

CONFIRMATION OF BACTERIAL PATHOGEN IN DISEASED SAMPLES

BY STRING TEST The string test is used to confirm the bacterial pathogen in disease sample of vascular wilt caused by bacterial pathogens. The test is particularly used to detect *Pseudomonas/Ralstonia* wilt pathogens in the roots of eggplant, tomato, tobacco, potato, and so on.

Material Required Wilted plant along with root, distilled sterile water, beaker, razor blade, tissue paper, and so on.

Procedure Collect the wilt diseased plant. Separate the root from the crown portion of the plant. Wash the root in tap water to remove the soil particles and dry it in blotter paper. Take distilled sterile water in a small beaker or test tube. Cut a portion of root at 45° angle and hang the cut end in water with the help of a thread and leave for 10 to 15 minutes. A string of bacterial ooze will be observed with the naked eye in the water. The string ooze confirms the involvement of bacteria as the disease causal agent.

BY STAINING OF CRUSHED DISEASE PORTION At times when the bacterial population is very low in the affected tissue, as in the case of young lesions of red stripe of sugarcane and *Xanthomonas* leaf spot of mung beans, distinct bacterial ooze may not be detected. In such cases, staining of smears from diseased tissue may reveal the presence of bacterial cells.

Material Required Infected leaf sample, glass slide, distilled sterile water, razor blade, crystal violet stain or carbol fuchsin stain, Bunsen burner for flame, tissue papers, microscope, cedar oil, and so on.

Procedure Take an infected disease sample and wash in running tap water to remove dirt and external microbes. Dry the sample with blotter paper/tissue paper. Take a piece of the diseased tissue and crush it in a drop of water with the razor blade on one end of the glass slide. Allow it to stand for 1 minute and then tilt the slide so that the water suspension flows toward the other end of the slide, leaving most of the crushed host tissue behind. The smear so prepared is dried and fixed by passing over a flame. Flood the smear with crystal violet solution or carbol fuchsin and allow to stand for 1 minute. Drain the stain and wash the slide thoroughly in running tap water. Blot dry and examine under oil immersion objective for presence of bacterial cells.

Observation If presence of the bacterium is not detected by the ooze test or by staining, there is no use trying isolation from such material.