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Isolation and Characterization of Plant Pathogens from *Phaseolus lunatus* (Lima Beans) and *Solanum melongena* (Brinjal)

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During an investigation of the disease profile of *Phaseolus lunatus* (Lima beans) and *Solanum melongena* (Brinjal). Repeated isolations from infected part of plant showed the association of the bacterial and fungal pathogens were identified *Xanthomonas oryzae, Bacillus subtilis, Pseudomonas putida, Lactobacilus, Corynebacterium, Bacillus cereus, Pseudomonas fluorescens Klebsiella, Staphylococcus aureus, Enterobacter, Fusarium solani, Candida albican, Aspergillus flavus, Penicillium. Trichoderma viride, Mucor, Aspergilus niger, Fusarium oxysporum.* The identified fungal hyphal development using slide culture technique .These pathogens were grown in groundnut plant and there was significant decrease in the crude protein and fibre contents with increasing disease severity.

Key words: *Phaseolus lunatus, Solanum melongena*, Plant pathogens, Slide culture technique.

The plant surface is a natural habitat, which represent a hetrogenous population of microbes comprising of both pathogens and non-pathogen. Qualitative assessment indicator that there microbes comprise a wide range of organism including yeast, filamentous fungi,bacteria, actinomycetes and blue green algae. The fluorescent pseudomonad *P. brassicacearum* can have both pathogenic and plant growth-promoting effects on tomato plants[1]. The sporulation characteristics formed one of the important criteria for identification of the pathogen up to genus or species level [2,3]. Diurnal pattern of release and survival of sporangia of Phytophthora phaseoli and the effect of leaf wetness duration and timing on the incidence of downy mildew of lima bean.

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MATERIALS AND METHODS

Collection of samples

The infected parts of plants like leaves were collected from naturally infected plants of *Phaseolus lunatus* and *Solanum melongena* from Thiruthuraipoondi ,Thiruvarur District .

Isolation[4]

The collection of infected leaves were sterilized with 1% Nacl for 1 minutes and washed thrice in sterile water and then samples were crushed in a pestle and mortar.Then the fresh sap was collected in sterile screw capped bottle[6]. Serial dilution were performed by using the collected leaves samples to isolate the bacterial and fungal pathogens ,From that the crushed leaves samples were diluted with tube containing 9ml of sterile distilled water and mixed thoroughly to make a 1:10 dilution (10^1) . Then 1ml of diluted sample was transferred to the next tube and serially diluted into the series of test tubes having 9ml of sterile distilled water with sterile pipettes, up to 10

dilutions.0.1ml of serially diluted sample was taken from 10^{-4}-10^{-7} dilution and was spreaded over the nutrient agar plates were incubated at 37°C for 24 hours. After incubation to form a colonies observed on the plates.0.1ml of serially diluted sample was taken from 10^{-2}-10^{-5} dilution and were spreaded over the potato dextrose agar (PDA) plated medium and the plate were incubated at 30°C for 2-3 days [5].

Identification bacterial and fungal pathogens[7]

After incubation isolated bacterial pathogens were identified by Gram staining technique, Motility test and Biochemical test [1]. A thin smear of bacterial isolates were separately made on a clean glass slide and heat fixed. Then the smear was stained by crystal violet for 1 minutes, and then washed with water followed by flooded with gram's iodine, After 1 minutes the slide was washed again in tap water and alcohol. After decolorizaion, the smear was counter stained with safranin for one minutes. The slide was washed and air dried , finally it was observed under microscope. Petroleum jelly was applied around the cavity slide. A loopful of bacterial isolate was placed in the center of clean coverslip. The petroleum jelly was applied on the concave slide surface facing down over the coverslip. Then the slide was observed under the microscope. The motility of the bacterial isolates was observed.

Slide culture technique [5]

A-V Shaped bent glass rod,a microscopis slide and two cover slip were placed in a petriplate, sterilized in a hot air oven at 160°C for 1 hour.A small squre agar block was cut from PDA plate using sterilized scapel and it was placed on the glass slide with the help of an inoculating needle,the growth from the colony with the supporting agar was removed and placed on the glass with the help of inoculating needles,it was teased into small bits and were transferred to the corners and edges of the block. A coverslip was placed over the agar and was pressed gently to seat firmly on the agar. These slides were placed inside the petriplates having water soaked cotton and incubated at room temp (25°C-35°C). The preparation was examined every 48 hours for the growth to occur over the coverslip and slide. When sufficient growth has occurred, the coverslip was removed with a sterile forceps and transferred to a drop of LPCB on a glass slide. The agar block was removed and discarded in the discarding jar. A drp of LPCB was added to the growth on the glass slide and covered with a coverslip. Two LPCB

Table 1. Morphological and cultural characteristics of bacterial isolates from *Phaseolus lunatus* (Lima beans) and *Solanum melongena*

S.No.	Isolated organism	Morphological characteristics	Cultural characteristics
1	*Xanthomonas oryzae*	Gram +ve,Rod shaped, nonmotile, flagellated,Whitish	Host resistence, common plant pathogen,34-64 kilobases,mucoid & smooth colonies
2	*Bacillus subtilis*	Gram+ve, Thick rods in chains, non motile, Spore former	Large, round, irregular mucoidal, fast growing colonies
3	*Pseudomonas putida*	Gram-ve ,rod shaped,motile,Non spore former	Bluish green, Small irregular opaque colony,16s r RNA analysis,
4	*Lacto bacillus*	Gram +ve , rod shaped ,Facultative anaerobic	Pink colour, Lactic acid bacteria group ,P^H-5.0
5	*Corynebacterium*	Gram +ve, rod shaped, Eubacteria, Non motile, Non pathogenic.	16s rRNS,high G-C content, Non spore forming ,gray colour
6	*Bacillus cereus*	Gram+ve, Thick rods in chains, non motile, Spore former	Large, round, irregular mucoidal, fast growing colonies.
7	*Pseudomonas auerоginosa*	Gram+ve ,rod shaped,motile,Non spore former.	Bluish green, Small irregular opaque colony,16s r RNA analysis.
8	*Klebsiella pneumoniae*	Gram –ve , Non motile,Encapsulated, Rod shaped bacterium.	Facultative anaerobics,Lactose fermenting.
9	*Staphylococcus aereus*	Gram+ve coccal bacterium,Virulance facter.	Golden grape cluster berry colour,Facultative anaerobic.
10	*Enterobacter aerogens*	Gram-ve rod shaped,Non motile, Non spore forming.	Entrobacteriaceae, Opportunistic pathogen,Variety of size.

mounts obtained by this method were examined at 10x, 45x for characteristics morphology. Gram character of bacterial isolates was noted.

RESULTS AND DISCSSION

The ten bacterial and eight fungal pathogens were isolated from the infected lima beans and brinjal plant and were identified by means of morphological, cultural and bio chemical characteristics features which was shown in table 1 and 2

The bacterial pathogens were isolated from infected leaves of Lima beans *Xanthomonas oryzae, Bacillus subtilis, Pseudomonas putida, Lactobacilus, Corynebacterium* and fungal pathogens like *Fusarium solani, Candida albicans, Aspergillus niger, Aspergillus flavus, Penicillium. Trichoderma viride.* Another bacterial pathogens were isolated from infected leaves of brinjal like *Bacillus cereus, Pseudomonas fluorescens, Klebsiella, Staphylococcus aureus, Enterobacter* and fungi isolate *Mucor, Aspergillus niger, Aspergilus flavus, Fusarium solani, Trichoderma viride, Fusarium oxysporum*[2].

Table 2. Morphological and cultural characteristics of fungal isolates from *Phaseolus lunatus* (Lima beans) and *Solanum melongena*

S .No	Isolated organism	Morphological characteristics	Cultural characteristics
1	*Fusarium solani*	Hyphae are hyaline,Branched and septate.Conidia are formed in slimy.	Condia are 2 types. Micro conidia and macro conidia. Micoconidai is one celled, oval or comma shape.Macro conidia is hyaline, spindle shaped with pointed end.
2	*Candida albicans*	Diploid fungus, Multi filamentous form, Oppurtunistis,Commensals.	Unicellular Yeast like form, Diamorphism.
3	*Aspergillus niger*	Globose vesicles has a concave under surface and long sterigmata.	Blachspores with salt pepper appearance
4	*Aspergillus flavus*	Globose, subglobose, Elliptical vesicle, Sterigmata cover entire surface or 3/4 th surface.	Yellowish green in colour, Velvety in texture.
5	*Trichoderma viride*	Conidia from globular clusters on conidiophores.	Colonies are Spreading at first white becoming either white or light green or deep green shades with age.
6	*Penicillium*	Long conidiospores,flask shaped phialids,tapered sterigmata.	Rapidly folded green to bluish green pigment spores.
7	*Mucor*	Fast growing,Globular sporangia, Column –shaped columella.	Asexual Reproduction, Moulds,White or gray,
8	*Fusarium oxysporum*	Commen vascular,Kidney shaped mycelia,asexual spores.	World wide fungus,Microconidia, Chlamydospores.

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