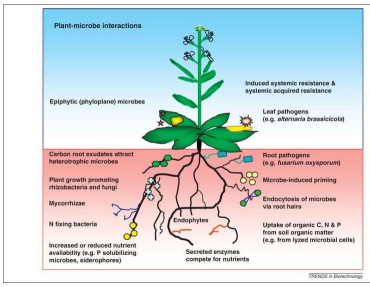


- **Microbiology of Rhizosphere:**
- The soil surrounding the plant root where root exudate migrate and microbiological activity is exceptionally high is called rhizosphere.
- The surface of root is called rhizoplane.
- Plant root produce and release various exudates containing sugar, aminoacids, organic acids, fatty acids, vitamins, nucleotides and other organic matters that promotes growth of microorganisms.
- Therefore rhizospheric soil is characterized by greater number of microorganisms than soil away from plant roots.
- The intensity of rhizospheric effects depends on the distance to which root exudates can diffuse. The number of microorganisms decreases continuously as the distance from the plant root increases.

- The term rhizosphere to soil ratio (R:S) indicates number of microbes in rhizospheric soil divided by number of microbes in soil free of plant root.
- R:S ratio is greater for bacteria (20:1) and less for fungi and actinomycetes.
- Effects of rhizosphere is almost negligible for algae and protozoa. It is because algae are photosynthetic and do not depends upon organic matter present in root exudates.
- On the other hand most bacteria cannot utilize relatively resistant to organic matter of soil and depends on easily available decomposable matter of root exudates. Therefore number of bacteria is exceptionally high in rhizosphere.

- This nutritional enrichment around roots creats unique environment for soil microorganism including the rhizosphere (that's volume of soil around roots influenced by roots exudation) and the rhizoplane (the immediate root epidermal surface that interfaces that rhizosphere soil.
- It includes releases come form secretion , exudates , lysates , mucilage and mucigel.
- The term rhizodeposition includes (Root exucates , mucilage , death & decay part of root).
- Mucilage:- Root mucilage form a gelatinous layer surrounding the root tip and is one of the few clearly visible signs of organic carbon excretion from roots. Its mainly composed of polysaccharides of 106-108 Da in size and its actively secreted by exocytosis from root cap cells. Alongside polysaccharides , its also contains proteins and some phospholipid.

- Rhizosphere can be divided into :
- Inner rhizosphere: the very root surface
- Outer rhizosphere: embracing the immediately adjacent soil

RHIZOSPHERE EFFECT

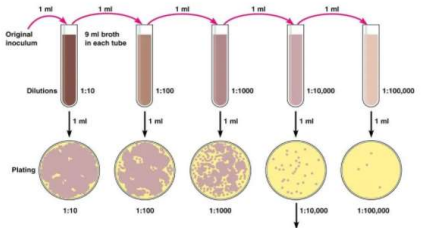
- Compared to non-rooted bulk soil , the rhizospheric soil around the plant root contains much larger population of microorganism.
- A stimulation that can be put on a quantitative basis by the use of the R : S ratio
- R : S ratio = number of microorganisms in the rhizosphere soil : number of microorganisms in the non-rhizosphere soil.
  - R/S > 1, good stimulation
  - R/S = 1, no stimulation
  - R/S < 1, inhibition

- 1.Collection of Soil Sample
- NR.= first
- RS = soil
- Plate
- Standard Plate Count
- 1 gm—100ml saline : 10 min

1.A:1:100—
2.A:1ml ----9ml=10ml: 1:1000
3.B 1ml ----9ml=10ml: 1:10000

The number of microorganisms present in the particular test sample is determined using the formula:

CFU/mL= CFU * dilution factor * 1/aliquot



Determination of rhizosphere effect (R : S ratio)

Rhizosphere effect is an indication of the degree of stimulation of microorganisms - in the root region of a plant and is determined by dividing the population in cfu/g in the rhizosphere soil by that obtained in the non – rhizosphere soil (Dubey and Maheshwari, 2005).

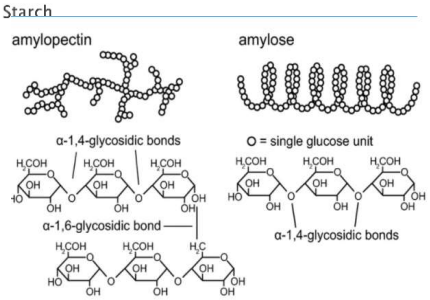
$$R : S \text{ ratio} = \frac{\text{Fungal population in the rhizosphere zone}}{\text{Fungal population in the non-rhizosphere zone}}$$

- CFU/Ml= 120x 1:10000x 1

Isolation of starch degrading bacteria.

- Starch hydrolysis test is used to determine if the organism is capable of breaking down starch into maltose through the activity of the extra-cellular α-amylase enzyme. Starch, the most important source of carbohydrate for humans, is a polysaccharide mixture of two polymers, **amylose,** and **amylopectin**, the latter being predominant.
- Amylose is a linear polysaccharide of several thousand α-D-glucose linked by 1,4-α-glycosidic bonds.
- Amylopectin is a branched-chain polysaccharide composed of glucose units linked primarily by α-1,4-glycosidic bonds but with occasional α-1,6-glycosidic bonds, which are responsible for the branching.

Starch



Principle

- In starch hydrolysis test (also known as **amylase test**), we use starch agar, which is a differential nutritive medium. The test organisms are inoculated onto a starch plate and incubated at 30°C until growth is seen (i.e. up to 48 hours). The Petri plate is then flooded with an iodine solution.
- If there is no enzyme present, and therefore no hydrolysis, the amylose and iodine react together to form a blue color. Depending on the concentration of the iodine used, **iodine turns blue, purple, or black in the presence of starch.**
- When bacteria capable of producing α-amylase and oligo-1,6-glucosidase are grown on starch agar, they secrete enzymes into the surrounding areas and hydrolyze the starch. As no amylose is present in the medium surrounding the bacterial colony, clearing around the bacterial growth is seen (there is no color development).

Requirements

- 1.Test culture
- 2.Starch agar: Composition
- Nutrient agar +1% Starch
- Iodine Solution 10%

Test procedure

1.Take a loopful of test culture aseptically using a sterile loop.

- Streak a starch plate in the form of a line across the width of the plate or spot inoculate .
- Several cultures can be tested on a single agar plate, each represented by a line or the plate may be divided into four quadrants for this purpose.
- Incubate plate at 37 °C for 48 hours.

2.Serial dil.----0.1 ml-Spread surface ----incubate---Record results

- Add 2-3 drops of 10% iodine solution directly onto the edge of colonies.
- Wait 10-15 minutes and record the results. Read plates immediately after the addition of iodine, as the blue color fades.

Amylase Test

Aim: Demonstration of i) Ammonification ii) Nitrification and iii) Denitrification

1. Ammonification:

- **Ammonification:**
- The nitrogen in proteins (as well as in nucleic acids) may be regarded as the end of the line as far as synthesis of nitrogenous compounds is concerned. The nitrogen in proteins is 'locked' and is not available as a nutrient to plants.
- In order to set this organically bound nitrogen free for reuse, the first process that
- must take place is the enzymatic hydrolysis of proteins (proteolysis).
- This is accomplished by microorganisms capable of elaborating extracellular **proteinases**
- that convert the protein to smaller units (peptides). The peptides are then attacked by **peptidases,** resulting ultimately in the release of individual amino acids.

- Among the most active in this respect are some of the clostridia, e.g., Clostridium histolyticurn and C. Sporogenes ; a lesser degree of activity is found in species of the genera Proteus, Pseudomonas, and Bacillus.
- Many fungi and soil actinomycetes are extremely proteolytic.
- Peptidases, however, occur widely in microorganisms . The end products of proteolysis are amino acids. Their fate in the soil may be utilization as nutrients by microorganisms or degradation by microbial attack.
- Amino acids are subject to a variety of pathways for microbial decomposition.

- Soil sample
- 2% peptone water
- Nessler's reagent

Study of Ammonification


Inocculate

Incubation – one Wk

test

1ml of culture + 2 drops Nessler's reagent----- yellow colour + test

Study of Nitrification


Inocculate

Incubation – one Wk

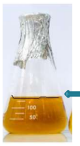
test

Suphanilic acid and alpha nepthalamine

1ml of culture + 2 drops GI reagent----- PINK colour +NITRITE test

Study of Nitrification


Inocculate

Incubation – one Wk

test

1ml of culture + 2 diphenyl amine reagent----- blue colour +NITRITE test

Isolation of Azotobacter from soil

Enrichment

- Glucose or mannitol Nitrogen Free Mineral Medium (NFMM) used to isolate nitrogen-fixing bacteria.
- The composition of the isolated medium was as follows (g/L): 1.0 K_2HPO_4, 1.0 $CaCl_2$, 0.5 NaCl, 0.25 $MgSO_4 \cdot 7H_2O$, 0.01 $FeSO_4 \cdot 7H_2$ or O, 0.01 $Na_2MoO_4 \cdot 2H_2O$, 0.01 $MnSO_4 \cdot 5H_2O$ and a carbon source was glucose (7 g/L).
- Solid medium was produced by adding 2.5% agar used for isolation

Inocculate

Incubation – one Wk

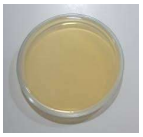
Pellicle

Isolation of Azotobacter from soil

Enrichment

- Glucose or mannitol Nitrogen Free Mineral Medium (NFMM) used to isolate nitrogen-fixing bacteria.
- The composition of the isolated medium was as follows (g/L): 1.0 K_2HPO_4, 1.0 $CaCl_2$, 0.5 NaCl, 0.25 $MgSO_4 \cdot 7H_2O$, 0.01 $FeSO_4 \cdot 7H_2$ or O, 0.01 $Na_2MoO_4 \cdot 2H_2O$, 0.01 $MnSO_4 \cdot 5H_2O$ and a carbon source was glucose (7 g/L).
- Solid medium was produced by adding 2.5% agar used for isolation

Isolation of Azotobacter



Nitrogen free mannitol agar plate

Streak loopful of pellicle on NFMA Plate

Incubation

Colonies of Azotobacter

Gram's staining of Azotobacter