Aim 39

To Isolate the *Rhizobia* from Root Nodules

Requirements

Root nodule, yeast extract, mannitol, agar medium , 0.1% $HgCl_2$, 70% ethyl alcohol 10 ml sterile water blank, sterile water blank, 90ml sterile water blank, magnetic shaker, inoculating needles, Bunsen burner.

Procedure

1) Preparation of YEMA medium constituent of which are as follow:-

K_2HPO_4	0.5 g
$AgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
Mannitol	10 g
Yeast extract	1 g
Agar	20 g

2) Dissolved weighted amount of all the constituent (except K_2HPO_4 which is to be dissolved separately) in distilled water mix in the agar solution. Make the volume to 1000ml and autoclave it. Conge red solution is to be sterilized separately and added to the medium at the time of pouring in petriplates.
3) Uproots the roots of leguminous plant and brought to the laboratory.

4) Wash the root system in running tap water to remove adhering soil particles.
5) Select healthy pink, unbroken and firm root nodules and wash in water.
6) Immerse the solution nodule in 0.1% $MgCl_2$ 5 min to surface sterilize these.
7) Repeatedly wash the nodule in sterile water for 3-4 times to get rid of sterilizing agent.
8) Place the nodule in 70% ethyl alcohol for 3 minutes.
9) Repeatedly wash the nodule in sterile water.
10) Crush a nodule in 1 ml of H_2O with a sterile glass rod.
11) Make a uniform suspension of Rhizobia with H_2O.
12) Make serial dilution of nodules extract.
13) Spread 1 ml each of suspension from various dilution on YEMA plates.
14) Incubate the plate at 26^0C for ten days.

Observation

Observe the plate 3 days after incubation and regularly after word for the development of rhizobia colonies.

Precautions

1) Pinkish nodule should be selected for isolation
2) Nodule should be surface sterilized properly and should be free from all the surface born microorganism.
3) Suspension of bacterium be carefully poured on a peplated YEMA medium.