

FROM DISEASE FIELD SOIL SAMPLE

Many plant pathogenic bacteria can be isolated from soil, too, as infected leaves and plant debris fall on the ground, are buried in the soil, and the decomposing infected material releases the bacteria in the soil of the plant canopy. Such soils can be used for the isolation of disease, causing bacterium when the symptoms are not available on the young growing leaves, for example, oily spot disease of pomegranate or bacterial wilt of solanaceous crops.

Material Required

Soil sample from diseased plant soil, nutrient agar plates, distilled sterile water, sterile water blank, sterile pipettes, inoculating needle, sterile glass rod, and so on.

Procedure Collect the soil sample from 1 to 3 inches in depth of soil under the plant canopy in case of leaf spot disease and from rhizosphere in case of wilt disease. Add 10 g of the soil sample in 100 ml of distilled water, shake well, and allow to settle. Take 1ml suspension from this to make serial dilution up to 10^{-8} .

Prepare the nutrient sucrose agar medium, sterilize, and add 500 ppm of aureofungin in the sterilized media before pouring into Petri plates. When media solidifies, keep the plates under UV radiation for 30 minutes. Use these plates for plating 0.1 ml suspension of individual serial dilutions in a separate plate. Replicate the plating of serial dilution for three replications. Incubate the plates at $28 \pm 2^{\circ}\text{C}$ in a BOD incubator. Observe the plates for the development of bacterial colonies.

Note: Most of the plant pathogenic bacterial colonies appear in the media after 3 days. Do not pick up the colonies that appear within 24 or 48 hours. The colonies obtained and selected should be assessed for their pathogenic nature.

FROM FIELD WATER SAMPLE

Bacterial plant pathogens can also be isolated from field water and irrigation channel waters. This is specifically true in cases where the bacterial spread is through irrigation water or standing water in the field, for example, the bacterial blight of rice.

Material Required

Field water sample, Whatman filter paper, nutrient agar plates, sterile water blanks, sterile pipettes, inoculating needle, spreading glass rod, and so on.

Procedure Collect the water sample from the field or irrigation channel in a plastic jar or bottle. Filter through a Whatman filter paper no.42 to remove dirt, fungal, and algal structures, if any. Collect the filtrate and use for plating.

Prepare nutrient sucrose agar medium, sterilize, and add 500 ppm of aureofungin in the sterilized media before pouring in the Petri plates. When media solidifies, keep the plates under UV radiation for 30 minutes. Use these plates for plating 0.1ml suspension of the water filtrate. Make five plates of this water filtrate. Incubate at $28 \pm 2^{\circ}\text{C}$ in the BOD incubator. Observe the plates for development of bacterial colonies.

Note: Most of the plant pathogenic bacterial colonies appear in the media after 3 days. Do not pick up the colonies which appear within 24 or 48 hours. The colonies obtained and selected should be assessed for their pathogenic nature.

- **FROM INFECTED SEED MATERIAL**

The bacterial plant pathogens which are seedborne or transmitted through seed (cotton bacterial blight, sesamum leaf spot, chili leaf spot, tomato leaf blight, halo bean blight, etc.) can be isolated from seed material.

- **ISOLATION OF EXTERNALLY SEEDBORNE BACTERIA**

Material Required

Test seed material, nutrient agar plates, sterile forceps, blotter paper, and so on.

Procedure

Collect the seed samples. Put them in sterilized water-soaked blotter paper for 4–8 hours. Transfer these seeds to nutrient sucrose agar plates and incubate at $28 \pm 2^{\circ}\text{C}$ in the BOD incubator. Bacterial growth formed on and around the seed is to be selected, sub cultured, and assessed for pathogenicity.

- **ISOLATION OF INTERNALLY SEEDBORNE BACTERIA**

Material Required Test seed sample, nutrient agar plates, mercuric chloride solution, sterile pestle and mortar, sterile water blank, sterile pipettes, and so on.

Procedure Collect the seed sample. Sterilize the seed in 0.1 percent HgCl_2 solution for 60 seconds followed by three washings of distilled sterilized water. Keep half of the seed on sterile NAS media in Petri plates. The other half of the seed is to be macerated with a sterilized pestle and mortar in 2 ml of distilled sterile water. Pipette out the macerate on NAS media in plate B.

Incubate the plates at $28 \pm 2^{\circ}\text{C}$ in the BOD incubator. Observe the plate and pick up the bacterial colonies which appear after 70 hours. Note: The bacterial colonies that appear in plate B indicate the internally seedborne bacterial infection. If colonies appear in both in both the plates, this indicates both external and internal seedborne Infection.