**DEMONSTRATION OF KOCH'S POSTULATES FOR PLANT PATHOGENIC 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.