Semester – V

15MBU511 APPLICATION ORIENTED PRACTICAL – V 5H – 3C

Instruction Hours / week: L: 0 T: 0 P: 5 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 9 Hours

SCOPE

Acquire knowledge to identify the common infectious agents with the help of laboratory procedures and use antimicrobial sensitivity tests to select suitable antimicrobial agents.To ensure Safety in a food and agricultural microbiology laboratory is important in the prevention of contamination that might be caused by the microorganisms being studied.

OBJECTIVES

To provide an understanding of the natural history of infectious diseases in order to deal with the etiology, laboratory diagnosis, treatment and control of infections in the community. To support the need based technique to develop entrepreneur skills.

1. Analysis of Blood grouping and Rh typing
2. Performance of WIDAL – slide and tube test
3. Identification of antibodies by Passive agglutination – RPR, ASO, RA and CRP
4. Ouchterlony's double immuno diffusion
5. Enumeration of leucocytes by Leishmann stain
6. Isolation of coliphages from sewage sample.
7. Demonstration of Cultivation of virus using embryonated egg.
8. Assessment of milk quality by MBRT
9. Identification of Bacteria and Fungus from spoiled food and vegetables.
10. Bacteriology analysis of food samples (TPC)
11. Isolation of free living and symbiotic organisms
12. Cultivation of Mushroom
13. Isolation of micro-organisms from air
14. Bacteriological examination of water by MPN test.
15. To determine dissolved oxygen of water.
16. To determine BOD
17. To determine COD
18. Demonstration of waste water treatment plant.

REFERENCES

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APPLICATION ORIENTED PRACTICAL – V (15MBU511)

Experiment No. 1
ANALYSIS OF BLOOD GROUPING AND RH TYPING

INTRODUCTION

In 1900, Karl Landsteiner discovered four different kinds of human blood group with respect to the presence or absence of agglutinogens. The four different groups are known as A, B, AB and O. Among these O group is characterized by the absence of A and B agglutinogens. Landsteiner and Weinner first reported about Rh factor. The D factor is responsible for the Rh positive conditions.

AIM

To determine the blood group of given sample.

PRINCIPLE

Blood typing is performed with anti sera containing high titre of anti A, anti B, anti D agglutinans. This test depends on the agglutination reaction with specific antigen and antibody present.

Agglutination occurs in the suspension to which anti A was added, the blood type A contains A antigen and B antibody. So anti A added will react with A antigen and produce agglutination. B group individuals contain B antigen and A antibody. The blood type AB and O groups do not have any antigens but only antibodies. The absence of agglutination in both the mixtures indicates the blood type O. The presence or absence of agglutination in anti D mixture represent the positive or negative for Rh factor respectively.

MATERIALS REQUIRED

Anti A, Anti B, Anti D, clean glass slide, micropipette, blood sample, dropper, applicator stick.

PROCEDURE

1. 3 clean glass slide were taken and labelled as anti A, anti B and anti D.
2. A drop of blood was added to each slide to that drop of anti A, anti B anti D serum was added using a dropper.
3. This mixture was mixed thoroughly using an applicator stick.
4. Presence or absence of agglutination was recorded.
5. The same procedure was performed with other blood samples.

RESULT

The given blood samples were identified and the results were tabulated.

Experiment No. 2
WIDAL SLIDE AND TUBE TEST

INTRODUCTION

In enteric fever specific agglutinans are usually detectable in patients blood after 7days of fever. Serum from individuals vaccinated with tab may also show moderately elevated titre of all 34 agglutinans. Salmonella agglutinating agents are used to detect, identify, quantify specific antibodies in serum sample from patient suffering from pyrexia of undetermined origin.

AIM

Invitro detection and quantitative estimation of specific antibodies present in serum by rapid slide and conventional test by tubes for enteric fever.

PRINCIPLE

Antibodies in serum produced in response to exposure to Salmonella will agglutinate bacterial suspension which carry homologous antigens.

MATERIALS REQUIRED

Glass slide and tube, Salmonella typhi H antigen, O antigen, Salmonella paratyphiA(H), B(H) antigens, positive control serum.

SAMPLE

Fresh serum or serum stored at 28 degree celciusincase of any delay. It should be cleaned, it should not be heated or inactivated.

RAPID SLIDE TEST(QUALITATIVE)

1.Clean the glass slide and make it free of water. Place one drop of undiluted serum in each of the first four circles and one drop of positive control serum in each of the last 2circles

2. Place one drop of antigen O,H.A(H),B(H) in the circles 1,2,3,4 respectively and O antigen in circle5.

3. Any one of the H antigen in the circle 6

4. Mix the content of the circle with the separate stick and rotate the slide for one minute and observe for agglutination.

5. If the agglutination is visible within one minute proceed for quantitative slide test or tube test for the estimation of the titre of appropriate antibody.

QUANTITATIVE SLIDE TEST(SEMI QUANTITATIVE)

1. Clean the glass slide and make it free of water.
2. 0.005 ml, 0.01 ml, 0.02 ml and 0.04 ml, 0.08 ml of undiluted serum is added to 1,2,3,4 and 5 circles respectively and add 1 drop of antigen suspension which showed agglutination in the screening test.
3. Mix the contents of each circle and rotate the slide slowly and observe for agglutination.

TUBE TEST(QUANTITATIVE)

1. Take a set of 8 clean dry test tubes.
2. Dilute each serum sample and set up the test as per the table.
3. Mix well and incubate the tube at 37 degree celcius for 16-20 hrs and examine for agglutination.

OBSERVATION

Agglutination was observed in the serum of the patient after reaction with ‘O’ antigen, ‘H’ antigen and no agglutination was observed in ‘AH’ and ‘BH’ antigen.

The agglutination seen within the serum of the patient when reacting with ‘O’ antigen is seen as chalk powder like deposit and the colony forms agglutination when the corresponding antibodies react with H antigen. No agglutination was seen in ‘AH’ and ‘BH’ antigen.

RESULT

SLIDE TEST:

The patient is diagnosed to be infected with Salmonella typhi and hence is suffering from typhoid fever and not paratyphoid fever.

TUBE TEST:

The patient is diagnosed to be suffering from typhoid fever because the antibody titre is 1:240 which is more than the significant amount of antibody titre.

DISCUSSION

The antibodies were produced in patients body because of the presence of Salmonella typhi which triggers the immune system to produce the corresponding antibodies.

The patient is said to be suffering from typhoid fever and not parartyphoid fever A and B because the antibody titre of O, H antigen is 1:240 which is significant.

The typhoid and parartyphoid fever are transmitted through fecally contaminated food and water. The incubation period is around 3-4 days and may even extended upto 10 days. The initial titre is increased the agglutination titre depends upon the stage of the disease. A simultaneous and moderate rise in the titre value of the antigen 'O','H', and 'AH', 'BH' suggestive of recent TAB vaccination.

Experiment No. 3

RAPID PLASMA REAGIN TEST

AIM

To perform a rapid screening procedure for the diagnosis of syphilis.

PRINCIPLE

Syphilis is a sexual disease caused by Treponema pallidum a spirochete. It is slightly coiled, highly motile organism that quickly loses viability outside the human body. It can be grown in rabbit tissuse culture or rabbit testis. Syphilis is both a systemic and congenital infection which mainly spreads through the sexual contact and from infected mother to child.

Syphilis if untreated spreads through three clinical stages 1. The first stage or primary syphilis is characterized by formation of painless papule at the site of infection 2. The secondary syphilis represents the extension of the infection and the formation of rash, malaise and lymphadenopathy. 3. Following this stage the disease become self limiting and the patient appears asymptomatic until the development of tertiary syphilis. In the final stage life threatningcompliacteins may develop as the result of extensive cardiovascular and nervous tissue damage.

After Treponema paliidum infection, two main tyoes of antibodies are produced in the host 1. Reaginantibodies 2. Treponemal antibodies.

The rapid plasma regain serological test is based on the conditional relationship between the lipid extracts of tissue that is the cardio lipin cholesterol lecithin antigen non-specific antibody called reagin that develops in the serum of the infected person. The regain appears two weeks of the post infection uin the patients plasma and will perisist at high concentration until the dieaese is eradicated.

In the Rapid plasma regain test blood serum of a patient is mixed with solubke antigen and two carbon particles within a circle on the diagnostic card. If the regain is present in the blood it will react with the antigen producing macroscopically visible black agglutination reaction due to the formation of antigen antibody complex false positive results may be obtained because normal persons can have regain in the serum.

MATERIALS REQUIRED

Syphilic serum (RPR kit), non syphilitic serum, patient serum, applicator stick.

PROCEDURE

1. The disposable test card with circles to lod the sample which was provided along with the kit was taken.
2. 0.5ml of the positive control, negative control and the sample were placed in the separate circle using a capillary pipette and mark respectively.
3. When the serum was spread around the circle using applicator stick.
4. One drop of antigen was added to the circles using antigenic stopper.
5. The card was then placed on a VDRL shaker which was set at 100 rpm for 8minutes and read the results macroscopically as black precipitate.

OBSERVATION

The card was observed by tilting back and forth the presence or absence of black clumps in each of the serum antigen.

RESULT

The black agglutination was observed which indicated that the patient is infected by Treponema pallidum (ie) syphilis positive.

DISCUSSION

In the serum sample if there is reactive regain antibodies it will produce antigen antibody complexes which produce a macroscopically visible black agglutination reaction giving positive result.

In the serum if there is no non specific regain antibodies so it will not react with the carbon particles and so there is absence of agglutination reaction giving rise to uniform suspension which is an indicator of negative result.

ANTISTERPTOLYSIN O TEST

INTRODUCTION

The group 2 beta haemolytic streptococci produces various toxins that can act as antigens. One of these exotoxins that is streptolysin 'O' was discovered by Tott in 1932.

A person infected with the group alpha, beta haemolytic streptococci produces specific antibodies against the exotoxins. One of which is antistreptolysin 'O'. The quality of this antibody in a patients serum will establish the degree of infection due to beta haemolytic streptococci.

The usual procedure for the determination of antistreptolysin titre is based on the inhibitory effect of the patients serum produces on the haemolytic power of pretitred and reduced streptolysin 'O'.

However the antigen antibody reaction occurs independently of the haemolytic activity of streptolysin 'O'. this property enabkes the establishment of a qualitative test for the determination of Anti streptolysin 'O' by the agglutination of latex particls on the slide.

AIM

To determine the qualitative measurement of antibody through streptococcal exoenzymes in human serum.

PRINCIPLE

The ASO reagent contains the latex particles coated with streptolysin 'O' antigen. When the reagent is mixed with the serum containing ASO at alevel greater than the 200 IU/ML the particles will agglutinate.

MATERIALS REQUIRED

SAMPLE : Patients serum

REAGENTS : REAGENT 1 – ASO latex reagent
REAGENT 2 – Positive control
REAGENT 3 – Negative control
Slides,stirrer,dropper.

PROCEDURE

QUALITATIVE TEST:

1. Serum was collected from the patients blood by centrifugation.
2. One drop of serum, positive control and negative control was added within the respective circle on the slide. Similarly ASO latex reagent was added to each ring of the slides.
3. Then the slide was rotated for 2 minutes and observed for agglutination.

OBSERVATION

Agglutination reaction occurs. Its due to the formation of an insoluble immune complex by cross linking of cells on particles.

RESULT

Agglutination indicates as ASO concentration is above 200IU/ML. The patient is positive for ASO.

DISCUSSION

Agglutination results from the reaction of the patients serum with streptolysin O, antibodies which has reacted the antistreptolysin O latex antigen

Hence the patient serum shows the positive for ASO titre, H is diagnosed that the patient is suffering from streptococcal infection.

RHEUMATOID FACTOR

INTRODUCTION

Rheumatoid arthritis is an autoimmune disorder, where autoantibodies are produced against self antigens (IgG). The autoantibodies are termed as "Rheumatoid factor", which are immunoglobulins of predominantly IgM class, which combines with Fc portion of immunoglobulin IgG molecules. RF is present in sera of 80% cases of rheumatoid arthritis.

The idea of using inert particles coated with gammaglobulin to detect rheumatoid factor was developed from the sheep well agglutination tests like Rose waaler test. Suspension of stabilized polystyrene particles of uniform size coated with gamma globulin are extensively used in currently available qualitative or semiquantitative slide agglutination tests and quantitative immunoturbidometric and nephelometric assays. Most of these are based on the original work of Singer and Plotz.

PRINCIPLE

Rheumatoid factor latex test kit consists of polystyrene latex particles coated with specifically modified preparation of human gammaglobulin (IgG) inorder to avoid non specific agglutination. The suspension of coated latex particles visible when mixed with serum containing Rheumatoid factor. The sensitivity of the reagent has been adjusted to detect = 10IU/ML of RF calibrated against an international standard.

SAMPLE

No special preparation of the patient is required prior to sample collection. Serum must be used because fibrinogen in plasma may give non specific agglutination of the latex gammaglobulin reagent. The serum sample should be stored at +2 to +8 degree celcius after collection. It can be stored best at -20 degree celcius if prolonged storage is desired. Inactivation of the serum is not necessary.

REAGENTS AND ACCESSORIES

REAGENTS :

REAGENT 1 : latex gammaglobulin reagent, a uniform suspension of polystyrene latex particles coated specifically modified preparation of human agglutination.

REAGENT 2 : positive control serum

REAGENT 3 : negative control serum

ACCESSORIES

Glass slide, disposable applicator stick, disposable plastic dropper, rubber teasts.

PROCEDURE

A. QUALITATIVE TEST

1. Bring all the reagemts as well as the samples at room temperature
2. Using the disposable plastic dropper, place one drop of test serum within the circled area marked test on the special slide provided in the kit.
3. Shake the vial gently add one drop of the latex gamma globulin reagent to the above drop and mix well with the disposable applicator stick.
4. Rock the slide gently to and fro two minutes and examine for agglutination. Do not examine beyond 2minutes.

NOTES

1. For positive and negative controls, follow the same procedure as mentioned above by taking control serum from respective vials.
2. Controls are not to be diluted

RESULTS

Results should be read within 2minutes. Clearly visible agglutination indicates a positive result. Granularity of doubtful clumping should be ignored.

Experiment No. 4

PREPARATION OF OUTCHERLONY'S DOUBLE IMMUNO DIFFUSION TECHNIQUES

INTRODUCTION

In the immune system, an antigen will react only with its specific antibody because of the principle either antibody can be used to identify antigen or an antigen can be used to identify the antibody in patient serum. These characteristics is bases of all serodiagnostic procedure.

AIM

To determine the qualitative reaction which depends upon the interaction between the antigen and antibody.To detect the presence of a specific antigen or specific antibody.

PRINCIPLE

When the soluble antigen is mixed with the patient's serum, precipitation occurs. Precipitation due to the cross linking of antibodies in various amounts with the antigen to form visible aggregates.

The double diffusion agar assay is based on the principle that diffusionof both the antibody antigen through agar can form stable and observable immune complexes.

The complete confluent line formed indicates the immunological reaction between antigens. A Y shape spur indicates particularly located antigens formation of X shape or straight line or two separate lines of precipitation indicates the unrelated or non identical antigenic determinant.

MATERIALS REQUIRED

Slides, agarose, beaker, test serum, antigens.

PROCEDURE

1. A Clean slide was coated with a thin layer of 1%agar and was allowed to solidify.
2. A central well was made using the 3mm gel puncture

3. Around the central well 4 equidistant wells were in a concentric fusion with the distance between the central and peripheral wells. Not being greater than twice the diameter of the well.
4. Add 10microlitre of test serum in central well.
5. Add 10 microlitre of antisera (A1,A2,B1,B2) in the surrounding wells.
6. After incubation at 37 degree celcius the slides were examined for precipitation line in reflected light to detect capacity in the gel.

OBSERVATION

A confluent line of precipitation was seen in the zone of equivalent between the cells A1, A2 and the central well.

RESULT

The line of precipitation indicates that the antibodies in the test serum bind to the same antigenic determination in A1, A2. Thus A1and A2 antigenic sample have similar antigenic determinant.

DISCUSSION

The test solutions added to the separate wells meet due to the outward diffusion combine and precipitate forming an indicator.

At the zone of equivalent optimal proportion of antigen and antibody combines and hence the precipitation is maximal.

Experiment No. 5
ENUMERATION OF LEUCOCYTES

AIM:

To determine the differential count of various white blood cells (leucocytes) from given blood sample.

PRINCIPLE:

Examining the stained blood smear us a routine part of complex blood count. The procedure of studying the blood smear comparises of preparing a blood smear on microscopic slide which dried, fixed and stained. Study of blood smear helps in the diagnosis of various anaemias, leukemias and infection of dead parasites.

Differential count isa distribution of various white cells in the peripheral blood as determined in a blood smear attained with polychromatic stain (Leishman stain, wright's stain). Differential count is vital for the diagnosis of number of blood related disorders involving either red cells or white cells. Clinical terms in specific like (neutophilia, esonophilia, lymphocytes, monocytes), or their fall (neutropenia) are detected on differential count.

Three major steps involved in differential count

1) Preparation of smear
2) Staining of smear
3) Microscopic examination

   The smear is directly taken from the skin puncture which gives the true picture of blood morphology, but anticoagulated venous blood is satisfactory if smears are made within 2-2 hours of collection. The staining is done with a polychromatic stain i.e. includes methylene blue and eosin in its preparation. The polychromic stain induces multicolor when applied to cells. The cells are dissolved in methanol, which acts as a fixative and a solvent. The blood smear should be made within 4 hrs after the collection and if mixed with ethanol, if can be preserved at room temperature for a long period. Following staining basic components of white blood cells are stained by acidic eosin dye and they are described as acidophilic or basophilic, while the acidic components of cells take blue to purple shades by the basic methylene blue and they are called basophils. The neutral components of cells are probably stained by both of the dyes.

   The normal vaue of neutrophils in blood is 40-75%, esonophils 1-6%, basophils 1%, lymphocytes 20-45% and monocytes 2-10 %. Various types of white cells are identified on the basis of the following characters.

GRANULOCYTES:

These are cells with granulated cytoplasm which stains fade pink. These include neutrophils, esonophilis and basophilis.

NEUTROPHILIS:

Pale pink cytoplasm with fine granules include band and segmented forms (lobes) normally 3-4 lobes

ESONOPHILIS:

Cytoplasm stains fade pink and contains large rod and orange red granules.

BASOPHILIS:

Cytoplasmic granules, large dark and blue black which fill the cell.

LYMOHOCYTES:

Large sized, lymphocytes have clear blue cytoplasm on the margin of nucleus, in smaller lymphocytes.

MATERIALS REQUIRED:

Clean grease free glass slides, staining rack, spreader, slide, capillary tube or applicator stick.

REAGENTS:

Leishman stain- buffered water, disodium hydrogen phosphate (3.76gm), potassium dihydrogen phosphate (2.10gm), distilled water- 1000ml

PROCEDURE:

1. Transfer a drop of well mixed blood specimen (anticoagulated venous blood) within a pair of applicator stick to a clean grease free slide, approximately from the end.
2. With the thumb and index finger of the left hand hold 2 edges of a slide. With the right hand place the spreader slide just in front of a drop of blood such thet angle between 2 slides is approximately 30-45ºC. push the spreader to the end of the slide with a smooth quick movement.
3. Stain the smear with Leishman satin for 2 minutes.
4. Add buffered water of about doubled the volume of stain, allow the stain for 5-7 minutes.
5. Wash the slide using running tap water, air dry and observed under oil immersion objective

Experiment No. 6

ISOLATION AND TITRATION OF COLIPHAGES FROM SEWAGE SAMPLE

AIM

To isolate virulent coliphages from sewage.

INTRODUCTION

The virus infecting bacteria are called bacteriophages. Like other viruses, phages are also host specific multiplying in either of two cycles. In lytic cycles of the phages, the virus multiplying within the host which is lysed at the end of the infection cycle to release several hundreds of progeny particles.

In the lysogenic cycle the phages genome integrate within the bacterial genome and it is transmitted to the daughter cell as a part of the bacterial genome.

PRINCIPLE

Bacteriophages are widely distributed in nature. Sewage contains a large number of coliforms, it is bound to contain coliphages. The lysis of bacterial cells by phages can be demonstrated in the laboratory by the formation of the clear zone of lysis in the bacterial lawn culture on agar medium. The clear zone of lysis is called plaque and are due to disruption of bacterial cells by their phages. Choloroform is active against the host bacterial cell and dissolves the lipopolysaccharide and lipid bilayer of the cytoplasmic membrane. Some viruses are made up of protein and nucleic acids, they remain unaffected by choloroform. Chloroform is also a high density solvent, which can settle down easily during centrifugation leaving the phage particles in the supernatant.

MATERIALS REQUIRED

CULTURES : 24 hrs broth culture of E.coli, sewage sample.

MEDIA :1. 5ml tube of bacteriophage nutrient broth.

2.Tryptone agar plate

3. 3ml of tryptone soft agar tubes

EQUIPMENT : sterile centrifuge tubes, petriplates, Whatmann no.1 filter paper, funnel, sterile Eppendroff's tubes.

PROCEDURE

1. Aseptically add 5ml of bacteriophage nutrient broth, 5ml of E.coli broth culture and 45 ml of sewage to 250 ml beaker.
2. The sewage sample was filtered through a whatmann no.1 filter paper.
3. The filterate is collected in a sterile test tube.
4. Equal volume of chloroform is added to this and vortexing it to lyse the bacteria.
5. The homogenate was then centrifuged in centrifugal tubes at 500rpm for 10 minutes.
6. Pour the supernatant solution through the sterile membrane filter apparatus to collect the bacteria free phage containing filterate in the vacuum flask'
7. Melt the soft tryptone agar by placing the tubes in a boiling water bath and cool to 45 degree celcius.
8. Label the tryptone agar plates and tryptone tubes accordingly.
9. Using a sterile 1ml pippete add 0.1ml of the E.coli culture to all the molten soft agar tubes.
10. Using a sterile Pasteur pippete add 1,2,3,4 and 5 drops of filterate to the respectively labelled soft agar tubes.

11. Mix and pour each tubs of soft agar into its approximately labelled agar plates.
12. Allow agar to harden.
13. Incubate all the plates in an inverted position for 24 hrs at 37degree celcius.
14. The number of plaques in the plates are counted and the phage in the original samples are expressed as plaque forming units.

RESULT

Plaques are formed after 24hrs of incubation at 37 degree celcius. This indicates the presence of coliphages in the sewage.

Experiment No. 7

ANALYSIS OF MILK BY METHYLENE BLUE REDUCTION TEST (MBRT)

AIM:

To determine the methylene blue reduction time of the given milk sample to check the quantity of milk.

PRINCIPLE:

Milk is good medium for the growth of microorganisms. A variety of microorganisms can be found in both raw milk and pasteurized milk. These are actively growing microorganisms. Normally the milk is contaminated with microorganism such as *S.aureus, S. pyogens, .aeuroginosa, Enterobacter spp., Bacillus spp., etc,.* Contaminated milk is one of the important sources for transmission of diseases from animals to humans. The main reason for this contamination is the unproper handling of milk. Normally milk is contaminated during

the milking process by the animal, such as udder and adjacent areas unsterilized diary utensils such as milking machines. Milk is also a good source of contamination of microorganisms.

The principle of methylene blue reduction test depends on the fact that the colour imparted to the milk by adding a dye such as methylene will disappear more or less quickly, which depends on the quality of the milk sample to be examined. Methylene blue is a redox indicator, that lose its colour under the absence of oxygen and is though to be reduced. The depletion of oxygen in the milk is due to the expanded rate of bacterial metabolism. The dye reduction time refers to the microbial load, in the milk and the total metabolic reactions of the microorganisms.

MATERIALS REQUIRED:

- ⇨ SAMPLE: milk
- ⇨ REAGENTS: methylene blue indicator.
- ⇨ SOLUTION: 0.1 mg of methylene blue solution was atken and mixed in 250 ml distilled water 1:25000 dilution.
- ⇨ GLASSWARE AND EQUIPMENT: screw capped tubes, beaker, pipette, water bath, autoclave, hot air oven.

PROCEDURE:

1. Sterilized screw capped tubes, were taken and 10 ml of the given milk sample was transferred into each test tube and to that 1 ml of methylene blue dye was added.
2. A thin foil was placed and gently inverted to mix (avoid air bubbles).
3. The tubes were placed in the water bath at 37ºC and checked at intervals of every 5 minutes.
4. The time for the dye reduction to occur gives a clear picture about the number of organism present in the sample which is assumed with the help of a standard chart.

Experiment No. 8

IDENTIFICATION OF BACTERIA AND FUNGUS FROM SPOILED FOOD AND VEGETABLES

AIM:

To analyse and distinguish the types of spoilage caused by microorganisms to bread and vegetables.

PRINCIPLE

Foods are especially susceptible to microbial decomposition because they are rich in nutrients that support the growth of microbes contaminated foods are often unedible and may also be the source of human disease if the contaminating organism are pathogens or toxin produces some of the important foods poisons caused by bacteria are botulism(caused due to clostridium

botulism), staphylococcal(staphylococcus aureus) and enteric(E-coli) and salmonella typhymenium. There food preserving are serious and sometimes fatal.

MICROBIAL SPOILAGE OF FOOD:

Moulds are the most common cause for spoilage of food. The temperature attained in the banking procedure usually are high enough to kill the air during cooking from handling or from wrapper and usually initiate growth in the crease of the leaf and between the slice of the bread. Chief moulds involved in the spoilage of bread are the bread moulds, Rhizopus, stolonifer, other moulds are penicillium expansum or penicillium stoloniferum, Aspergillus niger etc. Ropiness of bread is common in home baked breads, especially during hot weather. It may be cause by bacterial species namely Bacillus or Bacillus litheniforms.

MICROBIAL SPOILAGE OF VEGETABLES:

Microbial spoilage of vegetables may be due to plant pathogens saprophyte. Organisms, which may be secondary invaders and may enter a healthy vegetables in the case of various rots and grows on its surface Bacterial spoilage is commonly encountered in most piled vegetables.

MATERIALS REQUIRED:

Sample: Spoiled Bread and Vegetables.

Medium: Nutrient agar medium for bacterial isolation, peptone broth, malt extract agar for fungal and mold isolation.

Equipments: Sterile petridishes, inoculation loop, Bunsen burner, s sterile pipettes and pestles etc.

PROCEDURE:

1. About 20gm of food samples to be tested was taken and placed in a clean mortor.
2. The sample was then ground to a fine pulp with the help of a pestle.
3. The sample was discharged into 20ml of sterile buffered peptone broth to obtain 10^-1 dilution, the sample was serially diluted upto 10^-2 dilution.
4. Then the pour plate technique and spread plate technique was performed using nutrient agar for technical isolation and malt agar for fungal and mold isolation.
5. After 24 hours of incubation of the plats at 37degree Celsius in the case of bacteria and 3-5 days of incubation at about 25degree Celsius incase of fungi. The colonies were counted and the sample of spoiled bread and vegetables can be used to judge the microbial quantity of the food sample.

RESULT:

Analysis of spoiled Bread samples:

The chief types of microbial spoilage of baked bread was analysed microscopically.

Microscopic observation of fungal methods:

The chief moulds involved in the spoilage of bread moulds, Rhizopus sps, with its white cottony mycelium and black dots of sporangia.

- Green spread penicillium sp., was observed.
- Aspergillus sp., was identified with its greenish or purplish brown to black conidial heads and yellow pigment diffusing into the blood.

Interpretation:

As the bacterial growth of 10^-2 is 58*10^2 CFU/g of the spoiled bread sample shows the presence of numerous micro organisms.

The fungal growth is 10^-2 dilution in 17 plaques/ml shows that the bread is spoiled by most of the fungal species also.

Analysis of spoiled vegetables:

Microscopic vegetables of fungal moulds:

Rhizopus sps., was observed with its white mycelium and black dots of sporangia Aspergillus sps., was observed within its spore becoming heads which are long, tightly packed and rough coloured from tuft to grey to blackish. The blue green bread mould was observed to be penicillium sp., which causes soft rot of the vegetables.

Geotrichum sps., was observed with its white yellowish growth appearing as a fir felt like mars and appears as a soft and creamy on the vegetables.

Trichodema sp., was observed on the vegetables as the bright green conidia glued to together and tuft of white hyphae stick up well above the conidiospore.

Microscopic observation of the fungal:

Moulds:

The fungal mycelia growth was teased off with the teasing needle and lactophenol cotton blue staining method wasperformed to be observed under microscopic for characteristic morphology.

Microscopic observation of bacterial species:

The greenish tinge produced on the surface of the vegetables was deduced to be pseudomonas sps., staphylococcus aureus was observed on the sliminess was caused by saprophyte bacteria in the vegetable sample.

Interpretation:

As the bacterial growth in 10^-2 dilution is 69*10^2 CFU/g of the spoiled vegetable sample shows the presence of numerous micro organisms. The fungal growth in 10^-2 dilution is 33 propagulated ml shows that the vegetables has been spoiled by most of the fungal species also.

Discussion:

As microorganisms are ubiquitous their presence in food is unavoidable and complete sterility in food products is inevitable. But when the number of microorganisms in the food crosses a limit, it is considered as unfit for consumption, when pathogenic microorganisms are involved its consumption may lead to a number of complication ranging from food poisoning like salmonellosia, many organisations like world health organisms has set the standard of food Quality.

Experiment No. 9

ISOLATION OF FOOD BORNE BACTERIA FROM FOOD PRODUCTS

AIM

To isolate and anlyse the type of food borne bacteria from the given sample of food.

PRINCIPLE

Foods are more susceptible to microbial decomposition because they are rich in nutrients that support the growth of microbes. Contaminated foods are often unedible and may also be the source of human diseases if contaminating organism are pathogens or toxin producers. Some of the important food poison produced by the bacteria is botulism(caused by Clostridium botulinum), staphylococcal poisoning (staphylococcus aureus) and enteric (E.coli) and Salmonella typhimurium. This food poisoning is serious and sometimes fatal.

MATERIALS REQUIRED

SAMPLE : Food products

MEDIUM :Nutrient agar medium

- Sterile petriplates
- Inoculation loop
- Sterile pipettes

- Mortor and pestle

PROCEDURE

1. About 1 gm of the food sample to be tested was taken an placed in a clean mortor.
2. The sample was then ground to a fine pulp with the help of a pestle.
3. The sample was then added to the 9ml distilled water and considered as the stock solution.
4. Then 1ml of the stock solution is added to the 9ml of sterile distilled water to obtain 10-1 dilution, the sample was serially diluted upto 10-7 dilution.
5. Then spread plate technique was performed during nutrient agar plates for the bacterial isolation. 0.1ml of the sample was used for the spread plate technique.
6. After 24hrs of incubation of the plates at 37 degree Celsius the colonies were counted and the samples of food products can be used to judge the microbial qualityof the food sample.

RESULT

The bacterial groth at 10-4 dilution is 125*10-4 and 76*10-5 in 10-5 dilution of soft drinks. The bacterial load in chocolate was observed at 10-3 with the total count of 80*10-3 and 90*10-3 in 10-3 dilution in ketch up indicates the presence of microorganism.

Experiment No. 10

ISOLATION OF NITROGEN FIXING BACTERIA-AZOTOBACTER SPS

AIM

To isolate the free living nitrogen fixing organisms, Azotobactersps., from soil sample.

PRINCIPLE

It is a free living aerobic, nitrogen fixing bacterium and is known as a non symbiotic nitrogen fixer which is capable of forming cysts.

Azotobacter where ‘azo’ means nitrogen. The aerobic bacteria capable of fixing nitrogen belong to entirely different families and have been isolated from different habitats viz soil, fresh and marine waters, animals and others.

Azotobacter was first isolate and described by Beijerinck in 1901. Several species of Azotobacter was

- *Azotobacterchroococum*
- *Azotobacteragilis*
- *Azotobacterbeijerinckii*

They are heterotrophic and depends on the energy derived from the degradation of plant residues. They are caharcterised by the relatively large size of the individual cells as seen in the wet preparations under the phase contrast microscope, often motile with peritrichous flagella and production of thick walled microcysts in some species.

They are obligate aerobes capable of fixing atmosphereicnitrogen when provided with suitable carbohydrates or energy source.

Azotobactersps can be isolated by the soil dilution plating method. Direct isolation or soil plate method on any one of the medium like Ashby’s media or Jensens’s medium or Beck’s or Beijeinck’s media.

In all these methods soil sample from which azotobacter must be isolated or soil is poured or spreaded on the nitrogen free nutrient media. Isolation through plating method is commonly used. They involve in the production of microbial polymers.

REQUIREMENTS

Jensen’s media, test tubes, distilled water, soil sample, sterile pipettes, sterile petriplates, cyclomixer, Bunsen burner, glass marking pencil.

PROCEDURE

1. Sterilepetriplates with Jensens media were prepared.
2. The given soil sample was serially diluted and was inoculated by pour plate technique.
3. The plates were then inverted and incubated at 35 degree celcius for 2days.

OBSERVATION

The plates were then observed after 3days at incubation for the appearance of colonies onto the agar plate.

RESULT

Azotobactercolonies appear flat, salt, milky, and mucoid.

DISCUSSION

Azotobactersps., is uesd as an important biofertelizer. Incubation of soil on seed with Azotobzcter is effective in increasing crop yield in well mannered soil with high organic matter content. Besides the ability to fix atmospheric nitrogen Azotobactersps., known to synthesize biologically active substance such as beta vitamins, indole acetic acid etc., in pure cultures.

Azotobactersps., was shown to increase yields of sorghum,maize, and cotton.

Azotobacterchroococum has shown to increase the fertility thereby minimizing the use of fertilizer nitrogen.

Experiment No. 11

MUSHROOM CULTIVATION

AIM:

To know cultivation technology of edible mushroom cultivation of oyster mushroom spawn production.

BACKGROUND INFORMATION:

Mushroom are fresh fungi, which constitute a major group of lower plant kingdom. The mushroom is a common fungal fruit body that produces basidiopores at the tip of the basil. The mushroom consisting of short stem and a cap which being to open like umbrella. India is the second most popular country of the world with a population of over 100 crores mushroom provide a rich addition to the diet in the form of proteins, carbohydrates, minerals and vitamins.

Nutrient value of mushroom include 91.1% moisture 29.9%, proteins 4.4%, carbohydrate 0.3, fat and 16K calories.

Presently about a dozen fungi are cultivated in over 100 countries with a production of 2-2 millions. *Agaricusbasidiospores*(56%), white button colonies, edodes(14%) spilt take *Volvaridlyvolvneay(8%)* paddy straw, *Pleurotus spp., Oyster flammulina(5%), Mushroom timmula spp.,(4.6%), Selvercarphiloter(1.1%)* are the mushroom varieties cultivated or human usage.

Species of the genus *Pleurotus* called oyster mushroom or Dhingri or wood fungus is ranked as the 4^{th} important cultivated mushroom with a productin of 15,000 tons per annum. The genus contains over 50 spp of these *S.sapidus, P.fossulates, P.sequarresules*and*P.florida*have been cultivated on India.

OYSTER MUSHROOM CULTIVATION:

The oyster mushroom are rich in protein mineral contents, devoid of starch and low calories and CHO. These are ideal food for diabetic and heart patiently and those who do not want to put on weight. The various substrates utilized for the cultivation of *Pluerotus* are banana, psuedolims wheat straw, paddy straw, raggi straw, compressed rice husk and karad hay. However the highest yields are obtained on rich straw. These can be grown in any container. Eg: earthern pot, cane gasket, polythene bags, iron baskets or in wooden trays.

SPAWN PRODUCTION:

Thatched hut/polyethylene chamber, mud/ pure house, dry paddy, straw (chopped) or other agrowaste 100kg horse grain powder, 4 kg spawn bottom of *Pluerotus spp.,* polyethylene bags 1 kg , water sprayer.

PROCEDURE:

1. Take dried paddy straw.
2. Chop the straw in to 1 to 2 cm bits.
3. Soak the chopped straw into water overnight.
4. Add horse grain powder at the rate of 8g/ kg.
5. Add spawn at the rate of 30 g /kg.
6. Mix all the constituents.
7. Fill the mixture into polyethylene bags with holes.
8. Incubate the fluid bags in a room at 21 to 35ºC with sufficient light and ventilation for 15-16 days for spawn running.

Experiment No. 12

BACTERIOLOGICAL EXAMINATION OF WATER BY MPN TEST

INTRODUCTION

The three basic test to detect coliform bacteria in water are presumptive. Confirmed and Complete. The tests are performed sequentially on each sample under analysis. They detect the presence of coliform bacteria (indicator and fecal contamination). The gram –ve, non spore forming Bacilli that ferment lactose with the production of acid, gas that is detectable following a 24hours incubation period at 31 degree Celsius.

PRESUMPTIVE TEST:

Determination of the most probable Number of Coliform bacteria :

PURPOSE:

To determine the presence of Coliform bacteria in water sample

To obtain some index as to the possible number of Organisms present in the sample under analysis.

PRINCIPLE:

The presumptive test is specific foe detection of Coliform bacteria measured adequate of the water to be tested are added to a lactose fermentation both containing on incubated gas vial. Because these bacteria are capable of using lactose as a carbon source(the other enteric organism are not) their detection facilitates by the use of this medium. In this experiment the lactose fermentation broth also contain a surface tension depressant, bile salt which is used to suppress the growth of organisms other than the Coliform bacteria.

Tubes of the lactose medium are inoculated with 10ml,1ml,0.1ml aliquots of the water sample. The series consist of atleast 3 groups. Each composed of a 5 tubes of the specified medium. The tubes in each groups are then inoculated with the designated volume of water sample as described under ‘procedure’. The greater the number of tubes per group. The greater the sensitivity of test development if gas in any of the tube is presumptive evidence of tge presence of Coliform in the sample. The presumptive test also enables the microbiological to obtain some ideas of the number of coliform organism by means of MPN test

MATERIALS REQUIRED:

Culture: Water Sample

Media: 15 double strength lactose fermentation broth and 30 single strength lactose fermentation broth.

Equipment: Bunsen burner, 45 test tuber, test tube rack, sterile 10ml pipette, sterile 1ml pipette and 0.1ml mechanical pipetting device and glassware marking pencil.

PROCEUDRE:

1. Set up 3 separate series consisting of 3 groups, a total of 15 tubes per series in a test tubes rack; for each tube labels the water source and volume of sample inoculates as illustrated
2. Mix the sewage plant water sample by shaking thoroughly. Excessive case handling sewage waste water sample because enteric pathogen may be present.
3. Flame bottle then using a10ml, pipette transfer to 10ml, aliquote of sample to 5 tubes labelled 1.32*10ml.
4. Flame bottle then using 1ml pipette transfer 1ml aliquote of water sample to 5 tubes labelled LB1*0.1ml.
5. Flame bottle then using o.1ml pipette transfer 0.1ml aliquote of water sample to 5 tubes labelled lB1*0.1ml
6. Repeat step 2 through 5 for the tap and pond water sample.
7. Incubate all tubes for 48 hours at 37degree celsius.

CONFIRMED TEST:

Purpose: To confirm the presence of Coliform bacteria in a water sample for which the presumption test was positive.

Principle: The presence of positive doubtful presumptive test immediately suggest that the water sample is non-portable confirmation of these results is necessary since positive presumptive tests may be the result of organisms of non-coliforms origin that are not recognised as indicator of focal pollution. The confirmed test required that selective and differential media such as eosin methylene blue(eMB) or endo agar be streaked from a positive lactose broth obtained from presumptive test. The nature of differential and selective media was discussed Eosio-methylene blue contains the dye methylene blue, which inhibits the growth of gram +ve organisms. In the presence of an acid environment. EMB forms a complex that precipitates out onto the coliform colonies producing dark centre and a green metalistic sheen. The reaction is characteristic of e-coli, the major indictor of fecal pollution.The endo-agar is a nutrient medium containing the dyr fuschin which is present in the decolourized state. In the presence of acid produced by coliform bacteria fuschin form a dark pinkcolour complex that turns the E-coli colonies and surrounding medium pink

MATERIALS REQUIRED:

Culture: One 24 hrs old positive lactose broth culture from each of the 3 series from the presumptive test.

Media: Three each per designated student group. Eosin methylene blue agar plate or endo agar plate.

Equipment: Bunsen burner, glassware, marking pencil, inoculation loop.

PROCEDURE:

1. Label the covers of the 3 EMB plates or 3 endo-agar plates with the source of water sample.
2. Using a +ve 24 hours lactose broth culture from the sewage water series from the presumptive test, streak the surface of one EMB or Endo agar plate.
3. Repeat step using a +ve lactose broth culture from the pond and tap water series to inoculate the remaining plates.
4. Inoculate all plate cultures in an inverted position for 24 hours at 31degree Celsius .

COMPLETED TEST:

Purpose: To confirm the presence of Coliform bacteria in a water sample, or if necessary, to confirm a suspicious but doubtful result of previous test.

Principle:

The completed test is the final analyse of the water sample. It is used to examine the coliform colonies that appeared on the EMB or Endo agar plates used in the confirmed test. An isolated colony is picked from the confirmatory test plate and inoculated into tube of lactose both and streaked on a nutrient agar slant to perform a gram stain following inoculation and incubation tubes showing acid and gas in the lactose broth and the presence of gram negative bacilli on microscopic examination are further confirmation of the presence of E-coli and they are indicative of the completed test.

MATERIALS REQUIRED:

Cultures: 24 hours Coliform +ve EMB or endo agar culture from each of the three series of the confirmed test.

Media: Nutrient agar slants and lactose fermentation broth.

Reagent: Crystal violet, Grams iodine, 95% ethyl alcohol and safranin.

Equipment: Bunsen burner, staining tray, inoculating loop, lens paper, bibulous paper, microscopic and glassware, marking pencil.

PROCEDURE:

1. Label each tube with the source of its water sample.
2. Inoculate one lactose broth and one nutrient agar slant from the same isolated E-coli colony obtained from an EMB or endo agar plate from each of the experiment water sample.
3. Incubate all tubes for 24 hours at 31degree Celsius.

RESULT:

PRESUMPTIVE TEST: Examine all tubes after 24 hour and 48 hours of incubation, the result were tabulated.