www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(10): 599-602

www.thepharmajournal.com
Received: 06-08-2022
Accepted: 17-09-2022

KS Chaudhari
Department of Plant Pathology and Agriculture Microbiology, PGI, MPKV, Rahuri, Maharashtra, India

SV Kolase
Department of Plant Pathology and Agricultural microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

MH Shete
Department of Plant Pathology and Agricultural microbiology, AICRP on Fruits, MPKV, Rahuri, Maharashtra, India

AV Chandanshive
Department of Horticulture, Tomato Improvement Project, MPKV, Rahuri, Maharashtra, India

MR Patil
Department of Statistics, PGI, MPKV, Rahuri, Maharashtra, India

Corresponding Author:
KS Chaudhari
Department of Plant Pathology and Agriculture Microbiology, PGI, MPKV, Rahuri, Maharashtra, India

Cultural and bio-chemical characterization of bacterial canker disease caused by *Xanthomonas axonopodis* pv. *Citri* in acid lime

KS Chaudhari, SV Kolase, MH Shete, AV Chandanshive and MR Patil

Abstract
The *Xanthomonas axonopodis* pv. *Citri* causing bacterial canker of acid lime was isolated from different acid lime growing locations of Ahmednagar district of Maharashtra State and were used for these tests. Biochemical tests and Cultural characteristics *viz.*, colony shape, margin, elevations and pigmentation help to identification of bacteria. In biochemical tests Gram staining, Potassium hydroxide (KOH) test, Catalase test, Starch hydrolysis test were performed to characterize the Xac. Different ten test isolates were studied using NA as basal culture medium. The results of all morphological, biochemical and cultural tests were confirmed the Xac a Gram-negative bacterium.

Keywords: Acid lime, bacterial canker, xac, morphology, cultural characters, biochemical tests

Introduction
Bacterial canker is of common occurrence and in severe proportions, where ever acid lime (*C. aurantifolia*) is grown on commercial basis and has become a crucial problem of acid lime orchards in the country. All cultivars of citrus are susceptible to canker but grape fruit, Mexican lime and lemon are highly susceptible, whereas sour orange and sweet orange are moderately susceptible. Mandarins are moderately resistant (Gottwald *et al*., 1993) [4]. The typical canker lesion caused by *Xanthomonas axonopodis* pv. *Citri* is erumpent with water-soaked spot with brown coloured margin surrounded by chlorate yellow halo. The disease is economically important because the lesion downgrade the appearance of fruit and when severe cause premature fruit drop. Heavy foliage infection causes severe defoliation, leaving only base twins (Singh, 2005) [8]. Confirmation of bacterium is very important for the application of right management practices. Therefore, studies on the isolation of bacterium Xac its cultural characters and biochemical characterization of this bacterium were carried out.

Material and Methods
Isolation and purification of *Xanthomonas axonopodis* pv. *Citri* (Xac)
Infected plant parts showing typical symptoms of bacterial canker of acid lime were collected from the different farmer's fields of Ahmednagar districts, where acid lime fields were infected with bacterial canker. Bacterial canker samples were collected separately in polyethylene bags and labelled. The different parts of the acid lime plant showing characteristic symptoms of bacterial canker infection were collected and subjected to isolation by following the method given by Schaad (1992) [7].

The infected samples were washed in running tap water, infected portion was cut into small bits with small portion of healthy tissue. These bits were surface sterilized with 0.1 per cent solution of mercuric chloride and washed in three series of sterile distilled water to remove traces of mercuric chloride. The bits were then crushed in 3 ml of sterile distilled water and allowed to diffuse for 5 min at room temperature. A loop full of crushed leachate was streaked on Nutrient Agar Medium (NA) plates aseptically and incubated at 28±2 °C in BOD incubator for 48 hrs. The inoculated plates were constantly monitored after every 24 hrs. For the development of bacterial colonies resembling *Xanthomonas* spp.

The suspected yellow- coloured bacterial colonies grown within 48 hrs. Were picked out and streaked on NA plates, discrete colonies were sub cultured on NA agar plates for further studies. The cultures were renewed by sub-culturing once in a fortnight on NA agar plates, then cultures were stored in the refrigerator at 5 °C, which served as a stock culture for further studies.

The ten bacterial cultures obtained upon isolation from the different diseased samples were designated as isolates from XacN1, XacSD2, XacK3, XacR4, XacSR5, XacPD6, XacJ7, XacS8, XacPR9 and XacSA10 and were maintained in the same way for further study.

Morpho-cultural variability

Morpho-cultural characters of *X. axonopodis* pv. *Citri* pathogen will be studied by microscopic and visual observation of Morpho-cultural characters, *viz.*, cell shape, flagellation, colony edge, elevation, pigmentation and surface appearance.

Biochemical tests

Biochemical characters of *X. axonopodis* pv. *Citri* pathogen were studied by subjecting the bacterial isolates to various biochemical tests as follows:

a. Gram Staining

The Gram-reaction of each isolate was determined by following the staining procedure in Schaad *et al.,* (2001) [10]. First a loop full of the bacterium suspension was smeared on clean glass slide, air fixed by gentle heating on flame of the spirit lamp. Aqueous Crystal violet solution (0.5%) was spread over this smear for 30 second and then washed with running tap water for a minute, this stained smear was later flooded with Gram iodine solution for one minute and rinsed in tap water. Later decolorized with 95 per cent of ethanol until colour runoff, washed with water and treated with Safranin as counter stain about 10 seconds, washed with water, air/blot dried and observed under research microscope (make: - Olympus) at 100 X using oil immersion technique.

b. Catalase test

A loop full of 24-28 hrs. Old culture of test bacterium was placed on the clean glass slide, and to this a drop of 3 per cent hydrogen peroxide (H_2O_2) was mixed and allowed to react for few minutes and observed for the production of gas bubbles.

c. KOH test (Potassium hydroxide)

A drop of 3 per cent potassium hydroxide was placed on clean glass slide and to this 48 hrs. Old bacterial culture was mixed with clean inoculation loop and stirred for 10 sec and observed for slime threads. When raised the wire loop, if strands of viscid material seen, then the bacterium is gram negative.

d. Starch hydrolysis

For each hydrolysis test 20 g Nutrient Agar (NA) was added to 80 ml of water and dissolved by successive heating and stirring similarly two-gram starch was then thoroughly dissolved in 10 ml distilled water separately and added to hot molten agar with through stirring. Amount of 100 ml of this basal medium was then transferred to conical flask (250 ml) and autoclaved at 115 °C for duration of 10 minutes. The medium was then poured into Petri plates. The plates were then inoculated with individual isolate aseptically, labeled and sealed to avoid chances of contamination. These plates were then incubated in upside down position at 27 °C for 7 days. After scraping bacterial growth to each plate Lugol's iodine was added which was prepared by mixing 1gm iodine and 2 gm potassium iodide in 300 ml distilled water, stirred for until dissolved completely. The appearance of cleared zones around the colonies was indicative of presence or absence of starch hydrolysis. (Rafi *et al.*, 2013) [11].

Results and Discussions

Cultural and morphological characteristics of *X. axonopodis* pv. *Citri* isolates

The morpho-cultural variability studies on following characteristics *viz*., pigmentation, colony shape, elevations and margin of different ten test isolates were studied by using NA as basal culture medium**.** Cell shape of *X. axonopodis* pv. *Citri* was observed under binocular microscope @ 40X.

The morpho- cultural characteristics presented in Table 1 and photograph shown in Plate 1 it was found that, out of ten isolates tested six isolates *viz.*, XacN1, XacSD2, XacK3, XacR4, XacPD6 and XacS8 exhibited yellow pigmentation while, rest of four isolates *i.e*., XacSR5, XacJ7, XacPR9 and XacSA10 light yellow. In all the ten test isolates showed fusiform colony shape, convex elevation and entire colony margin. Morphologically all the test isolates were found single rod shaped. Similar results were also reported earlier by many workers (Singh and Thind 2014) [9]; Abhang *et al.*, 2018) [1].

Table 1: Cultural and morphological characteristics of different isolates of *X. axonopodis* pv. *Citri* on acid lime

Name of isolate	Pigmentation	Colony shape	Elevation	Margin	Cell shape
XacN1	Yellow	Filiform	Convex	Entire margin	Single rods
Xac*SD*2	Yellow	Filiform	Convex	Entire margin	Single rods
XacK3	Yellow	Filiform	Convex	Entire margin	Single rods
XacR4	Yellow	Filiform	Convex	Entire margin	Single rods
XacSR5	Light yellow	Filiform	Convex	Entire margin	Single rods
XacPD6	Yellow	Filiform	Convex	Entire margin	Single rods
XacJ7	Light yellow	Filiform	Convex	Entire margin	Single rods
XacS8	Yellow	Filiform	Convex	Entire margin	Single rods
XacPR9	Light yellow	Filiform	Convex	Entire margin	Single rods
XacSA10	Light yellow	Filiform	Convex	Entire margin	Single rods

Biochemical tests

Biochemical characteristics of *X. axonopodis* pv. *Citri* isolates were studied by various biochemical tests including Gram staining, Catalase test (H_2O_2), Potassium hydroxide test (KOH) and Starch hydrolysis test.

From the Table 2 and photograph shown in Plate 2 revealed that, all the isolates were found to Gram negative staining test while, and observed positive to Catalase test (H_2O_2), Potassium hydroxide test (KOH) and Starch hydrolysis test. The results obtained were as follows.

a. Gram staining

Microscopic examination of Gram-stained *X. axonopodis* pv. *Citri* isolates mount elucidated that the test bacterium did not retain violet colour of the primary stain (Crystal violet) but cells appeared pink coloured due to counter staining with the stain safranin. Hence the test bacterium was Gram negative and straight rod shape which is the characteristic feature of the plant pathogenic bacteria of *X. axonopodis* pv. *Citri* isolates given in Table 2 and photograph shown in Plate 2.

b. Catalase test (H_2O_2)

Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water. To find out if a particular bacterial isolate was able to produce catalase enzyme, a small inoculum of bacterial isolates was mixed into hydrogen peroxide solution (3%) and was observed for the rapid elaboration of oxygen bubbles. The lack of catalase was evident by a lack of or weak bubble production. Catalytic activities of all the ten isolates of *X. axonopodis* pv. *Citri* were found positive, when culture was produced bubbles of oxygen within one minute after addition of H_2O_2. (Table 2 and Plate 2).

c. Potassium hydroxide test (KOH)

For conducting the test of KOH formation of slime threads or loop is an indication of being Gram-negative because gram negative bacteria have relatively fragile cell walls, bounded by an outer membrane. This was readily disrupted by exposure to 3 per cent KOH releasing the viscous DNA. All the ten test isolates of *X. axonopodis* pv. *Citri* were showed to form mucoid thread after added KOH and found positive test in all isolates of *X. axonopodis* pv. *Citri* isolates given in Table 2 and photograph shown in Plate 2.

d. Starch hydrolysis

Results presented in Table 2 and photograph shown in Plate 2 the test bacterium produced colourless zone around bacterial growth on starch agar medium flooded with lugol's iodine and showed positive for starch hydrolysis test. The test bacterium was hydrolysed starch by exoenzyme amylase and broken down to dextrin's, maltose and glucose/alpha-amylase. Similar results were also reported earlier by many workers (Al – saleh *et al.*, 2014; Mubeen *et al.*, 2015; Katkar *et al.*, 2016; Abhang *et al.*, 2018; Bhure *et al.*, 2019) [2, 6, 5, 1, 3].

Table 2: Biochemical characteristics of different isolates of *X. axonopodis* pv. *Citri* on acid lime

Name of isolate	Gram staining	Catalase test (H_2O_2)	Potassium hydroxide test (KOH)	Starch hydrolysis
XacN1	- ve	+ ve	+ ve	+ ve
XacSD2	- ve	+ ve	+ ve	+ ve
XacK3	- ve	+ ve	+ ve	+ ve
XacR4	- ve	+ ve	+ ve	+ ve
XacSR5	- ve	+ ve	+ ve	+ ve
XacPD6	- ve	+ ve	+ ve	+ ve
XacJ7	- ve	+ ve	+ ve	+ ve
XacS8	- ve	+ ve	+ ve	+ ve
XacPR9	- ve	+ ve	+ ve	+ ve
XacSA10	- ve	+ ve	+ ve	+ ve

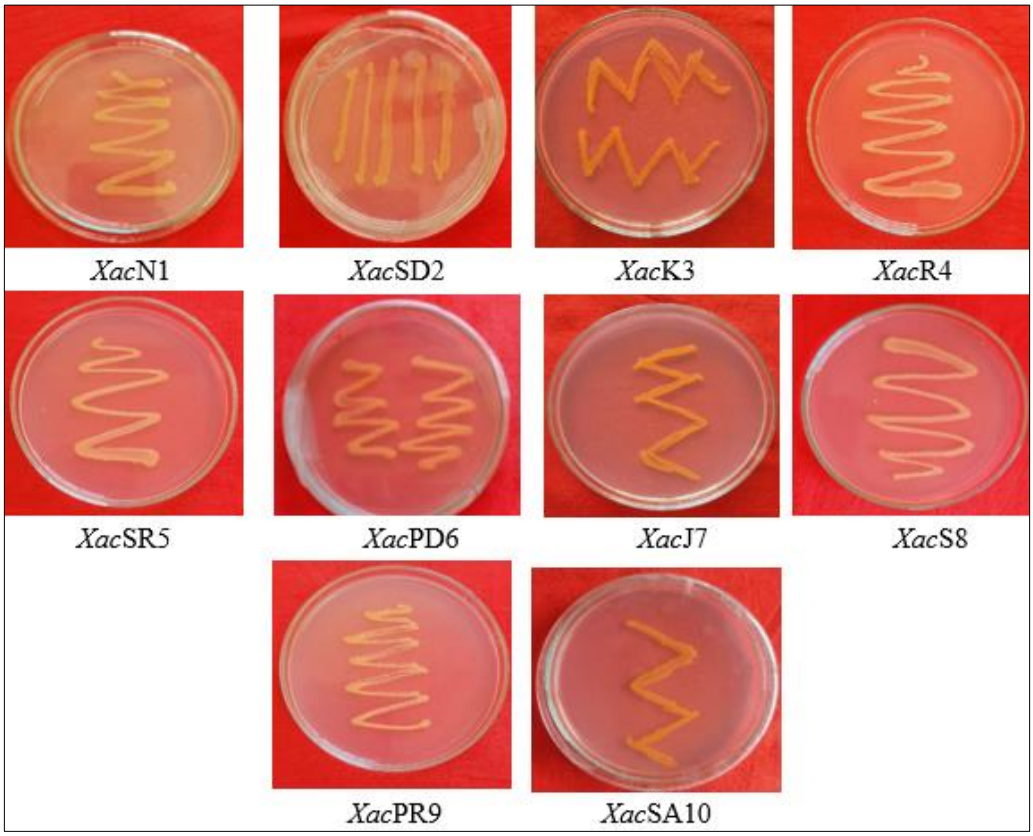

Plate 1: Photographs showing cultural variability amongst *X. axonopodis* pv. *Citri* Isolates

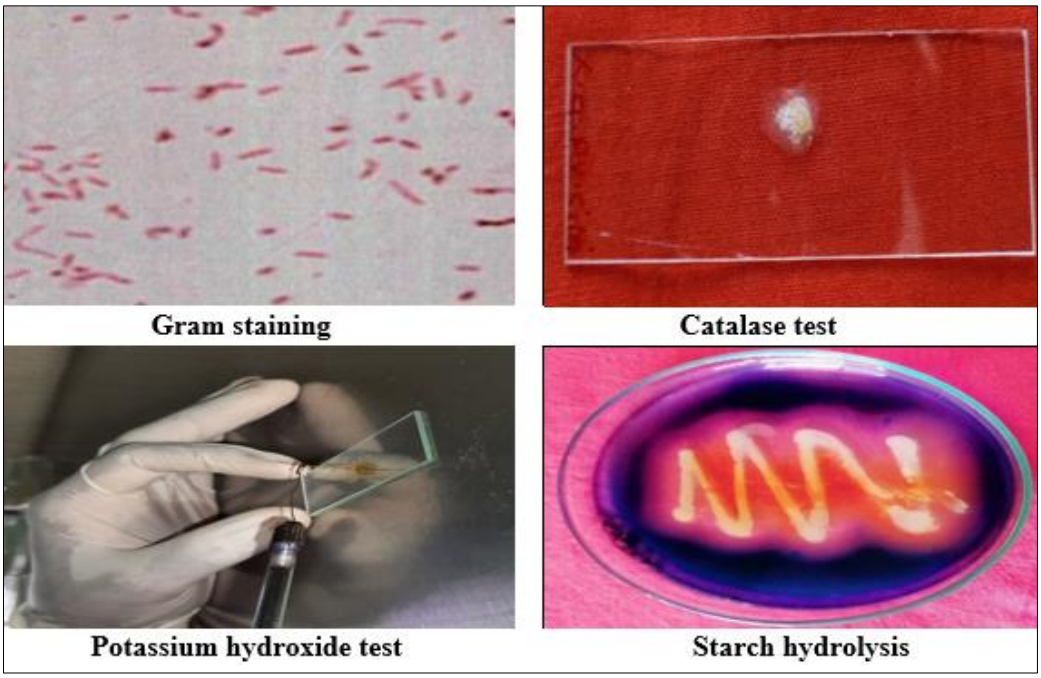

Plate 2: Photographs showing biochemical tests of *X. axonopodis* pv. *Citri*

References

1. Abhang PB, Totawar MV, Katkar MM, Atram PC, Mane SS. Efficacy of fungicides botanicals bio agents against *Xanthomonas axonopodis* pv. Citri. Int. J Chem. Studies. 2018;6(2):1108-1111.
2. Al-Saleh MA, Widyaman A, Saleh AA, Ibrahim YE. Distribution and pathotype identification of *Xanthomonas* Citri. Sub sp. Citri. Recovered from south western region of Saudi Arabia. African J of Microbiol. Res. 2014;8(7):673-679.
3. Bhure SS, Bramhankar SB, Thakur KD, Wasnik DG, Pawar RD, Labhasetwar AA, *et al.* Physiological and biochemical characterization of *Xanthomonas axonopodis* pv. *Citri:* A gram negative bacterium causing citrus canker. Int. J Chemical Studies. 2019;7(1):1941-1944.
4. Gottwald TR, Graham JH, Civerolo EL, Barret HC, Hearn CJ. Differential host range reaction of citrus and citrus relatives to citrus canker and citrus bacterial spot determined by leaf mesophyll susceptibility. Pl. Dis. 1993;77(10):1004-1009.
5. Katkar M, Raghuwanshi KS, Chimote VP, Borkar SG. Pathological, Bio-chemical and Molecular diversity amongst the isolates of *Xanthomonas axonopodis* pv. *Citri* causing Citrus canker in acid lime from different agro-climatic region of India. Int. J Environ, Agril. Biotec. 2016;1(2):1-14.
6. Mubeen MA, Hafiz MI, Iftikhar Y, Irfan UM, Bilqees I. Bio-chemical characterization of *Xanthomonas axonopodis* pv. *Citri*: a Gram-negative bacterium causing citrus canker. Internat. J Sci. Nature. 2015;6(2):151-154.
7. Schaad NW. Laboratory guide for the identification of plant pathogenic bacteria. 2nd ed. American Phytopathol. Soc; c1992. p.138.
8. Singh RS. Plant Diseases, 8th Edn. Oxford IBH, New Delhi, p. 48. Effect of thymine on bacterial canker in iran. J PI. Pathol. 2005;91(3):691-696.
9. Singh D, Thind SK. Prevalence, isolation and standardization of growth media for *Xanthomonas axonopodis* pv. *Citri* causing citrus canker. Pl. Dis. Res. 2014;29(2):188-192.
10. Schaad LJ, Hess BA. Dewar resonance energy. Chemical Reviews. 2001 May 9;101(5):1465-76.
11. Rafi HK, Karthik NV, Gong H, Starr TL, Stucker BE. Microstructures and mechanical properties of Ti6Al4V parts fabricated by selective laser melting and electron beam melting. Journal of materials engineering and performance. 2013 Dec;22(12):3872-83.