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Biochemical and Pathological Diversity of *Xanthomonas axonopodis* pv. *citri* In different Agro Climatic Zone of Marathwada Region

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Abstract: *Xanthomonas axonopodis* pv. *citri* (Xac) is the phytopathogen responsible for bacterial citrus canker. Twenty two strains of *Xanthomonas axonopodis* pv. *citri* (Xac)*)* were isolated from different Citrus growing areas of Marathwada region of Maharashtra state. Morphological, biochemical and pathogenicity tests were carried out to identify, characterize and determine the strains of bacteria responsible for Citrus canker. All the 22 bacterial isolates produced mucoidal colonies on Yeast extract-dextrose-CaCO3 (YDC) agar media selective media for *Xanthomonas*. Biochemical tests of the 22 bacterial isolates indicated that all the isolates were Gram-negative short rods, positive for Hydrolysis of Aesculin, Gelatin liquefaction, Starch hydrolyse, Milk Hydrolysis Tween80 lipolysis, Catalase, H2S production and Urease production , Oxidase test were showed variation negative weakely positive and positive . All isolates produced acid from Glucose,,Fructose where all isolates were fail to produced acid from Manose. The results of the study suggest variation in the pathogenic nature of these isolates 06 were highly virulent, 11 were moderately virulent and 05 were less virulent from different agro climatic zones of Marathwada region of Maharashtra state .

Key Words: *Xanthomonas axonopodis* pv. *citri* (Xac), Citrus canker, Citrus, Biochemical, pathogenicity, Pathogenic variability.

I. INTRODUCTION

Citrus belongs to family *Rutaceae* have high economic, nutritional and medicinal value. In India, citrus occupies third position among fruits after mango and banana. Canker is one of the major constraints of its cultivation (Minhaj et al., 2014) . In Marathwada the citrus fruit belongs to *Citrus aurentifolia* (Lime), *Citrus lemona* (Lemon), *Citrus sinensis* (Sweet lime). *Citrus lemona* is cultivated over an area of 87100 hectare in Marathwada region alone, Premalinae, Vikram, Sai sharbati and Local are common varieties cultivated in this region (Minhaj et al., 2012). Citrus canker is one of the most feared of citrus diseases, affecting all types of important citrus crops which is caused by *Xanthomonas axonopodis* pv. *citri* (Xac).The disease caused raised necrotic lesions on leaves, twigs and fruits hampering the quality. The disease causes extensive damage to citrus and severity of this infection varies with different species and varieties and the prevailing climatic condition (Gade et al., 2018).

Bacteria gain entry in to the leaves through stomata, wounds/injuries and penetrate into the intercellular spaces of leaves and attaches to the mesophyll cells using type III secretary system and releases effecter proteins (toxins) which causes hypertrophy, hyperplasia and and causes multiple infection and produces water soaked lesion with yellow hollow. Epidermis gets raised and began to rupture. After two weeks of infection, the watery lesions began to dry and form necrotic lesions (Hameed et al., 2019). On the basis host range, symptomatology, and geographical distribution citrus canker bacterium *X. axonopodis* is divided into three major types .These pathotypes named as forms A or *X. a.* pv. *citri*, B, and C. Of these pathotypes form A which causes true or Asiatic canker disease is the most devastating form and can infect most commercial citrus varieties and common in Asia (Schoulties et al.,1987, Gotwald et al., 1993, Vauterin et al.,1991, Stall et al.,1991 Lin et al.,2008and Khodakaramian et al., 2011,). The present study is based on physiological, biochemical and pathological characterization of *Xanthomonas axonopodis* pv. *citri* (*Xac*)isolated from different districts of Marathwada region of Maharashtra state.

II. MATERIAL AND METHOD

Collection of symptomatic samples :

Infected leaves and fruit of citrus collected from Nanded, Hingoli, Parbhani, Jalna, Aurangabad, Latur, and Beed.different citrus growing areas of Marathwada region of Maharashtra state India.

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Isolation of bacterial strains from infected leaves:
Infected portion of leaf and fruit were cut, surface sterilized with 0.1% HgCl2 and 30% alcohol washed with distilled water twice teased with saline streak suspension on YDC (Yeast, Dextrose, Calcium carbonate) agar plates by using flame wire loop incubated at 28-37 °C for 24 hrs. Pure cultures were maintained on YDC slants (Minhaj et al., 2012)

Morphological and Biochemical Tests:
Morphological characteristics such as Gram staining, Colony colour & Cell motility were recorded for all these strains9 Bradbury et al., 1984). Biochemical tests like Starch Hydrolysis, Gelatin liquefaction, Aesculin hydrolysis, Tween80 lypolysis, Catalases, Starch Hydrolysis, Milk Hydrolysis, Oxidase test, H2S Production, Urease production, and utilization of carbon from different sources includingGlucose, fructose, sucrose, maltose and manose were used to identify the pathogen by performing standard procedures given in the laboratory manuals Goszczynska et al., 2000)

Pathogenicity Test:
The detached leaf method was used to test the pathogenicity of *Xanthomonas axonopodis* pv.*citri* on citrus leaves healthy leaves sample were collected separatly and washed under running water for about 10 min to remove the dirt on the leaves then leaves were soaked in 1% sodium hypochloride for 1 min., after that leaves were washed for 3 times with sterilized distilled water to remove the traches of chemical and leaves were kept for air drying. For –ve control 10µl of sterilized distilled water was placed aseptically onto three leaves of each cultivar at six different sites on each leaf with the help of sterilized syring. For +ve control 10µl of each bacterial suspension was placed onto three leaves of each cultivar at six different sites on each leaf with the help of sterilized syring. Separate syring was used for each isolate. plate incling was wrap and plates were placed at 27±20C in a growth cabinate equipped with white light for 12 hrs exposure to white light and 12 hrs for dark. Observations were recorded from 2nd day of inoculation upto 20th day of inoculation to record development of symptoms , reisolation of the pathogen was done and culture so obtained was compared with the original culture (Katkar et al., 2016)**.**

III. RESULT AND DISCUSSION

Collection of diseased samples
The disease sample of citrus canker showing disease symptoms such as small, reddish, raised spots and expanded into slightly raised necrotic areas with little water soaking or chlorosis and raised corky lesions surrounded by an oily or water-soaked margin were collected from different citrus growing areas of Marathwada like Nanded, Hingoli, Parbhani, Aurangabad, Jalna, Latur and Beed districts

Isolation and Identification of *Xanthomonas axonopodis* pv.*citri* strains:
Total 22 isolates of *Xanthomonas axonopodis* pv. *citri* were isolated from infected sample by streak plate method on Yeast, Dextrose, Calcium Carbonate (YDC) agar media from each location was designated according to name of region as presented in Table 1 similar isolation method was adopted for the isolation of *Xanthomonas citri* subsp. *citri* by using nutrient agar medium by Kharat *et al.* 2020, Manyam et al., 2020) and Ismail et al.,2014 isolated *Xanthomonas axonopodis* pv. citri on nutrient agar and further purified on YDCA Katkar *et al*. (2016) obtained 14 isolates of of *Xanthomonas axonopodis* pv. citri from dfferent agro climatic zone of India while (Isokar et al.,2020) isolated from different agro climatic zone of Maharashtra Isolates.

Morphological and Biochemical Characterization:
All the isolates were studied with respect to their colony color, shape and Grams staining reaction. Yellow mucoid colonies on YDC agar plates were observed and bacterial cells appeared short rod and Gram negative Table 2. All twenty two isolates were positive for, Aesuline hydrolysis, Gelatin liquefaction test, Milk hydrolysis starch hydrolysis, forming a clear hallow around the colony by hydrolyzing the starch, all the 22 strains found to be positive for Tween 80 lipolysis, Urease production and Catalase test, only Oxidase test showed positive by only *Xac-02, Xac-03, Xac-06, Xac-15, Xac-18, Xac-21* while *Xac-04, Xac-09, Xac-16, Xac-22* +w weakly positive reaction and other *Xac-01, Xac-05, Xac-07, Xac-08, Xac-10, Xac-11, Xac-12, Xac-13, Xac-14, Xac-17, Xac-19, Xac-20* showed negative reaction, all isolates produce acid from Glucose, Fructose,Sucrose and Maltose. Whereas all isolates fails to produceacid from Manose. Bhardwaj *et al.2014* performed different physiological and biochemical for *Xanthomonas axonopodis* pv. *citri* properties viz. H2S production, gelatin liquefaction, KOH test, catalase test, acid production from different sugars for identification of isolates and Khalid *et al*.,(2010) revealed that biochemical and molecular characterization of *X. axonopodis pv. citri* is necessary for the identification and control measures of citrus canker disease

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Pathogenicity Test:

The disease symptoms produced after 2-20 days after inoculation, appearing as water soaked areas followed by chlorosis and necrosis. On the basis of necrotic lesion size the isolates were categorized into three different virulence groups highly virulent, moderately virulent and less virulent. +++ = highly virulent, producing necrotic lesions of 9 to12 mm in diameter which belonged to, Nanded, Parbhani, Hingoli and Jalna district, moderately virulent strain produced 4to8 mm size necrotic lesion belonged to Nanded, Parbhani, Hingoli Jalna, Latur, Aurangabad, and Beed district. Less virulent, produced necrotic lesions of 1 to3 mm in diameter and belonged to only Nanded, Latur and Beed districtTable-4 Katkar *et al*. (2016) reported that *Xanthomonas axonopodis* pv. *citri* showed varied reaction in the symptoms development highly virulent strain developed symptoms at the point of inoculation within 7-9 days after inoculation less virulent strain developed symptoms after 3-16 days of inoculation. Ismail *et al.* (2014) studied the pathogenicity of *Xanthomonas axonopodis* pv. *citri* on 5 different host of *Rutaceace* family by detached leaf assay and reported that the pathogen also produced water soaking, followed by necrosis around the wound inoculated surface on grape fruit, Rough lemon followed by Lime. Most strains belonged to the prevalent lineage 1 pathotype A that has a wide host range among Rutaceae species. restricted, whereas pathotype A is found in several citrus-producing regions.Pruvost et al 2021 reported that on citron (*Citrus medica*) the first occurrence of genetically unrelated, nonepidemic lineage 4 pathotype A* strains with a host range restricted to Mexican lime and related species) in Mauritius, Moheli and Réunion.

IV. CONCLUSION

The present investigation clearly c suggest variability in the pathogenic behaviour of citrus canker isolates which were isolated from different citrus growing areas of Marathwada region of Maharashtra State there exist the pathological, biochemical and molecular variation amongst the different isolates of *Xanthomonas axonopodis* pv . *citri* collected from the different areas of Marathwada regions .

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Table 1: Isoaltion of *Xanthomonas axonopodis* pv.*citri* from different areas of Marathwada Region

Sr. No.	Location	Isolates
1	Nanded	*Xac-01*
2	Nanded	*Xac-02*
3	Nanded	*Xac-03*
4	Nanded	*Xac-04*
5	Hingoli	*Xac-05*
6	Hingoli	*Xac-06*
7	Hingoli	*Xac-07*
8	Parbhani	*Xac-08*
9	Parbhani	*Xac-09*
10	Parbhani	*Xac-10*
11	Jalna	*Xac-11*
12	Jalna	*Xac-12*
13	Jalna	*Xac-13*
14	Aurangabad	*Xac-14*
15	Aurangabad	*Xac-15*
16	Aurangabad	*Xac-16*
17	Latur	*Xac-17*
18	Latur	*Xac-18*
19	Latur	*Xac-19*
20	Beed	*Xac-20*
21	Beed	*Xac-21*
22	Beed	*Xac-22*

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Table 2: Morphological Characters of *Xanthomonas axonopodis* pv.*citri*

Serial No.	Characteristics	Results
1	Gram's Nature	Gram-negative short rods
2	Colour of colony	Creamish yellow to dark yellow
3	Surface of colony	Smooth
4	Consistency	Sticky
5	Size of colony	2mm-4mm
6	Motility	Motile having single flagella

Table 3 : Biochemical characteristics of *Xanthomonas axonopodis* pv.*citri*

Biochemical Characteristics	***Xac* 01**	***Xac* 02**	***Xac* 03**	***Xac* 04**	***Xac* 05**	***Xac* 06**	***Xac* 07**	***Xac* 08**	***Xac* 09**	***Xac* 10**	***Xac* 11**	***Xac* 12**	***Xac* 13**	***Xac* 14**	***Xac* 15**	***Xac* 16**	***Xac* 17**	***Xac* 18**	***Xac* 19**	***Xac* 20**	***Xac* 21**	***Xac* 22**
Hydrolysis of Aesculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	+	+	+W	-	+	-	-	+W	-	-	-	-	-	+	+W	-	+	-	-	+	+W
Gelatin Liquifaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrolysis of Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Milk Hydrolysis	+	-	-	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+	-	+	+	+
Tween 80 Lipolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H2S Production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid production from 1) Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2)Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3)Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4)Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5)Manose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\+ : Positive Reaction
+W : weakly Positive Reaction
\- : Negative Reaction

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Table 4. Pathogenicity reaction of isolates *Xanthomonas axonopodis pv.citri* By Detached Leaf Assay

Strains	Location	Virulence
Xac-01	Nanded	++
Xac-02	Nanded	+++
Xac-03	Nanded	+
Xac-04	Nanded	++
Xac-05	Hingoli	+++
Xac-06	Hingoli	++
Xac-07	Hingoli	++
Xac-08	Parbhani	++
Xac-09	Parbhani	+++
Xac-10	Parbhani	++
Xac-11	Jalna	++
Xac-12	Jalna	+++
Xac-13	Jalna	+++
Xac-14	Aurangabad	++
Xac-15	Aurangabad	++
Xac-16	Aurangabad	++
Xac-17	Latur	++
Xac-18	Latur	++
Xac-19	Latur	+
Xac-20	Beed	++
Xac-21	Beed	++
Xac-22	Beed	+

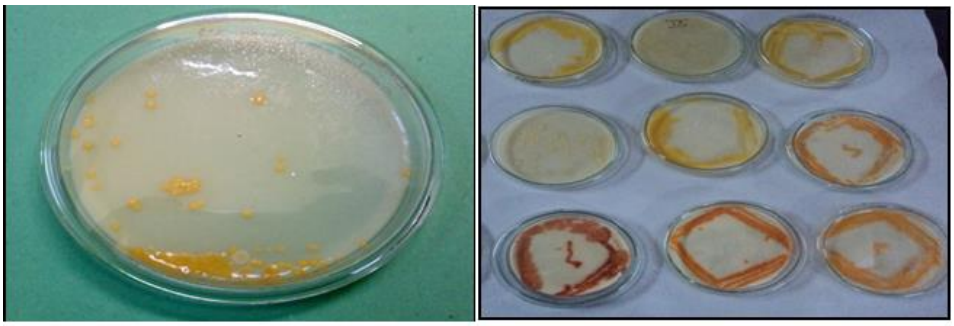

Fig.1 Isolation Of *Xanthomonas axonopodis* pv.*citri*

Fig.2 Pathogenicity Test of *Xanthomonas axonopodis pv.citri*