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BIOCHEMICAL CHARACTERIZATION OF *XANTHOMONAS AXONOPODIS* PV. *CITRI*, CAUSAL AGENT OF CITRUS CANKER

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ABSTRACT
Citrus canker caused by *Xanthomonas axonopodis pv. citri* is one of the most important disease of citrus. In the present study, 20 isolates of *Xanthomonas axonopodis* pv. *citri* were collected from various regions of Varanasi. Isolates were characterized with the help of morphological, pathogenicity and biochemical analysis. All the isolates showed similar morphological and biochemical characteristic and all were found pathogenic on citrus, thus confirming the identity of isolates as belonging to those of *Xanthomonas axonopodis* pv. *citri*.

INTRODUCTION

Citrus is the third most important fruit crop of the world. The genus citrus is diverse in species, cultivars and clones (Singh, 2001). Several species and varieties of citrus are prone to different diseases caused by fungi, bacteria, viruses and phytoplasma. Citrus canker caused by *Xanthomonas axonopodias* pv. *citri* (Syn. *Xanthomonas citri* subsp. *citri*) is one of the most destructive disease of citrus throughout the world including Africa, Asia, Australia, South America and USA (Graham and Gottwald, 1991). The bacterium, *Xanthomonas axonopodis* pv. *citri* causes different symptoms ranging from pustules to necrotic lesions consisting of erumpent corky tissue surrounded by water soaked tissues and yellow halo on leaves, stems and fruits (Zekri *et al*., 2005; Graham *et al*., 2004; Schubert and Sun, 2003). The bacterium has been divided in five different forms or pathotypes. Among all pathotypes form A which is also known as true or Asiatic canker disease is the most devastating form and can infect most commercial citrus varieties and citrus relatives (Vauterin *et al*., 1991, 1995). Several approaches have been used in the past to study the taxonomy and strain differentiation of *Xanthomonas axonopodis* pv. *citri*. These include biochemical tests and metabolic fingerprints (Goto *et al*., 1980), bacteriophage sensitivity (Goto *et al*., 1980; Civerolo, 1984), fatty acid profile (Stall and Hodge, 1989) and genomic DNA fingerprinting (Hartung and Civerolo, 1987) Various workers around the world have tried to isolate the citrus canker pathogen from diseased samples and characterize it through available biochemical tests as a preliminary pathogen detection technique (Dhakal *et al*., 2009; Lin *et al*., 2005; Chand and Pal, 1982; Goto, 1969,1992; Falico de Alcaraz, 1980; Rangaswami and Soumini, 1957; Hamlin, 1967; Khalid *et al*., 2010; Mohammadi *et al*., 2001; Verniere *et al*., 1991; Rezaei *et al*., 2012).). Proper identification and characterization of *X. axonopodis* pv. *citri* strains through biochemical tests is helpful in accurate detection of pathogen and devising effective management strategies as the pathogen is responsible for considerable loss of fruit quality as well as its market value (Khalid *et al.,* 2010). So, the present study was conducted to characterize the isolates of *X. axonopodis* pv. *citri* collected from various areas of Varanasi district of Uttar Pradesh.

MATERIALS AND METHODS

Bacterial isolation and pathogenicity testing:

Diseased samples of citrus plants were collected from Varanasi district of Uttar Pradesh, India and isolated as described by Broadbent *et al*., (1992). Briefly, small piece of tissue from typical lesions margin were excised from samples using a sterile razor and pieces were chopped in a drop of sterile distilled water. The resulting suspension was streaked on plates containing Sucrose Peptone Agar medium (SPA)[Sucrose-20g/L; Peptone-5g/L; K_2HPO_4-0.5g/L; $MgSO_4.7H_2O$-0.25g/L; Agar-20g/L; Deionized distilled water-1L], followed by incubation at 28^{0}C for 72 hours. The isolated bacterial cultures were tested for their pathogenicity on Kagzi lime(*Citrus aurantiifolia*) using a suspension of approximately 1×10^8 viable cells/ml as described by Broadbent *et al*., (1992).

Biochemical tests

A total of 20 isolates of *X. axonopodis* pv. *citri* were compared on the basis of their biochemical characteristics as follows:

Gram's reaction (Schaad, 1988), Starch hydrolysis (Fahy and Persley, 1983), Nitrate reduction and catalase (Lelliott and Stead, 1987), Milk proteolysis (Schaad, 1988), Arginine dihydrolase (Fahy and Persley, 1983), Gelatin liquefaction (Dickey and Kelman, 1988) and Salt tolerance (Fahy and Persley, 1983).

RESULTS AND DISCUSSION

Table 1 shows the isolates, host plant, affected part and locations of *X. axonopodis* pv. *citri* isolates used in the study. Host plant was *Citrus aurantifolia*. Affected parts were leaves and fruits. In this study, all the isolates formed circular, convex, mucoid, yellow colonies with smooth margin on Sucrose Peptone Agar (SPA) medium. All isolates formed typical symptoms of citrus bacterial canker on the leaves of Kagzi lime (*Citrus aurantifolia*) 2-3 weeks following inoculation. On the leaves, lesions appeared firstly on the lower leaf surface and then on the upper surface. Lesions gradually merge into one another and formed erumpent callus like tissue with water soaked margin. The bacterium was re-isolated from the inoculated leaves and grown in culture and in this way Koch's postulates were proved. Broadbent *et al*., (1992) and Mohammadi *et al*., (2001), have also carried out similar inoculation experiments with *Xanthomonas axonopodis* pv. *citri* and they have also found that symptoms were produced on the susceptible hosts 2-3 weeks following inoculation.

A total of 20 isolates of *Xanhomonas axonopodis* pv. *citri* were compared for their biochemical characteristics (Table 2). All the isolates were found to be Gram negative, catalase positive, unable to reduce nitrate, arginine dihydrolase negative, hydrolysed tween 80, starch hydrolysis positive, gelatine liquefaction positive, milk proteolysis positive and able to tolerate 1,2 and 3% salt concentration. Thus, all the

Table 1: Isolates, host plant, affected part and locations of *Xanthomonas axonopodis* pv. *citri* used in the study

Name of isolate	Place of collection	Host	Affected part
X-1	Horticulture farm, BHU	*Citrus aurantifolia*	Leaf
X-2	Delhana, Varanasi	*C.aurantifolia*	Leaf
X-3	Faridpur, Varanasi	*C.aurantifolia*	Leaf
X-4	Bhagwanpur, Varanasi	*C.aurantifolia*	Leaf
X-5	Chandpur, Varanasi	*C.aurantifolia*	Leaf
X-6	Ramna, Varanasi	*C.aurantifolia*	Leaf
X-7	Madhopur, Varanasi	*C.aurantifolia*	Leaf
X-8	Tariya, Varanasi	*C.aurantifolia*	Leaf
X-9	Maruadih, Varanasi	*C.aurantifolia*	Leaf
X-10	Bhikharipur, Varanasi	*C.aurantifolia*	Leaf
X-11	Rampur, Varanasi	*C.aurantifolia*	Leaf
X-12	Chitaipur, Varanasi	*C.aurantifolia*	Leaf
X-13	Narayanpur, Varanasi	*C.aurantifolia*	Leaf
X-14	Dafi, Varanasi	*C.aurantifolia*	Leaf
X-15	Kotwa, Varanasi	*C.aurantifolia*	Leaf
X-16	Bandepur, Varanasi	*C.aurantifolia*	Leaf
X-17	Sandaha, Varanasi	*C.aurantifolia*	Leaf
X-18	Lamhi, Varanasi	*C.aurantifolia*	Leaf
X-19	Dinapur, Varanasi	*C.aurantifolia*	Leaf
X-20	Vishunpur, Varanasi	*C.aurantifolia*	Leaf

Table 2: Biochemical characteristics of the isolates

Isolate	Gram's reaction	Catalase test	Starch hydrolysis	Nitrate reduction	Milk proteolysis	Arginine dihydrolase test	Gelatin liquefaction	Tween 80 hydrolysis	NaCl tolerance (1%, 2% & 3%)
X-1	-	+	+	-	+	-	+	+	+
X-2	-	+	+	-	+	-	+	+	+
X-3	-	+	+	-	+	-	+	+	+
X-4	-	+	+	-	+	-	+	+	+
X-5	-	+	+	-	+	-	+	+	+
X-6	-	+	+	-	+	-	+	+	+
X-7	-	+	+	-	+	-	+	+	+
X-8	-	+	+	-	+	-	+	+	+
X-9	-	+	+	-	+	-	+	+	+
X-10	-	+	+	-	+	-	+	+	+
X-11	-	+	+	-	+	-	+	+	+
X-12	-	+	+	-	+	-	+	+	+
X-13	-	+	+	-	+	-	+	+	+
X-14	-	+	+	-	+	-	+	+	+
X-15	-	+	+	-	+	-	+	+	+
X-16	-	+	+	-	+	-	+	+	+
X-17	-	+	+	-	+	-	+	+	+
X-18	-	+	+	-	+	-	+	+	+
X-19	-	+	+	-	+	-	+	+	+
X-20	-	+	+	-	+	-	+	+	+

isolates showed similar biochemical characteristics properties which suggests that all the isolates belongs to form A or Asiatic form of *Xanthomonas axonopodis* pv. *citri*. Further these biochemical characteristics were in consistence with those described previously for Asiatic form of *Xanthomonas axonopodis* pv. *citri* (Witeside *et al*., 1993; Verniere *et al*., 1998; Khodakaramian *et al*., 1999; Mohammadi *et al*., 2001; Dhakal *et al*., 2009; Sujata and Sai Gopal, 2010). Similar studies on characterization have been done on other pathogens like *Xanthomonas axonopodis* pv. *punicae* (Raghuwanshi *et al*., 2013) and *Xanthomonas campestris* pv. *magniferaeindicae* (Dayakar and Gnanamanickam, 1996) also. From the present study it was concluded that isolates collected from various locations of Varanasi belongs to those of *Xanthomonas axonopodis* pv. *citri* Asiatic(A) form and these biochemical tests accompanied with pathogenicity testing can be effectively employed in accurate detection of the *Xanthomonas axonopodis* pv. *citri* which will help in deciding effective management practices for this devastating pathogen.

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