Practical No. 4

DEMONSTRATION OF KOCH'S POSTULATES FOR BACTERIA

Erwinia carotovora is the etiologic agent of soft-rot of several plants. *E.carotovora* - carrot system will be used to demonstrate Koch's postulates.

Materials

Infected carrot with *E.carotovora,* Healthy carrots, Nutrient agar plates, Scalpel, Potato peeler, Forceps, alcohol, sterile Petri plate with filter paper, Sterile water, Disinfectant, Gram- stain reagents, Inoculation loop, Bunsen burner

Procedure

1. Isolate the bacterial pathogen from the infected carrot
2. Purify the bacterial culture, if contaminated, following purification technique.
3. Prepare a bacterial smear from the pure culture and gram stain it.
4. Wash the healthy carrot well
5. Peel the carrot
6. Allow it to dry
7. Surface sterilize it with disinfectant rinse with 2-3 changes of sterile water
8. Cut the carrot into slices (5 to 8 mm thick) with alcohol dipped and flamed scalpel.
9. Using flamed forceps, transfer four carrot slices into sterile Petri plate lined with filter paper
10. Inoculate the centre of three slices each with a loopful of bacterial culture; the fourth carrot slice should be kept as un-inoculated control.
11. Saturate the filter paper with sterile water.
12. Incubate the plate at ' room temperature (25°C) for 3-5 days or until soft rot appears.
13. Streak an inoculums from the diseased carrot on the nutrient agar plate.
14. Incubate the inoculated plate in an inverted position for 48 hours at room temperature.
15. Prepare a smear from the nutrient agar culture
16. Make a smear from the disease carrot too
17. Gram stains both the smears.

Observations and Results

Observe the infected carrot and artificially inoculated carrot pieces for soft-rot symptoms. Observe the gram stain preparations for the morphology and gram reactions, all the three smears.

If the disease symptoms are produced on artificially inoculated carrot pieces and the morphology and Gram-stain reaction in the three smears are the same (i.e. the pathogen is the same, *Erwinia carotovora*) the Koch's postulates are proved.