Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-6: 1682-1687

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-6 pp. 1682-1687**
Journal homepage: http://www.ijcmas.com

Original Research Article

Cultural and Bio-Chemical Characterization of *Xanthomonas axonopodis* pv. *citri*: Causing Citrus Canker

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Keywords

Kagzi lime, Bacterial canker, *Xac*, Morphology, Cultural characters, Biochemical assays

A B S T R A C T

The *Xanthomonas axonopodis* pv. *citri* causing bacterial canker of kagz lime was isolated from different kagzi lime growing locations of Marathwada and were used for these tests. Cultural characteristics *viz*., colony shape, margin, elevations, surface, and pigmentation of Biochemical tests help to identification of bacteria. Gram staining, Potassium hydroxide (KOH) solubility test, Catalase test, Starch hydrolysis test were performed to characterize the *Xac.* Different 9 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.

Introduction

Bacterial canker of Kagzi lime is one of the major causes of yield losses in citrus growing areas of world (Graham *et al.*, 1990). Reported that different pathovars and variants of the bacterium *Xanthomonas axonopodis (Xac)* were the cause of citrus canker disease, all cultivars of citrus are susceptible to canker, but grapefruit, Mexican lime and lemon are highly susceptible, whereas sour orange and sweet orange are moderately susceptible. Mandarins are moderately resistant (Gottwald *et al.*, 1993).

All young, above-ground tissues of citrus are susceptible to *Xanthomonas axonopodis*. In fact, bacterial pathogens infects into the plant tissues through natural openings (stomata) and mechanical injuries (wounds) (Graham *et al.*, 2004.) Conformation of bacterium is very important for the application of right management strategies. Therefore studies on the isolation of bacterium *Xac* its cultural characters and biochemical characterization of this bacterium were carried out.

Materials and Methods

Isolation and purification of *Xanthomonas axonopodis* pv. *citri (Xac)*

The collected diseased samples were checked for bacterial infection by ooze test. The leaves were washed and small pieces of infected leaves were cut aseptically from the

edge of typical spots with a little portion of healthy tissue. The leaf bits were surface sterilized with 0.1 per cent mercuric chloride for 30-60 sec., washed with sterilized distilled water for 3-4 times to remove traces of mercuric chloride. On a clean slide 1-2 drops of sterilized distilled water were placed, a small piece of diseased leaf portion was kept on water drop and teased well with a sterilized scalpel. After 3-5 minutes, the slide was observed under microscope for the presence of ooze. Samples giving cloudy discharge were treated as positive samples of the bacterial ooze.

The water drop from positive slide was taken with a bacterial inoculation loop and streaked on sterile solidified nutrient agar media which was already kept ready for inoculation in a Petri plate. The inoculated Petri plates were kept for incubation at 27 + 1^0 C in BOD incubator for 48-72 hours. After incubation period observations were made for the development of well separated, typical yellow colure bacterial colonies resembling *Xanthomonas* sp.

The suspected bacterial colonies were picked up with the help of sterilized inoculation loop and streaked on the Petri plates with nutrient agar media. The Petri plates were kept for incubation at 27 ± 1^0C for 48 hours. Observations were made for the development of well separated typical yellow, mucoid colonies, such pure colonies were further streaked onto the Nutrient Agar (NA) slants and the culture were stored/maintained in the refrigerator at 5 ^{0}C which served as a stock culture for further studies.

The nine bacterial cultures obtained upon isolation from the different diseased samples were designated as isolates from Xac l to Xac 9 and were maintained in the same way for further study.

Cultural characteristics of *Xanthomonas axonopodis* pv. *Citri*

Cultural characteristics *viz*., colony shape, margin, elevations, surface, and pigmentation of different 9 test isolates were studied using NA as basal culture medium.

Biochemical characters of *Xanthomonas axonopodis* pv. *citri* pathogen were studied by subjecting the bacterial isolates to various biochemical tests, *viz.* Gram staining, Potassium hydroxide (KOH) solubility test, Catalase test, Starch hydrolysis test.

Gram staining

The Gram-reaction of each isolate was determined following the staining procedure. 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 Grams iodine solution for one minute and rinsed in tap water.

Later decolorized with 95% 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 100X using oil immersion technique.

Catalase oxidation 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% hydrogen peroxide (H_2O_2) was mixed and allowed to react for few minutes and observed for the production of gas bubbles.

KOH test (Potassium hydroxide)

A drop of 3 per cent potassium hydroxide was placed on clean glass slide and to this 48 hr 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.

Starch hydrolysis

The autoclaved and cooled starch agar medium was poured in sterile glass Petri plate and on solidification of the medium, streaked pure culture of the test bacterium and incubated for 96 hrs at 28^0C. Then these plates was flooded with lugol's iodine and allowed to react for few minutes. Reddish coloured zones indicate negative reaction and appearance of yellowish, clear zones around the bacterial growth indicates positive reaction.

Results and Discussion

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

Cultural characteristics *viz*., colony shape, margin, elevations, and pigmentation of different 9 test isolates were studied using NA as basal culture medium**.** Of the nine isolates tested, seven isolates *viz.*, Xac 2, 4, 5, 6, 8 and 9 exhibited yellow pigmentation and rest two (Xac 3, 7) exhibited light yellow pigmentation. In all the test isolates isolated colony shape was filiform, convex elevation and entire colony margin (Table 1).

Similar results were also reported earlier by many workers (Hingorani and Sing 1959; Kanwar, 1976; Graham *et al.*, 2004).

Biochemical tests

Biochemical characters of *Xanthomonas axonopodis* pv. *citri* pathogen were studied by subjecting the bacterial isolates to various biochemical tests, *viz.*, Gram staining, potassium hydroxide (KOH) solubility test, catalase test, starch hydrolysis test.

All isolates are found negative to Grams reaction while as positive to Catalase oxidation test, KOH test, and Starch hydrolysis test. The results obtained (Table 2) are being narrated herein as follows.

Gram staining

Microscopic examination of Grams stained *X. axonopodis pv. citri* mount elucidated that the test bacterium did not retained violet color of the primary stain (Crystal violet) but cells appeared pink colored due to counter staining with the stain safarin. Hence the test bacterium was gram negative, straight rods, which is the characteristic feature of the plant pathogenic bacteria.

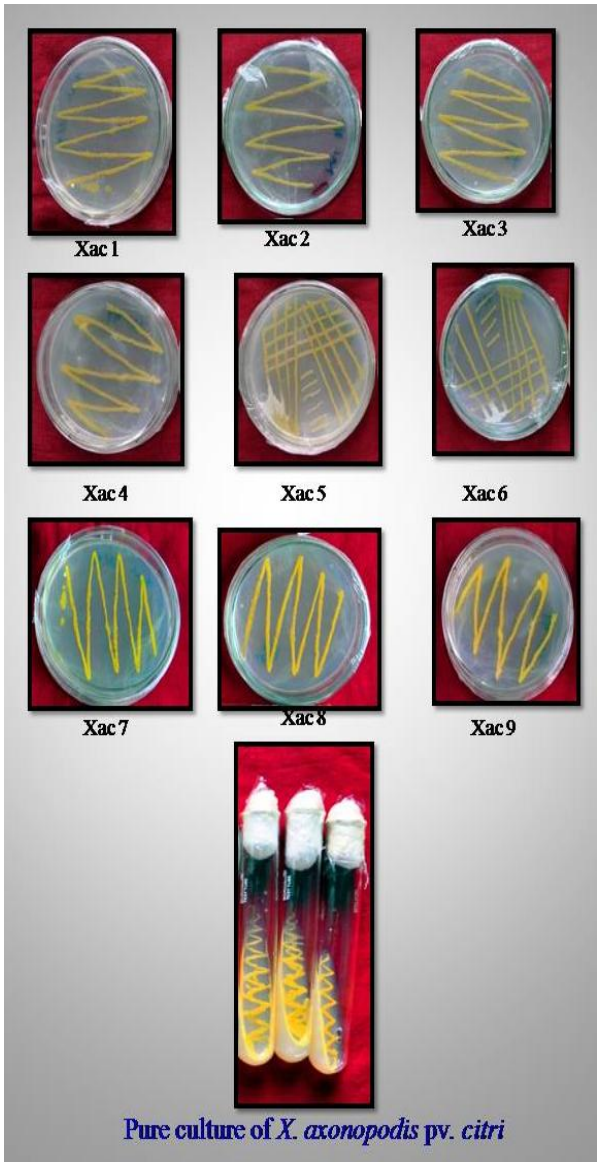
Table.1 Cultural and morphological characteristics of *X. axonopodis* pv. *citri* isolates

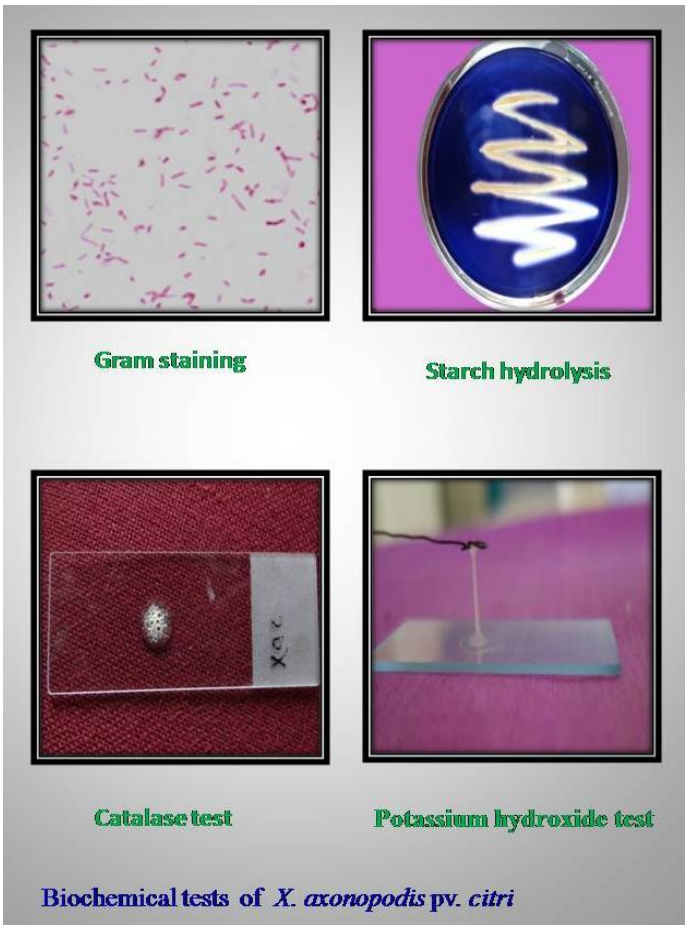
S.N.	Isolates	Pigmentation	Colony shape	Elevation	Margin	Cell shape
1	Xac1	yellow	Filliform	convex	Entire margin	Single rods
2	Xac2	yellow	Filliform	convex	Entire margin	Single rods
3	Xac3	Light yellow	Filliform	convex	Entire margin	Single rods
4	Xac4	yellow	Filliform	convex	Entire margin	Single rods
5	Xac5	yellow	Filliform	convex	Entire margin	Single rods
6	Xac6	yellow	Filliform	convex	Entire margin	Single rods
7	Xac7	Light yellow	Filliform	convex	Entire margin	Single rods
8	Xac8	yellow	Filliform	convex	Entire margin	Single rods
9	Xac9	yellow	Filliform	convex	Entire margin	Single rods

Table.2 Biochemical characteristics of *X. axonopodis* pv. *citri* isolates

S.N.	Isolates	Gram's reaction	Catalase Oxidation test	KOH test	Starch hydrolysis test
1	Xac1	-ve	+ve	+ve	+ve
2	Xac2	-ve	+ve	+ve	+ve
3	Xac3	-ve	+ve	+ve	+ve
4	Xac4	-ve	+ve	+ve	+ve
5	Xac5	-ve	+ve	+ve	+ve
6	Xac6	-ve	+ve	+ve	+ve
7	Xac7	-ve	+ve	+ve	+ve
8	Xac8	-ve	+ve	+ve	+ve
9	Xac9	-ve	+ve	+ve	+ve

-ve: Negative +ve: Positive





Catalase test

Results (Table 2) revealed that the test bacterium produced gas bubbles when mixed on glass slide with a few drops of 3 % hydrogen peroxide, which indicated positive catalase test.

Potassium hydroxide (KOH) test

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 is readily disrupted by exposure to 3 % KOH releasing the viscous DNA. But gram- positive bacteria by contrast possess a thicker, more rigid cell wall which resists the disruptive effect of KOH. The present study (Table 2) revealed that the test bacterium showed positive reaction to KOH test.

Starch hydrolysis

Results revealed that (Table 2) the test bacterium produced colorless zone around bacterial growth on starch agar medium flooded with Lugol's iodine and showed positive for starch hydrolysis test. The test bacterium is hydrolyzed starch by exoenzyme amylase and broken down to dextrins, maltose, and glucose/alpha-amylase.

Similar results were also reported earlier by many workers (McGuire *et al.*, 1988; Goto 1992, Manjula *et al.*, 2002; Myung *et al.*, 2009; Sujatha and Sai Gopal, 2010; Mubeen *et al.*, 2015).

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