Practical 2-3

DEMONSTRATION OF KOCH'S POSTULAES FOR FUNGI

The etiologic agent is the cause of a disease. Microorganisms are the etiologic agents of a wide variety of infectious plant diseases. Microbes can be parasites, living in or on and getting nutrients from its host, another living organism or pathogens, a parasite able to cause a disease in a particular host or range of hosts. All parasites may not be pathogens. Parasites may be obligate (biotrophs) if restricted to living tissues or facultative when they also colonise dead tissues in addition to living. The sequence of processes in disease development from initial contact between pathogen and host to completion of syndrome is called pathogenesis. The ability of pathogen to cause disease is called pathogenicity that depends upon the susceptibility of the host to the pathogen and on the virulence, the degree of pathogenicity of the pathogen.

The actual cause of many diseases is difficult to determine. Although many organisms can be isolated from a diseased tissue, their presence does not prove that any or all of them caused the disease due to the fact that the isolated microbe may be part of the normal flora or transient flora of that area or a secondary invader. The pioneering German microbiologist, Robert Koch, identified a set of four conditions which has to be satisfied to establish that a particular organism is the causative agent of a particular disease. These conditions known as Koch's postulates are:

1. The suspected pathogen must be found associated with the disease in all the diseased plants examined.
2. The organism must be isolated from the diseased tissue and grown in pure culture on nutrient media and its characteristics described (non-obligate parasites) or in a susceptible host plant (obligate parasites), and its appearance and effects recorded.
3. When a healthy plant, of the same species or variety, is inoculated with this culture, it must produce the disease and show the characteristic symptoms.
4. The organism must be re-isolated from the inoculated plants and must be shown to be the same pathogen as the original. If all the above steps have been followed and proved true, then the isolated pathogen is identified as the organism responsible for the disease.

a. Demonstration of Koch's postulates for fungal phyto-pathogens

Drechslera oryzae is the etiologic agent of brown leaf spot of paddy and will be used to demonstrate Koch's postulates

Materials required

Diseased paddy leaves infected by brown leaf spot, Paddy seeds susceptible to brown leaf spot disease, pure culture of *Drechslera oryzae*, sterile Petri-dishes, sodium hypochlorite (1%), sterilized distilled water, sterilized soil, hand sprayer, glass slides, needles, lacto phenol and microscope.

Procedure

1. Take diseased paddy leaf infected by brown leaf spot along with some healthy tissue.

2. Cut into small pieces (2-5 mm) containing both the' diseased and healthy tissue and keep in sterile Petri dishes
3. Dip the pieces into 1 % sodium hypochlorite solution for about one minute.
4. Transfer the pieces to Petri - dishes containing sterile distilled water and wash thoroughly in two changes of sterile water to free them from the chemicals if any.
5. Wash hands with rectified spirit and wipe the table top of inoculation chamber with rectified spirit.
6. Lit the burner
7. Hold the flask containing sterile Luke warm PDA in the right hand and remove plug near the flame. Lift the lid of Petri dish gently with left hand and pour about 20 ml of medium. Close the mouth of the flask with plug near the flame
8. After solidification of the medium, place four sterilized pieces at different distance in a single PDA plate.
9. Incubate the Petri dishes in an inverted position at 25° C and examine for 3- 5 days.
10. Select seeds of variety of paddy susceptible to brown leaf spot disease
11. Surface sterilize the seeds by dipping in 1 % sodium hypochlorite solution for one minute.
12. Wash thoroughly with sterile distilled water.
13. Sterilize the soil in an autoclave at 20 Ibs pressure for 2 hours consecutively for three day
14. Fill up the pots with sterilized soil.
15. Sow the seeds in soil contained in pot
16. Keep the soil moist by regular watering with sterilized water.
17. Keep the pots under controlled conditions to avoid any type of contamination.
18. When the plants have put forth five leaves, use the plants for inoculation.
19. Prepare a suspension of the spores from pure culture of the test fungus,
20. *D. oryzae* at desired concentration.
21. Spray the spore suspension on the leaves with a hand sprayer (atomizer).
22. Place the pots in moist chamber and incubate at 25° C for 48 hours. Cover the
23. Incubated plant with a bell jar /polythene bag if moist chamber is not available.

24. After 48 hours remove the pots from the moist chamber and place on the greenhouse bench. The disease manifests itself within a few days.

Observation and results

a) Examine the plants daily, note the symptoms and its development and study the morphology of the pathogen in advance stage. Compare the developed symptoms with original diseased specimen from which the test pathogen was isolated.

b) For, confirmation, re-isolate the pathogen and compare with, the culture isolated from the original specimen.