

CONFIRMATION OF BACTERIAL PATHOGEN IN DISEASED SAMPLES

Before proceeding to isolate the bacterium from a diseased plant, it is desirable to confirm the presence of the bacterium in the affected tissues. This can be done through the following tests.

BY OOZE TEST This test is more appropriate to detect the localized bacterial infection of leaves, stem, buds, flower, and fruit.

Material Required Disease sample, glass slide, cover slip, distilled sterile water, microscope, razor blade, tissue paper, and so on.

Procedure Wash an infected diseased portion in running tap water to remove the dirt and external microbes. Dry the sample with blotter paper/tissue paper. Cut a piece of the infected tissue portion with the razor blade on a sterilized glass slide. Put a few drops of water on the cut portion and put on the cover slip. Keep the slide at room temperature for 5 minutes to allow the bacterial ooze to come out. Examine under low power objective.

Observation If the infection is due to bacteria, a cloudy mass of bacterial cells will be seen oozing through the cut ends of the plant tissue pieces.

Important Guidelines Some idea is also obtained as to whether the infection is parenchymatous or vascular. In vascular infections, bacteria oozes out forcefully at distinct points corresponding to the vascular strands; in parenchymatous infections, the oozing of the bacteria is slow, diffused, and throughout the cut ends. This test works well in a majority of the bacterial diseases. Difficulty may be encountered in diseases like crown gall, hairy root, and leafy gall, where all the affected tissue may not contain the bacterial cells. In tissues that contain large quantities of starch, dispersal of starch grains in water may mask the bacterial ooze and make it difficult to recognize. However, with some experience it is possible to distinguish bacterial ooze from starch grains.

BY MILKY WATER TEST This test is more appropriate to detect the systemic and vascular infections in leaves, stem, and roots. This test is generally performed for detection of bacterial blight of rice bacterium.

Material Required Disease sample, distilled sterile water, test tube, razor blade, tissue paper, and so on.

Procedure Take an infected disease sample and wash in running tap water to remove the dirt and external microbes. Dry the sample with blotter paper/tissue paper. Take a sterilized test tube with 10 ml of distilled water in it. Cut a piece of infected leaf, stem, or root sample. Immerse a cut portion of the infected sample in test tube water and allow it to stand for 3 to 5 minutes.

Observation Observe a strand of bacteria vigorously coming out of the infected cut portion, which turns the clear water to a whitish turbid or milky appearance.