ADARSH COLLEGE

OMERGA, DIST. OSMANABAD

DEPARTMENT OF MICROBIOLOGY

CERTIFICATE

Practical paper – XVIII

Certified that Shri / Miss_____

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

Staff member incharge **Head of the department**

Date: Date:

Signature of the Examiner

Date:

Exam Seat No. _____

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B.Sc. III

P- XVIII

Expt. 01 **Date: -**

Preparation of buffers

Aim: - To prepare buffers

Theory / Approach: -

Each microbial species has a definite pH growth range and pH growth optimum. Microorganisms change the pH of the medium by producing acidic or basic metabolic waste products. Buffers are included in the media to prevent growth inhibition by large pH changes. pH is the negative logarithm of hydrogen ion concentration. The pH scale extends from pH 0.0 (1.0 M H^+) to pH 14.0 (1.0 x 10^{-14} M H^+), and each pH unit represents a tenfold change in hydrogen ion concentration.

Autoclaving of the media frequently changes the pH by removal of CO_2 or due to the precipitation of phosphates. During growth of bacteria, due to anaerobic fermentation or by dissimulation of salts of organic acids, large changes in pH may occur. The pH may be controlled by use of buffer.

Buffers are mixtures of weak acids and their conjugate bases. If protons (H^+) are added to the mixture, they combine with the salt to yield weak acid. An increase in alkalinity (OH^-) is resisted because the weak acid will neutralise hydroxyl ions through proton donation to give water.

1. Acetate buffer

Solutions required: -

Solution A: - 0.2 M solution of Acetic acid (11.55 ml in 1000 ml D. water)

Solution B: - 0.2 M solution of Sodium acetate (16.4 gm of CH_3COONa or 27.2 gm of $CH_3COONa \cdot 3H_2O$ in 1000 ml)

X ml of A + Y ml of B, diluted to total of 400 ml.

X	Y	pH
185.2	14.8	3.6
176.0	24.0	3.8
164.0	36.0	4.0
147.2	52.8	4.2
122.0	78.0	4.4
102.0	98.0	4.6
80.0	120.0	4.8
59.2	140.8	5.0
42.0	158.0	5.2
35.2	164.8	5.4
19.2	180.8	5.6

2. Phosphate buffer

Solutions required: -

Sol. A: - 0.2 M Solution of Monobasic Sodium Phosphate

(3.12 gm NaH_2PO_4 . $2H_2O$ in 100 ml D/W)

Sol. B: - 0.2 M Solution of Dibasic Sodium Phosphate

(2.83 gm Na_2HPO_4 or 7.17 gm of Na_2HPO_4 **.** 12 H_2O in 100 ml of D/W)

X ml of A + Y ml of B, diluted to total of 200 ml.

X	Y	pH
92.0	8.0	5.8
87.7	12.3	6.0
81.5	18.5	6.2
73.5	26.5	6.4
62.5	37.5	6.6
51.0	49.0	6.8
39.0	61.0	7.0
28.0	72.0	7.2
19.0	81.0	7.4
13.0	87.0	7.6
8.5	91.5	7.8
5.3	94.7	8.0

Procedure: -

In both Acetate and Phosphate buffer, Solution A and Solution B are prepared by measuring chemicals accurately and are mixed accordingly to prepare required pH.

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

Study of catalase enzyme

Aim: - To study the enzyme catalase

Theory / Approach: -

Most of aerobes and facultative anaerobes have the characteristic of showing catalase activity. Actually these organisms utilize oxygen to produce hydrogen peroxide. The hydrogen peroxide is toxic to their enzyme system. Hence, these organisms produce an enzyme called catalase, which converts the hydrogen peroxide to water and oxygen. It is also that the reason of not surviving anaerobes in the presence of oxygen is H_2O_2 production and absence of enzyme catalase.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \uparrow$$

In many cases gas can be readily seen as effervescence. A little bit of growth of bacterial colony is added in 3% H_2O_2 solution. Effervescence of oxygen gas is found.

Requirements: -

1. Bacterial culture (*Staphylococcus sp.)*
2. Inoculation loop
3. 3% H_2O_2 solution

Procedure: -

1. 1 ml of 3% H_2O_2 solution is taken in sterile test tube.
2. A little bit of growth from bacterial colony is picked up with the sterile wire loop and dipped in H_2O_2 solution.
3. Effervescence of gas is observed.

Observation: -

1. Effervescence of gas is observed / not observed.

Result: - The given bacterial culture has ability / has no ability to produces Catalase enzyme.

Conclusion: - Catalase enzyme is produced / not produced by the given culture and test is positive / negative.

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

Study of oxidase enzyme

Aim: - To study the enzyme oxidase

Theory / Approach: -

To differentiate certain group of bacteria, oxidase activity is one of the tests. Certain bacteria are oxidase negative e.g. members of the family ***Enterobacteriaceae,*** while ***Pseudomonas*** shows oxidase positive. To perform the test, a dye tetramethyl-p-phenylene diamine dihydrochloride (NNNN) is used. It donates the electron to cytochrome C, becomes oxidised, and produces a colour.

Requirements: -

1) 1% aqueous solution of tetramethyl-p-phenylene diamine dihydrochloride
2) Given microbial culture.

Procedure: -

1. Whatman paper strip is dipped in to NNNN reagent and is placed in petri plate.
2. With the help of nichrome wire loop a part of the bacterial colony is rubbed on the wet filter paper in the plate.
3. It is observed for the development of purple colour.

Result:

A purple colour is produced / not produced within 5 to 10 seconds by oxidase positive culture.

Conclusion: -

The test is positive / negative. Hence oxidase is present / absent in the culture.

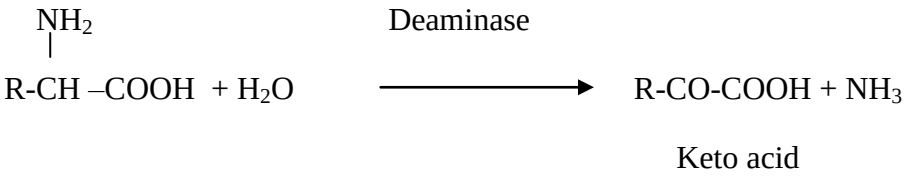
Expt. No. 02C **Date: -**

Study of Deaminase enzyme

Aim: - To study the Deaminase enzyme.

Theory: -

Deamination of an amino acid is the enzymatic splitting of the amino group to yield NH_3 and generally the corresponding keto acid. The reaction is



Deamination can readily be determined by measuring formation of NH_3. NH_3 is extremely soluble in aqueous media and does not accumulate as a gas in the tube. Among the enterobacteriaceae, only proteus strain has this ability to deaminase phenylalanine.

Requirements: -

1) Phenylalanine agar (Nutrient Agar 100ml + Phenylalanine 1gm)

2) Bacterial culture

3) 10% Ferric chloride solution

Procedure: -

1. Inoculate the test organism on phenylalanine agar and incubate over night at 37^0 C.
2. Pour a few drops of 10 % ferric chloride solution over the growth. A green colour indicates the deamination of phenylalanine to phenyl pyruvic acid.

Result: -

The green colour is observed / not observed.

Conclusion: -

NH_3 is produced / not produced. Hence the test is positive / negative.

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

Study of Desulphurase enzyme

Aim: - To study the Desulphurase enzyme.

Theory: -

The activity of some bacteria on sulphur containing amino acids frequently results in the liberation of the H_2S gas. Common examples of this reaction are found in smell of rotten egg and in the blackening of the certain spoiled foods. The mechanism of H_2S formation is as follows—

Cystein / Methionine + 2 H $\longrightarrow$ Alanine + H_2S

The production of H_2S by bacterial culture may be results from the decomposition of organic sulphur compounds or reduction of inorganic sulphur compounds.

Requirements: -

1) Cystein broth (Nutrient broth 100 ml + 0.1 gm cystein / Methionine)

2) Bacterial suspension

3) Lead acetate paper

Procedure: -

1. A loopful of bacterial suspension is inoculated in cystein broth.
2. Lead acetate paper is inserted in a tube and tube is plugged so that the paper will hang in the test tube.
3. The tube is incubated at 37^0 C for 24 hours.
4. The blackening of the lead acetate paper is noted.

Observation: -

Blackening of lead acetate paper observed / not observed.

Result: -

The enzyme desulphurase is produced / not produced by given bacterial suspension.

Conclusion: -

H_2S produced reacts with lead acetate to produce black coloured lead sulphide. Enzyme desulphurase produces H_2S, which results in blackening of the paper.

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

Study of Lecithinase enzyme

Aim: - To study the Lecithinase enzyme.

Theory / Approach: -

Certain bacteria produce enzyme lecithinase, which splits lipoprotein complexes in the human sera and egg yolk to produce opalescence reaction.

Requirements: -

1) Egg yolk medium [Nutrient agar 100 ml + Egg yolk (yellow part) 10 ml]

2) Bacterial culture (*B. subtilis, E. coli, S. aureus, Pseudomonas spp.)*

Procedure: -

1. Surface of the egg is washed with alcohol. Egg yolk is taken in a sterile petri plate. It is added to sterile molten nutrient agar of 55 ^{0}C and mixed well. Plate is poured immediately and allowed to solidify.
2. Plate is spot inoculated by test organism and is incubated at room temperature for 24-48 hours.

Result: -

Zone of opalescence is observed / not observed around the colony.

Conclusion: -

Licithinase activity is present / absent in a given organism.

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

Study of Caseinase enzyme

Aim: - To study the caseinase enzyme.

Theory: -

Casein is the chief protein present in milk, some organisms are capable of hydrolysing the proteins to smaller units. This is known as peptonization.

Skimmed milk is an excellent medium for supporting the growth of bacteria since it contains sugar lactose and protein casein as well as vitamins, minerals and water.

Some of the bacteria hydrolyse casein by producing an Extracellular enzyme caseinase. This activity is detected by production of clear zone around the colonies.

Requirements: -

1) Skimmed milk
2) Nutrient agar
3) Bacterial suspension

Procedure: -

1) Milk is heated and cooled and the cream layer is separated.

2) Skimmed milk is sterilized.

3) Sterile Nutrient Agar is prepared.

4) 10 ml of skimmed mil is mixed with 100 ml of sterile nutrient agar and plate is poured.

5) The test organism is inoculated and plate is incubated at 37^{0} C fro 24 hours.

Observation: -

Clear zone around the colony is observed / not observed

Result: -

Caseinase enzyme is produced / not produced.

Conclusion: -

Clear zone around the colony indicates caseinase activity.

Expt. No. 03 A **Date: -**

Effect of Temperature on amylase activity

Aim: - To determine the effect of temperature on amylase enzyme activity

Theory/Approach: -

The rate of enzyme catalyzed reactions generally increases with the increase in temperature within a certain range. The enzyme and substrate concentrations are kept constant and only the temperature is changed like reaction at 10, 37, 50^0 C. OD is measured at 546 nm and graph is plotted between OD and temperature. From graph optimum temperature at which maximum enzyme activity takes place is found out.

Requirements: -

1. Test tubes
2. Starch solution 1 % (10 mg/ml)
3. Amylase Enzyme solution 1 % (10 mg/ml)
4. Distilled water
5. Pipettes
6. Spectrophotometer / Colorimeter
7. Freeze, Incubator
8. DNS reagent (3, 5 Di-Nitro Salycilic acid)

Composition of DNS reagent

Water	100 ml
3,5 Di-Nitro salicylic acid	1 gm
Phenol	0.2 gm (200 mg)
Na_2CO_3	0.05 gm (50 mg)
NaOH	1 gm
Sodium potassium tartarate	20 gm

9. Buffer of pH 7

Composition of buffer

A] 0.2 M solution of Monobasic Sodium dihydrogen phosphate (NaH_2PO_4)

127.8 gm in 1000 ml D/W

B] 0.2 M solution of Sodium Dibasic hydrogen phosphate (Na_2HPO_4) 53.65

Gm in 1000 ml.

X ml of A + Y ml of B diluted to 200 ml with D/W

X ml of A	Y ml of B	pH
39.0 ml	61.0 ml	7

Procedure: -

1. 4 test tubes are taken and marked them as Blank, 10^0 C, 37^0 C and 50^0 c
2. 1 ml starch solution and 1 ml buffer of pH 7 is added to 10^0 C, 37^0 C and 50^0 C marked test tubes. Starch solution is not added to test tube marked as Blank, instead 2 ml buffer is added in it.
3. Tubes are incubated at different temperature ranging from 10^0 C, 37^0 C and 50^0 C for 15 minutes to reach appropriate temperature.
4. 1 ml amylase enzyme solution is added in all tubes.
5. Tubes are shaken well and incubated at 10^0 C, 37^0 C and 50^0 C for 15 minutes
6. 2 ml of DNS reagent is added in all tubes and tubes are kept in boiling water bath for 15 minutes.
7. Tubes are cooled and 6 ml D/W is added in each tube to make final volume 10 ml and shaken well.
8. OD is adjusted to zero by using Blank and OD of solution of other test tubes is measured at 546 nm.
9. A graph is plotted between OD and Varying temperature and optimum temperature is found out at which maximum enzyme activity has taken place.

Protocol: -

Test tube marked as	**1 % Starch solution in ml**	**Incubation for15 min at**	**Amylase sol^{n}**	**Incubation for 15 min at**	**DNS Reagent in ml**		**D/W in ml**	**OD at 550 nm**
Blank	NIL (Add 2 ml buffer)	Room temp	1 ml	Room temp	2 ml		5	
10^0 C	1 ml starch and 1 ml buffer of pH 7	10^0 C	1 ml	10^0 C	2 ml		5	
37^0 C	1 ml starch and 1 ml buffer of pH 7	37^0 C	1 ml	37^0 C	2 ml	**Keep in boiling water bath for 20 minutes**	5	
50^0 C	1 ml starch and 1 ml buffer of pH 7	50^0 C	1 ml	50^0 C	2 ml		5	

Observation: As per observation table and graph.

Result: - The optimum temperature for amylase is _____.

Conclusion: - The amylase showed maximum activity at 37^0 C and less activity at 10^0 C and 50^0 C indicating that temperature affects the activity of amylase.

Expt. No. 03 B **Date: -**

Effect of pH on amylase activity

Aim: - To determine the effect of pH on amylase enzyme activity

Theory/Approach: -

The nature of binding between enzyme and substrate is mainly electrostatic and depends upon the charge available on the substrate and the active site. Since active site is composed of amino acids, charges vary with pH and binding of enzyme with substrate is affected by pH of the medium.

The enzyme and substrate concentrations and temperature are kept constant and only the pH is changed like 6, 7, 8. OD is measured at 546 nm and graph is plotted between OD and pH. From graph optimum pH at which maximum enzyme activity takes place is found out.

Requirements: -

1. Test tubes
2. Starch solution 1 % (10 mg/ml)
3. Amylase Enzyme solution 1 % (10 mg/ml)
4. Distilled water
5. Pipettes
6. Spectrophotometer / Colorimeter
7. Freeze, Incubator
8. DNS reagent (3, 5 Di-Nitro Salycilic acid)

Composition of DNS reagent

Water	100 ml
3,5 Di-Nitro salicylic acid	1 gm
Phenol	0.2 gm (200 mg)
Na_2CO_3	0.05 gm (50 mg)
NaOH	1 gm
Sodium potassium tartarate	20 gm

OR

1 gm of 3,5 Di-nitro salicylic acid is moistened with few drops of water in 200 ml volumetric flask. 20 ml of 0.1 N NaOH is added slowly under constant shaking and finally 50 ml of water is added. When 3,5 Di-nitro salicylic acid dissolves completely, 30 gm of potassium sodium tartarate is added. The solution is made up to 200 ml with D / W. Shake well and filter.

9. Phosphate buffer of pH 6, 7 and 8.

Composition of buffer

A] 0.2 M solution of Monobasic Sodium dihydrogen phosphate (NaH_2PO_4) 127.8 gm in 1000 ml D/W

B] 0.2 M solution of Sodium Dibasic hydrogen phosphate (Na_2HPO_4) 53.65 gm in 1000 ml.

X ml of A + Y ml of B diluted to 200 ml with D/W

X ml of A	Y ml of B	pH
87.7 ml	12.3 ml	6
39.0 ml	61.0 ml	7
5.3 ml	94.7 ml	8

Procedure: -

1. 4 test tubes are taken and marked them as Blank, pH 6, pH 7 and pH 8.
2. 1 ml starch solution and 1 ml buffer of respective pH is added to pH 6, pH 7 and pH 8 marked test tubes. Starch solution is not added to test tube marked as Blank; instead 2 ml D/W is added in it.

3. Tubes are incubated at 37^0 C temperatures for 15 minutes to reach appropriate temperature.
4. 1 ml amylase enzyme solution is added in all tubes.
5. Tubes are shaken well and incubated at 37^0 C for 15 minutes.
6. 2 ml of DNS reagent is added in all tubes and tubes are kept in boiling water bath for 15 minutes.
7. Tubes are cooled and 5 ml D/W is added in each tube to make final volume 10 ml and shaken well.
8. OD is adjusted to zero by using Blank and OD of solution of other test tubes is measured at 546 nm.
9. A graph is plotted between OD and Varying pH and optimum pH is found out at which maximum enzyme activity has taken place.

Protocol: -

Test tube marked as	**1 % Starch solution in ml and buffer solution**	**Incubation for15 min at**	**Amylase sol^n**	**Incubation for 15 min at**	**DNS Reagent in ml**		**D/W in ml**	**OD at 550 nm**
Blank	NIL (Add 2 ml d/w)	37^0 C	1 ml	37^0 C	2 ml	**Keep in boiling water bath for 20 minutes**	5	
pH 6	1 ml starch and 1 ml buffer of pH 6	37^0 C	1 ml	37^0 C	2 ml		5	
pH 7	1 ml starch and 1 ml buffer of pH 7	37^0 C	1 ml	37^0 C	2 ml		5	
pH 8	1 ml starch and 1 ml buffer of pH 8	37^0 C	1 ml	37^0 C	2 ml		5	

Observation: As per observation table and graph.

Result: - The optimum pH for amylase is _____.

Conclusion: - The amylase showed maximum activity at pH ___ and less activity at pH __ indicating that pH affects the activity of amylase.

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

Demonstration of nitrate reduction

Aim: - To demonstrate the nitrate reduction activity of soil bacteria

Theory/ Approach: -

Certain bacteria use nitrate (NO_3) in place of oxygen as an external terminal electron acceptor. In case of aerobic bacteria, oxygen is first used and then nitrate is used as electron acceptor. The nitrate is reduced to nitrite (NO_2). The NO_2 may further give rise to nitrogen, ammonia, nitrogen oxide. The enzyme reaction is catalyzed by *nitrate reductase* as follows:

$$NO_3^- + 2\,H^+ + 2\,e^- \longrightarrow NO_2^- + H_20$$

Requirements: -

1. Nitrate broth

Water	100 ml
KNO_3	1.0 gm
Peptone	5.0 gm
Meat Extract	3.0 gm

2. Nitrite test reagent

D/W	100 ml
Zinc Chloride	2.0 gm
Starch	0.4 gm
Potassium Iodide	0.2 gm

3. Dilute Sulphuric acid (H_2SO_4)

Procedure: -

1. Nitrate broth is inoculated with given culture and incubated at 37^0 C for 24 hours.
2. 5 ml broth is taken in a test tube and 3 drops of nitrite test reagent and 1 drop of dilute sulphuric acid is added.
3. Blue colour indicates nitrite is produced.

Observation: - Blue colour appeared / not appeared

Result: - Nitrate is reduced / not reduced.

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

Demonstration of decarboxylation of amino acid

Aim: - To demonstrate decarboxylation of amino acid

Theory: - Most of the sugars fermenting bacteria have a property of decarboxylation or removal of carboxyl group from specific amino acids such as lysine. Such bacteria produce an extracellular enzyme called lysine decarboxylase, which converts lysine into CO_2 and cadaverine.

Due to accumulation of cadaverine, the pH of medium becomes alkaline. In such case a pH indicator dye bromocresol purple turns into purple colour or phenolphthalein into pink colour.

Requirements: -

1. Culture of *P. vulgaris* or *E. aerogenes*
2. Lysine medium

Composition

Water	100 ml
Lysine	0.5 gm
Glucose	0.1 gm
Yeast extracts	0.3 gm
Peptone	0.5 gm
Phenolphthalein	20 mg
pH	6.8

Procedure: -

1. Lysine medium is inoculated with given culture.
2. Incubation is done at 37^0 C for 24 hours.
3. Pink colour development indicates decarboxylation.

Observation: - Pink colour developed / not developed

Result: - Decarboxylation of lysine occurred / not occurred.

Conclusion: - Pink colour indicates the formation of alkaline pH due to cadaverine formed after decarboxylation of lysine.

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

Screening of starch degrading (Amylase producing) micro-organisms from soil

Aim: -

To screen out starch degrading (Amylase producing) micro-organisms from soil.

Theory: -

Starch serves as plant's storage product and as such it is major reserve carbohydrate. Starch has two components, amylose 10 to 30 % and amylopectin 70 to 90 %. The amylose contains several hundreds of glucose units linked together by α (1 – 4) linkage (glycosidic bonds). In amylopectin, the individual glucose units are linked together by α (1 – 4) linkage but the molecule is branched and has side chains attached to α (1 – 6) linkage.

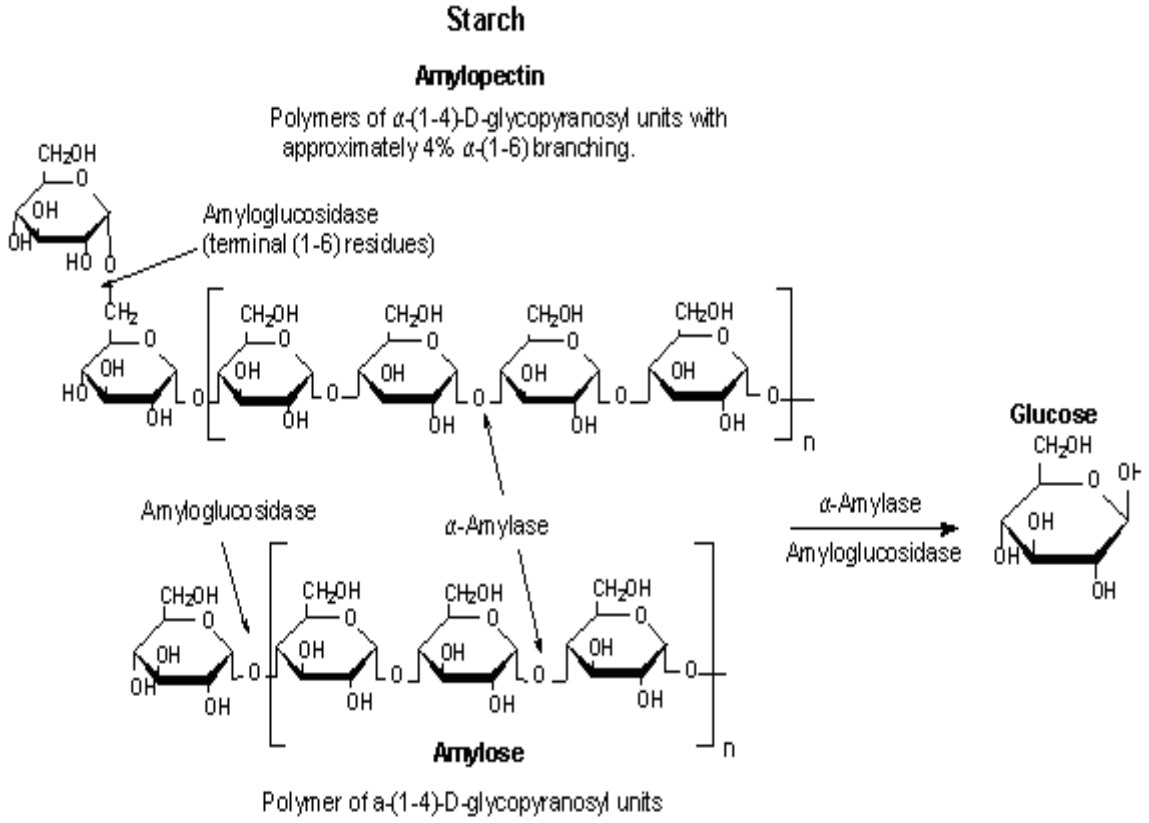
Bacteria, fungi and actinomycetes have the capacity to hydrolyze starch. Some microbial genera utilizing starch are –

1. Bacteria: - Bacillus, Chromobacterium, Clostridium, Cytophaga, Flavobacterium, Micrococcus, Pseudomonas spp. Etc.
2. Fungi: - Aspergillus, Fomes, Fusarium, Rhizopus spp. Etc.
3. Actinomycetes: - Micromonospora, Nocordia, Streptomyces spp. Etc.

Amylase is extracellular enzyme acting on a homopolysaccharide starch. There are three types of amylases.

1. Alpha (α) Amylase
2. Beta (β) Amylase
3. Glucoamylase

Alpha amylase can hydrolyze any alpha 1 – 4 linkage randomly; therefore limit dextrins are the end products. Beta amylase removes two glucose units each time from the end of molecule. Hence, maltoses are liberated. Glucoamylase produces glucose units from maltose.



Requirements: -

1. Sterile Starch agar plate (Nutrient agar + 1 % Starch)
2. Soil sample
3. Iodine solution

Procedure: -

1. One gram of soil sample is added in a 10 ml of distilled water in a test tube.
2. This soil suspension is boiled for few minutes.
3. After cooling, one loopful from this tube is streaked on starch agar plate.
4. The plate is incubated at 37 ^{o} C for 24 hours.
5. After incubation, iodine solution is poured on the plate and observed for colourless zone around the colonies.

Observation: -

Colourless zone is observed around some of the colonies.

Result: -

The colonies showing colourless zone around them are of amylase producing.

Conclusion: -

Starch gives blue colour with iodine; the colourless zone around the colonies on the plate indicates starch hydrolysis. From this observation we can conclude that the colonies showing colourless zone around them degrade starch extracellularly.

Expt. No. 06B Date: -

Screening of organic acid producers

Aim: - To screen out organic acid producing bacteria from soil

Theory / Approach: -

Several bacteria produce number of organic acids by partial oxidation of carbohydrates. Bacterial action on carbohydrate may be either aerobic or anaerobic. This may result in conversion of aldehyde group of carbohydrate to carboxylic group. Bacteria produce variety of organic acids such as pyruvic acid, acetic acid, formic acid, gluconic acid etc. The acid base indicators such as bromothymol blue, phenol red, and neutral red can be used to detect acid production. The acids produced can be determined by observing the colour change in the medium around colony.

Alternative method uses $CaCO_3$ in a medium which is hydrolysed by acid producers resulting in clearing around the colony.

Requirements: -

1. Soil sample
2. Sterile D/W tubes
3. Sterile pipettes
4. Sterile nutrient agar plates with neutral red and 1 % glucose

Procedure: -

1. 1 Gram soil sample is added in 10 ml of D/W and dilutions are prepared from 10^{-1} to 10^{-6} .
2. 0.1 ml of each dilution is spread over the nutrient agar plates.
3. Plates are incubated at 37^{0} C for 24 hours. And observed for pink colour formation around the colony.

Observation: -

Pink colour formation around the colonies are observed / not observed.

Result: -

Acid producing bacteria are detected / not detected.

Conclusion: -

Acid producing bacteria are screened from the soil sample which have produced pink colour around the colonies due to presence of acid indicator dye in the medium.

Expt. No. 6C Date: -

Screening of antibiotic producers from soil

Aim: - To screen out antibiotic producing microorganisms from soil

Theory / Approach: -

Antibiotics are the organic compounds produced by one type of microorganisms, which inhibit or kill the other type of microorganism at minute concentration.

Many bacteria, fungi and actinomycetes produce antibiotics. e.g. Penicillium species produce Penicillin, Streptomyces species produce Streptomycins, Chloramphenicol and Tetracyclines, Bacillus species produce Bacitracin etc. These antibiotics are used to treat the various types of diseases caused by microorganisms.

Crowded plate technique is used to detect and isolate antibiotic producers.

Requirements: -

1. Soil sample
2. Sterile nutrient agar
3. Sterile D/W tubes
4. Sterile petri plates and pipettes

Procedure: -

1. 1 gm of soil sample is added in 10 ml sterile D/W tube (10^{-1} dilution)
2. 1 ml from 10^{-1} diluted tube is added to the next tube containing 9 ml of sterile D/W (10^{-2} dilution).
3. Further dilutions like 10^{-3}, 10^{-4}, 10^{-5} etc are prepared as above.
4. 1 ml from each dilution is added in sterile empty petri plates and sterile nutrient agar ($45\ ^{0}$ C) is poured and plates are shaken well to mix the sample and allowed to solidify.
5. Plates are incubated at room temperature for 2 to 4 days and observed for inhibition zone around the colonies.

Observation: -

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

Result: -

Antibiotic producers are screened / not screened.

Conclusion: - Inhibition zone around the colonies indicates antibiotic production.