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VARIABILITY OF DIFFERENT ISOLATES OF *XANTHOMONAS AXONOPODIS* PV. *CITRI* CAUSING CITRUS CANKER DISEASE

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ABSTRACT

Acid lime (*Citrus aurantifolia*) is grown different agro-climatic situation in West Bengal. The crop is commonly affected by citrus canker caused by *Xanthomonas axonopdis* pv. *citri*. There was variation in severity of the disease in different orchards. *Xanthomonas axonopodis* pv. *citri* isolated from five different orchard showed variation in respect to virulence and enzymatic activity. Isolates of the pathogen were Xac_{JP} from Jagannathpur, Paschim Medinipur, Xac_{J} from Jaguli, Nadia, Xac_{K} from Kalyani, Nadia, Xac_{MON} from Mondouri, Nadia, Xac_{MHP} from Mohanpur, Nadia. Modouri isolate (Xac_{MON}) took minimum time (4 days) for expression of canker symptom on artificial inoculation and the lesion size was maximum. While maximum time (6 days) was taken for symptom expression by Xac_{JP} and lesion size was minimum. Others three isolates took 5 days for symptom expression. Highest cellulase activity was observed in Xac_{MON} followed by Xac_{K}, Xac_{J} and Xac_{MHP} isolate. The lowest cellulase activity was observed in Xac_{JP}. Similarly highest pectinase and protease activity was recorded in $Xac_{.MON.}$ isolate. But the highest pectinase activity observed in Xac_{MHP} isolate. However, highest lipase activity was observed on $Xac_{.JP.}$ Xac_{MON} isolate was found to be highly virulent and virulence had positive correlation with cellulase, pectinase and protease activity.

INTRODUCTION

In West Bengal citrus occupies an area of 11.3 thousand hectare with a production of 100.9 thousand metric tones (Indian Horticulture Database, 2010). Citrus canker is one of the most feared diseases of citrus, affecting all types of important citrus crops. The disease causes extensive damage to citrus and severity of this infection varies with different species and varieties and the prevailing climatic conditions. Among the commercial cultivars, acid lime (*C. aurantifolia*) is the most susceptible one and up to 50-60% yield reduction has been reported (Das, 2003). Citrus canker is mostly a leaf spotting and rind blemishing disease (Sahi *et al*, 2007). It is one of the biggest problems in citrus production worldwide (Stall *et al*, 1988). The occurrence of citrus canker disease in the plains of subtropical region in West Bengal is also a serious concern like many other citrus growing areas in India but little information are so far available about the serious disease under west Bengal condition. There was variation in severity of the disease in different orchards. Keeping this view, the present research programme was framed to study variation among the isolates *Xanthomonas axonopodis* pv. *citri* in respect to virulence and enzymatic activity.

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MATERIALS AND METHODS

Citrus canker infected leaves were collected from five different orchards. The causal pathogen, *Xanthomonas axonopodis* pv. *citri* (*Xac*)was isolated in Potato sucrose peptone agar medium following standard bacteriological technique and maintained in the same medium. For pathogenicity test selected leaves were inoculated by leaf infiltration technique (Klement, 1963). Observations were recorded at 24 hours interval for development of disease. Enzymatic Assay

Cellulase activity

A medium containing CMC-5g, Agar-17g, and Water-1000 ml was sterilized in conical flask by autoclaving and allowed it to cool up to 45^0C. It was then poured to Petriplate and allowed to solidify. Such plates were spot inoculated with 48 hours old bacterial culture and kept in BOD incubator for 4 days at 28^0C. The plate was then flooded with congo red solution (1mg/ml) for 15 min. The Congo red solution was then poured off and the plates were further de-stained by flooding with 1(M) NaCl solution for 15 minutes. The bacterial growth surrounded by clear orange zone indicated hydrolysis of cellulose.

Lipase activity

A medium containing (Peptone-8g, $Cacl_2H_2O$-0.1g, Agar-20g, Water-1000ml) in one flask and Tween (Polyoxyethylene sorbitan monolaurate 20 or 80) in another flask were sterilized by autoclaving and allowed it to cool up to 45^0C. These were then mixed together. Mixed medium was then poured to petriplate and allowed to solidify. Such plates were spot inoculated with 48 hours old bacterial culture and kept in BOD incubator for 4 days at 28^0C. A whitish zone was seen where the bacterium able to hydrolyse lipase

Protease activity

A medium containing, Skim milk agar- 51.5g and distilled water-1000 ml was sterilized in flask by autoclaving and allowed it to cool at 45^0C. It was then poured in sterilized petriplate and allowed to solidify. The plates were inoculated like the earlier experiment. After four days incubation, a clear halo zone was seen surrounding the bacterial growth where bacterium could able to hydrolyze protease.

Pectinase activity

A medium containing, Pectin-5g, yeast extract-5g, Agar-16g, water-1000ml, petriplate was spot inoculated *Xanthomonas axonopodis* pv. *citri* and incubated at 28^0C for five days.

Table 1. Pathogenecity test of *Xanthomonas axonopoidis* pv. *citri* isolates

Isolates	Place of collection of the isolates	Mean length of lesion (mm.) A	Mean breath of lesion (mm.) B	Size of lesion A x B (mm^2)	No. of days taken to initiate symptom
Xac $_K$	Kalyani Nadia	13	9	117.0	5
Xac $_J$	Jaguli, Nadia	10.5	10	105.0	5
Xac $_{MHP}$	Mohanpur, Nadia	15	9	135.0	5
Xac $_{MON}$	Mondouri, Nadia	17	10	170.0	4
Xac $_{JP}$	Jagannathpur, Paschim Medinipur	11	5	55.0	6

Table 2. Cellulase activity of *Xanthomonas axonopoidis* pv. *citri* isolates

Name of the *Xac* isolate	Bacterial growth (mm)	Halo zone(mm)	Halo zone - Bacterial growth (mm)	Halo zone /Bacterial growth (mm)
Xac $_K$	0.7	3.4	2.7	4.86
Xac $_J$	0.6	2.9	2.3	4.84
Xac $_{MHP}$	0.7	3.0	2.3	4.29
Xac $_{MON}$	0.6	3.0	2.4	5.0
Xac $_{JP}$	0.9	3.1	2.2	3.45

Table 3. Lipase activity of *Xanthomonas axonopoidis* pv. *citri* isolates

Name of the *Xac* isolate	Bacterial growth (mm)	Halo zone(mm)	Halo zone - Bacterial growth (mm)	Halo zone /Bacterial growth (mm)
Xac $_K$	1.4	2.6	1.2	1.86
Xac $_J$	1.7	2.9	1.2	1.71
Xac $_{MHP}$	1.4	2.4	1.0	1.72
Xac $_{MON}$	1.3	2.0	0.7	1.54
Xac $_{JP}$	0.7	2.4	1.7	3.43

Table 4. Protease activity of *Xanthomonas axonopoidis* pv. *citri* isolates

Name of the *Xac* isolate	Bacterial growth (mm)	Halo zone(mm)	Halo zone - Bacterial growth (mm)	Halo zone /Bacterial growth (mm)
Xac $_K$	0.9	1.4	0.5	1.56
Xac $_J$	0.9	1.8	0.9	2.0
Xac $_{MHP}$	1.0	2.6	1.6	2.6
Xac $_{MON}$	1.1	3.2	2.1	2.99
Xac $_{JP}$	0.7	1.4	0.7	2.0

Table 5. Pectinase activity of *Xanthomonas axonopoidis* pv. *citri* isolates

Name of the *Xac* Isolate	Bacterial growth (mm)	Halo zone(mm)	Halo zone - Bacterial growth (mm)	Halo zone /Bacterial growth (mm)
Xac $_K$	0.6	2.4	1.8	4.0
Xac $_J$	1.0	3.7	2.7	3.7
Xac $_{MHP}$	0.7	3.6	2.9	5.15
Xac $_{MON}$	0.7	2.8	2.1	4.0
Xac $_{JP}$	0.9	3.3	2.4	3.67

It was then flooded by with 1% tri-methyl ammonium bromide for 48 hrs. Pectinase activity will be positive if the clear zone observed surrounding the bacterial colonies.

RESULTS AND DISCUSSION

Canker lesion was formed on artificially inoculated leaves. Minimum time for expression of canker symptom was taken by Xac_{MON} (4days) isolate while it was maximum for isolate Xac_{JP} (6 days), others were within 5 days (table 1). Maximum Lesion size was produced by Xac_{MON} (170.0 $mm^{2)}$ followed by Xac_{MHP} (135.0 $mm^{2)}$, Xac_{K} (117.0 $mm^{2)}$, *and* Xac_{J} (105.0 $mm^{2)}$ isolates respectively. Minimum lesion size was recorded in Xac_{JP} (55.0 mm^2). Based on the size of lesions produced on artificial inoculation Xac_{MON} isolate appeared most virulent (4 days & 170.0 mm^2) and Xac_{JP} isolate (6 days & 55.0 mm^2) being the least one.

Enzymatic assay of different isolates of *Xanthomonas axonopodis* pv. *Citri*

The qualitative studies of enzymatic activities of different *Xac* isolates were done under *in vitro* condition. Highest cellulase activity was observed in Xac_{MON} followed by Xac_{K} and Xac_{J} isolate (Table 2). The lowest cellulase activity was observed in Xac_{JP}. Similarly highest protease activity was recorded in Xac_{MON} isolate .But the highest pectinase activity observed in Xac_{MHP} isolate. However, highest lipase activity was observed on Xac_{JP}.

The pathogenic variability studies indicated that Xac_{MON} isolate was found to be highly virulent as compared to other isolates and Xac_{JP} isolate found to be the least virulent among all the isolates. Thus from the enzymatic studies it may be concluded that protease and cellulose activities of *Xac* might be associated in the pathogenic fitness of *Xac* in citrus canker system.

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