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Research Article

ISOLATION AND IDENTIFICATION *XANTHOMONASSP* FROM VEGETABLE CROP FIELD

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ABSTRACT

Phytopathogenic bacteria and fungi utilise the vegetables and plant cells as privileged food sources and cause devastation under appropriate environmental conditions. In the present study, investigation deals with the isolation and identification of the phytopathogens from okra field of Mylaudy in Kanyakumari District, Tamil nadu., Different groups of microbes on okra can have diverse effects on human health and economic loss to the farmers.

Isolation of the bacteria was performed by leaf imprinting techniques on okra leaf. The pathogenic bacteria namely *Xanthomonas species* was identified on the basis of morphological, cultural characteristics and biochemical profile

INTRODUCTION

Vegetables are the important food as they supply sufficient amounts of phenolics, flavonoids, ascorbic acid, riboflavin, folic acid, carotene, and minerals (Khanzadi, 2011). Of the vegetable okra is an annual, sometimes biannual crop, requiring warm grows well in warm weather and should be planted in full sun. Okra starts yielding about 60 days after planting. Its medicinal value has also been reported by Siesmonsma and Hamon (2002). These vegetables are susceptible to phytopathogen along with the number of destructive pest both in the field and the storage The total loss of vegetable on this account has been estimated up to 20-30% but if the pathogens are allowed to develop, this loss may increase up to 80-90% (Glazebrook, 2005). Bacterial and fungal diseases cause heavy losses in vegetables. Hence the present study was deals with the isolation and identification of phytopathogenic bacteria from the leaf of okra .

MATERIALS AND METHODS

Source of plant material

From the okra cultivated field, affected leaves was collected in sterile polythene bags and brought to the laboratory.

Isolation of phytopathogen by leaf imprinting method

Infected leaf were surface sterilized in 70% alcohol and washed in three series of sterile water to remove traces of alcohol. Infected leaf was placed onto sterile nutrient agar plates and kept at 37^0C for 4-7 days for the development of colonies. (Abdul *et al*., 2014).

Identification of pathogen

Bacteriological tests were performed according to the determinative schemes described by Dye 1962. Phytopathogen bacteria was isolated based on colony morphological. Identification was made by performing various bio chemical characteristics, microscopic observation.

Pathogenecity test

Pathogenecitytest was conducted by following steps outlined in Koch's postulates. This involved artificial inoculation of sterile healthy okra crop leaf (2 mo old) in a pot with (10^{8}CFU ml^{-1}) cell suspension of *Xanthomonassp*in water with a plastic spray bottle until run -off and incubated at 37^oC. Negative control plants were sprayed with sterile water. Re-isolation was made from artificially infected leaf and the isolates was compared with the artificially introduced inoculum.

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RESULTS

Bacteria isolated by imprinting method

Isolated colonies was observed in Nutrient agar plates/Potato Sucrose agar after 4-7 days of incubation by leaf imprinting technique.

Identification of bacterial isolates from okra leaf

Bacteria *Xanthomonassp* was identified based on their morphological characteristics, Gram's staining, Further Biochemical tests were conducted to ascertain the genus of the bacteria. The test results depicted in (Table 1,2).The results were compared with the Bergey's manual of determinative bacteriology

Table 1 Gramreaction and cultural characteristics of bacterial isolates

S.No	Source	Isolated Organism	Gram Reaction and shape	Culture Characteristics on nutrient agar
1	Okra leaf	*XanthomonasSp*	Gram negative, rod	Smooth, circular, yellow colonies with a yellow pigment

Table 2 Biochemical characteristics of bacterial isolates

S.no	Test	*Xanthomonassp*
1	Motility	Motile
2	Mucoid growth	+
3	Indole	+
4	TSI	+
5	Nitrate Reduction test	+
6	Gelatin	+
7	Aesculin	+
8	Starch	+
9	Milk proteolysis	+
10	Catalase	+
11	Oxidase	-
12	Urease	-
13	Growth at 36^{O}C	+
14	Growth at 4 ^{O}C	+
15	Growth on 2%NaCl	+
	Acid from:	
16	Arabinose	+
17	Mannose	+
18	Galactose	+
19	Trehalose	+
20	Cellobiose	+
21	Fructose	+

+ indicate positive reaction - indicate negative

Pathogenecity test

Small yellowish -green to brown spots on the leaves and yellowing and stunding of the plant was observed followed by wilting and rolling of the leaves in okra. Artificially inoculated plates also showed the presence of yellow or orange colonies and it confirms koch postulates.

DISCUSSION

Furthermore, the common sites of bacterial colonization on leaf surface are base of trichomes, stomata, cell wall junctions, vein endings and even beneath the cuticles (Mansvelt and Hattings 1987, Mariano and McCarter 1993, Knief*et al.* 2010).

In my present study also observed the bacterial colonization from the surface of leaves. It is apparent that bacterial colonies in such sites are less likely to be recovered in a leaf imprint method made on the nutrient agar. Leaf washings, on the other hand, can possibly remove cells from the colonies situated even under cuticle through pores and cracks (Corpe and Rheem 1989).

The efficacy of our sterilization was confirmed by imprinting randomly selected surface sterilization leaf disc MEA in seperate plate (Schultz and Boyle 2005)results of leaf imprint method showed the highest population on the leaves of *C. incanus*.The ability to detect presence of bacterial colony on leaf surface is higher for the leaf imprints(Ram*et al.*,2011).In our present study results of leaf imprint method showed the highest population on the leaves of okra.

Penetration of stomata allows the bacteria to enter the interior air spaces of the mesophyll, where they start to multiply and produce biofilms consisting of high amounts of xanthans. In our present study bacteria was observed through gram staining technique.

The soft -rotting xanthomonads obtained in the study could be readily differentiated from other yellow or orange bacteria were identified as *Xanthomonassp* on the basis of 10 physiological test (Liao and Wells 1986). In our present study *Xanthomonassp* was identified based on various biochemical test.

Invasion of *Xanthomonas* via hydathodes of leaves has been visualized after air-pressurized spraying of the abaxial surface of the leaves fewer lesions were found. Small yellowish -green to brown spots on the leaves and yellowing and stunding of the plant was observed followed by wilting and rolling of the leaves in okra(Abdul *et al.,* 2014). In our present study yellowish -green to brown spots on the leaves was observed followed by wilting and rolling of the leaves in okra.

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