with chloroform. 10 ml sewage sample is mixed with 0.2 ml of chloroform and allowed to act for 15 minutes. The chloroform is evaporated by keeping the sample in oven or water bath adjusted at 45^{0} C.

1 ml of bacteria free sewage sample and 1 ml of fresh culture of E. coli is added to 5 ml of melted and cooled at 45^{0} C MacConkey's agar in a test tube. This mixture is shaken thoroughly and poured on sterile MacConkey's agar

plate. The plate is then incubated at 37^0 C for 24 hours and then observed for plaque formation.

Observation: -

Plaques are observed / not observed.

Result: -

Coliphages are present / absent in given sewage sample.

Conclusion: -

Bacteriophages exist in nature in association with their respective host. The coliforms generally present in sewage sample are from fecal matter of animals. The coliphages are also associated with them. The bacteriophages grow intracellularly causing the death of host bacteria which is observed on the pl ate as a plaque.

Expt. No. 07 **Date: -**

Isolation of enteric pathogens from domestic sewage
Isolation of *Salmonella species*

Aim: To study the morphological, cultural and biochemical study of ***Salmonella*** species.

Introduction: The genus ***Salmonella*** consists of Gram negative bacilli that parasitise the intestine of man (and large number of vertebrate species) leading to gastroenteritis, enteric fever, septicemia and the carrier state. The important member of the genus is ***Salmonella typhi***, which causes typhoid fever. Salmonellae comprise over 1800 species. All are potentially pathogenic. They may be divided into (1) Enteric fever group (2) The food poisoning group. ***S.typhi, S. paratyphi*** **A** and usually ***S. paratyphi*** **B** are confined to man. ***Salmonella typhimurium*** is common food poisoning salmonellae.

Clinical significance

● ***Salmonella typhi***

This species causes typhoid fever. The reticuloendothelial system, gallbladder and kidneys become infected when the bacteria pass from the small intestine into blood through lymphatic system. These organisms invade the intestine causing inflammationa and ulceration. Symptoms of the infection include fever, headache, toxemia, enlargement of spleen and low pulse rate.

S. tphi and S. paratyphi B produce black coloured colonies due to production of H_2S, while S.typhi produce green coloured colonies due to non ability to produce H_2S.

Requirements:

1) Blood or stool sample
2) Sterile Wilson and Blair's medium plate
   Composition of the medium: -

Solution A: - Bismuth sulphite Glucose Phosphate mixture

D. Water	100 ml
Bismuth Ammonio citrate	3 gm
Sodium sulphite	10 gm
Na_2HPO_4 . 12 H_2O	10 gm
Glucose	5 gm

Dissolve Bismuth Ammonio citrate in 25 ml boiling water and

Sodium sulphite in 50 ml boiling water. Mix two solutions and boil. Add the Disodium phosphate crystals while boiling the mixture. Cool. Add the glucose dissolved in 25 ml boiling water and cooled.

Solution B: - Iron Citrate Brilliant Green Mixture

Ferric Citrate solution	200 ml
(1% in sterile D/W)	
Brilliant Green solution	25 ml
(1% in sterile D/W)	
Mix together	

Complete Medium: -

Sterile Nutrient Agar	100 ml
Solution A	20 ml
Solution B	4.5 ml

5. Biochemical media

a. Glucose Phosphate Broth

d. Tryptone water

e. Koser's citrate medium

f. Christensen's Urea medium

g. Sugars: Xylose, Glucose, Lactose, Mannitol, Sucrose, Maltose,

6. Reagents:

a. Oxidase reagent

b. Hydrogen peroxide

c. Methyl Red

d. Kovac's reagent

e. α-Naphthol reagent

f. Sulphanilic acid

Procedure:

1) Streak a loop-full of the sample on Wilson and Blair's medium Agar.

4) Keep in the incubator at 35^{o}C for 24 hrs.

5) Observe for colony characters form the plates.

6) Perform Gram staining and motility.

7) Inoculate various biochemical media from a single colony of the organisms.

7) Keep in the incubator at 35^{o}C for 24 hrs.

8) Read the results.

Expected observations

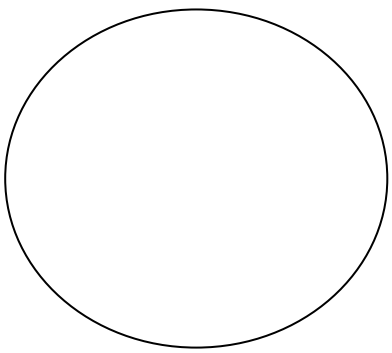
(1) Gram staining : Gram negative bacilli

(2) Motility : Motile organisms

(3) Cultural characters (colony characters)

● **Organisms**

Size		
Shape		
Colour		
Margin		
Elevation		
Opacity		
Consistancy		
Gram nature		



Biochemical reactions:

	S. typhi reactions	**S. paratyphi A reactions**	**S. paratyphi B. reactions**
Oxidase	-	-	-
Urease	-	-	-
TSI	Acid butt and alkaline slant and little H_2S	Acid butt and alkaline slant and no H_2S	Acid butt and alkaline slant and excessive H_2S
Indol	-	-	-
Methyl Red	+	+	+
Vogus Prauskauer	-	-	-
Citrate	-	-	Different stains gives different result
Nitrate	+	+	+
Gelatin	-	-	-

Sugar fermentation test:

	S. typhi reactions	S. paratyphi A reactions	S. paratyphi B. reactions
Xylose	+ Acid	-	+Acid, Gas
Glucose	+ Acid	+Acid, +Gas	+Acid, Gas
Sucrose	-	-	-
Maltose	-	-	-
Lactose	-	-	-
Mannitol	+	+ Acid	+Acid, Gas

Result & Conclusion: -From the cultural, morphological and biochemical characters the isolated bacterium is *Salmonella typhi / Salmonella paratyphi A / Salmonella paratyphi B.*