

PURIFICATION OF PLANT PATHOGENIC BACTERIAL CULTURES

During the isolation of bacterial plant pathogen from diseased plant samples, several nonpathogenic and saprophytic bacterial colonies also grow in the isolation plates. Generally, such colonies appear much earlier than plant pathogenic bacterial colonies. Sometimes the saprophytic bacterial colonies are intermingled with plant pathogenic bacterial colonies. Therefore, picking the pathogenic bacteria and their purification is an important step to obtain pure culture of bacterial plant pathogen. Further, during the preservation of bacterial cultures, sometimes the cultures are contaminated with other microbes and therefore purification is a necessary aspect in the maintenance of these cultures.

Material Required

Plates of isolated bacteria, nutrient agar plates, inoculating needle, marker, incubator, and so on.

Procedure

SELECTION OF SINGLE BACTERIAL COLONIES

With well-chosen diseased tissue, it is often possible that only the colonies of the pathogen develop. However, many times the colonies of saprophytic bacteria develop along with plant pathogenic bacteria. If more than one type of colonies are seen, it is preferable to select the representative colonies of the bacteria. Further, the colonies that came up more slowly are likely to be that of pathogenic bacteria. For instance, in isolation from neem leaves infected with *Pseudomonas azadiractae*, numerous white, bold, saprophytic colonies develop frequently within 48 hours and it is not until 4–5 days later that a few white, glistening, convex colonies of the pathogen become visible. In general, the colonies that appear before 48 hours are not likely to be that of a pathogen. In isolation of previously undescribed pathogens, sometimes it may be desirable to select two or three types of colonies. After multiplication on nutrient agar medium, these are tested for hypersensitive reaction and pathogenicity. The one which proves pathogenic is retained and the others discarded.

PICKING OF SINGLE COLONIES AND SUBCULTURE

After selecting the right type of colonies, transfer them to the nutrient agar slants. Touch as small wire loop of a bacterial inoculation needle to a well isolated colony and streak it on the agar slant in a tube. Incubate the tube at $28 \pm 2^{\circ}\text{C}$ for 48 hours to obtain sufficient bacterial growth.

CHECKING THE PURITY OF THE ISOLATED CULTURE

The cultures obtained by single colony transfer are checked for purity. Make a dilute suspension of the culture in distilled sterilized water and streak on the nutrient agar plates. If the culture is pure only one type of colony with original characteristic should develop. Only the culture obtained from pure colonies should be maintained and used for further investigations.