

## **ISOLATION OF BACTERIAL PLANT PATHOGENS**

Isolation of bacteria from infected disease plant material is an important step in the determination of the cause of the plant disease, and further, to prove Koch's postulate for confirmation of the cause of the disease as bacteria. This is also important to obtain a pure culture of the bacterial plant pathogen for its further identification up to genus and species level.

### **FROM DISEASED PLANT SAMPLE**

For successful isolation of bacterial plant pathogens, the selection of material is important. From young developing lesions the bacteria can be easily isolated. In advanced stages of lesion development, isolation of true pathogenic bacteria becomes difficult due to the overwhelming population of saprophytes, which overgrow the pathogen in isolation plates. If the diseased specimen does not contain young lesions, it is desirable to inoculate macerate of the diseased tissue on to the healthy host plant and resulting young lesions be used for isolation. Before proceeding for isolation, a part of the tissue from the lesion should be subjected to ooze test and staining to ensure the presence of the bacterium.

**Material Required** Diseased plant sample, nutrient agar plates, distilled sterile water, mercuric chloride solution, sterile Petri plates, glass slide, razor blade, dissection needle, inoculating needle, spirit lamp, and so on.

**Procedure** Rinse the selected lesion with the spirit and immediately dip in mercuric chloride solution (1:1000) for 15 seconds and then pass through three changes of sterile water. Rinse a slide in spirit, flame, and allow it to cool. Place the surface-sterilized lesion tissue on the slide in a few drops of water. In the initial ooze test, if the affected tissue shows abundant bacterial streaming, the lesion is cut into halves with a heat-sterilized razor blade and the slide is set aside for 2–3 minutes to permit the diffusion of bacteria into the water drops. The tissue showing feeble bacterial ooze should be teased apart with a sterilized blade to get the bacteria into suspension. The suspension thus prepared is used for isolation by any of the following methods.

#### **1. Streak plate method**

Prepare nutrient agar medium with the following constituents: Nutrient agar (NA) medium: Peptone, 10 g; beef extract, 5 g; agar, 20 g; water, 1000 ml; pH, 7.0. Pour about 25 ml of sterilized lukewarm nutrient agar in Petri plates and allow to solidify for 1 hour; then invert the plates. After 2–3 hours these plates are used for isolation purposes. Streak a loopful of

the suspension prepared as in the preceding section over the agar surface by the to-and-fro method with the help of the inoculation needle. Streak two more plates without recharging the wire loop with bacterial suspension. Label the plates and incubate in an inverted position at 25°C and examine daily. Most bacterial plant pathogens develop colonies within 3–5 days, but some of them may take as long as 10 days. Single colonies are usually obtained on second or third plate.

### **Spreading diseased tissue macerate on NA medium**

In those plant samples where oozing is not visible, the isolation of bacteria is done by the tissue macerate method. Sterilize the disease sample in 0.1 percent mercuric chloride solution for 1–2 minutes, wash with distilled sterilized water thrice, and macerate with a pestle and mortar in sufficient sterilized water. Allow the macerate to settle down. Pipette out 0.2 ml of supernatant of macerate and place on NA medium on the previously divided plates. Streak the supernatant in each compartment of the plate. Incubate the plate and observe for colony development.

### **Spreading dilutions on solid agar**

Prepare nutrient agar medium plates as in the streak plate method. Dilute the bacterial suspension prepared from the infected tissue serially in 5 ml of sterile water blanks. Usually three dilutions will suffice. Put one drop of each of the original suspension and three serial dilutions in four agar plates separately. In each plate spread the drop uniformly with a sterile bent glass rod. Incubate the plates and observe for single colony development.

### **Pour plate method**

The nutrient agar medium for this method should contain 1.5 percent agar. Sterilize the medium in test tubes in 20-ml quantities and allow to cool to 45°C. Inoculate one tube with 0.1 ml of the suspension from diseased tissue macerate. Mix thoroughly by rotating the tube between your palms. Transfer one loopful of this mixture to the second tube and mix thoroughly. Remove one loopful of the mixture from the second tube, transfer to the third tube, and mix thoroughly. Pour the above three dilutions in three separate Petri plates. Label the plates and incubate in inverted position after the agar has solidified.