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Cultural Variability within Different Isolates of *Xanthomonas axonopodis* pv. *citri* Collected from Various Species of Citrus in Different Areas of Subtropical Zone of Himachal Pradesh

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A B S T R A C T

Citrus is the most important fruit crop of Himachal Pradesh and citrus canker caused by *Xanthomonas axonopodis* pv. *Citri* is major disease in all citrus growing areas in subtropical zone of Himachal Pradesh. To find out the amount of variability present in the bacterium, nine isolates collected from different species of citrus in subtropical areas were studied *in vitro* in relation to various cultural traits of the pathogen. It was found that all isolates exhibited considerable amount of variability in terms of all cultural parameters under study. Furthermore, Wakimoto growth medium was found to be the best for the growth of pathogen with pH 7.0 incubated at 25˚C temperature. All isolates were found to produce light yellow, yellow, pale and dark yellow circular colonies with varied elevations and colony diameter ranging between 1.00 to 6.00 mm on five different media under study.

Introduction

Citrus is the most important fruit crop of Himachal Pradesh cultivated on an area of 24649 hectares having maximum area under K. lime with a total production of 26853 metric tonnes (Anonymous, 2018). Citrus crop is threatened by number of diseases and among them citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin *et al.*, is one of the most devastating diseases in nursery and young orchards. The disease is of great economic importance all over the citrus growing area of the world including India. The disease has played a significant role in causing extensive losses in nurseries as well as orchards (Gottwald and Irey, 2007). The bacterium has been divided

in five different forms or pathotypes by Vauterin *et al*., (1991, 1995) *viz.,* A (*X. axonopodis* pv. *citri*), B/C/D (*X. axonopodis* pv. *aurantifolii*) and E (*X. a.* pv. *citrumelo*). Pathotype A (Asiatic form) of bacterium has a wide host range and is pathogenic on almost all citrus varieties. The existence of extensive genotype and phenotype variations within pathotype A of *X. axonopodis* pv. *citri* was unexpected and further complicates the systematics of this species (Verniere *et al*., 1998).

Das (2002) reported the existence of pathogenic variability within the A strain of *X. axonopodis* pv. *citri*. Pulsed field and plasmid profile analyses of strains of *X. a.* pv. *citri* showed considerable degree of diversity with regard to their extrachromosomal genetic element (Carvalho *et al*., 2005).

Relatively high amount of genetic diversity among 25 samples of citrus canker restricted to West Malaysia has been reported by Arshadi *et al*., (2013). Colour variation in the colonies of *X. a.* pv. *citri* from pale to dark yellow and variable reaction towards gelatin liquefaction and starch hydrolysis has been reported from the 15 isolates of citrus canker collected 14 agroclimatic zones of India (Katkar *et al*., 2016). Keeping in view the variability present in pathotype A of the bacterium, present studies were conducted to assess the cultural variability present among different isolates collected from different areas of subtropical zone of Himachal Pradesh.

Materials and Methods

The infected samples of various *Citrus* spp. from different areas of the subtropical zone of the state were collected and the associated pathogen was isolated. In all nine isolates of *X. axonopodis* pv. *citri* were maintained and mentioned in Table 1.

Growth of different isolates of *X. axonopodis* pv. citri on various nutrient liquid media

Different purified isolates were allowed to grow on various liquid media *viz.,* nutrient sodium chloride broth (NB), potato sucrose peptone broth (PSPB), Wakimoto broth (WB), nutrient dextrose broth (NDB) and yeast extract peptone dextrose broth (YEPB) to find out the best liquid medium for the growth of different isolates of *X. axonopodis* pv. *citri*. Three replications for each medium per isolate were maintained and growth of each isolate was recorded in terms of colony forming units per ml.

Erlenmeyer flasks of 150 ml capacity containing 50 ml of liquid medium each were autoclaved at 121ºC temperature and 15 p.s.i. pressure for 20 minutes. After autoclaving, flasks containing different liquid media were inoculated with 1ml of 48 h old bacterial suspension and incubated at 28 ± 2˚C in shaking incubator for 48 h. After 48 h of incubation, 1ml of bacterial suspension from each flask was serially diluted up to a dilution factor of 10^{-7}. One ml of the suspension from each dilution was pour plated on nutrient agar medium and colony forming units of the bacterium were recorded after 48 h of incubation at 28 ± 2˚C by using the formula.

$$\text{cfu / ml} = \frac{number\ of\ colonies}{volume\ plated\ (ml) \times dilution\ factor}$$

Cultural characteristics of different bacterial isolates on various solid media

Purified isolates were grown on best liquid media selected during previous experiment by inoculating 48 h old bacterial culture in 50 ml broth each and after 48 h of incubation, this suspension was serially diluted up to 10^{-7}. One ml suspension from this dilution was pour plated on five different solid media *viz*., nutrient sodium chloride agar (NA), nutrient

dextrose agar (NDA), potato sucrose peptone agar (PSPA), yeast extract peptone dextrose agar (YEA) and Wakimoto agar (WA) and incubated at 28 ± 2˚C for 48 h. Colony characters like size, colour, shape and elevation were observed after 48 h of incubation.

Effect of different temperatures on the growth of *Xanthomonas axonopodis* pv. citri

One ml of 48 h old bacterial suspension of each isolate was inoculated into the best liquid medium and incubated at five different temperatures *viz*., 15, 20, 25, 30 and 35˚C for 48 h. Thereafter, bacterial suspension from each flask was serially diluted up to 10^{-7} and pour plated on nutrient agar medium and incubated at 28 ± 2˚C for 48 h to observe colony forming units per ml at each temperature.

Effect of different pH levels on the growth of *Xanthomonas axonopodis* pv. *citri*

The best liquid medium was adjusted to different pH regimes *viz*., 5.0, 6.0, 7.0, 8.0 and 9.0 and inoculated with 1 ml of 48 h old bacterial suspension of each isolate and incubated at best temperature for respective isolate. After that, bacterial suspension from each flask was serially diluted up to 10^{-7} and pour plated on nutrient agar medium and incubated at 28 ± 2˚C for 48 h. The data in terms of colony forming units per ml were recorded.

Results and Discussion

Growth of different isolates of *Xanthomonas axonopodis* pv. *citrion* various nutrient liquid media

It is clear from the Table 2 that irrespective of the isolates under study, the average growth of all isolates was recorded to be maximum (8.47×10^7cfu / ml) in WB which was statistically at par with the growth of all isolates in PSPB (7.97×10^7cfu / ml). However, the average growth of all isolates was recorded to be significantly minimum in YEPB (2.83×10^7cfu / ml) followed by NDB (4.73×10^7cfu / ml) and NB (6.57×10^7cfu / ml). As far as the average growth of individual isolates was concerned, isolate 4 exhibited maximum average growth (10.78×10^7cfu / ml) after 48 h of incubation, irrespective of the media used which was significantly followed by isolate 6 (9.18×10^7cfu / ml), isolate 2 (7.76×10^7cfu / ml) and isolate 9 (8.04×10^7cfu / ml). Isolate 7 exhibited significantly minimum average growth (0.86×10^7cfu / ml) irrespective of the media used significantly followed by average growth of isolate 3 (1.68×10^7cfu / ml).

Body of the table revealed that significantly maximum growth (19.86×10^7cfu / ml) was recorded in isolate 9 in WB after 48 h of incubation followed by isolate 5 (16.03×10^7cfu / ml) in NB and isolate 2 (14.43×10^7cfu / ml) in NB. In YEPB, isolates 1, 2, 3, 5, 7 and 9 and in NDB, isolates 1 and 7 did not grow at all. However, significantly minimum growth was recorded in isolate 5 (0.10×10^7cfu / ml) in NDB which was statistically at par with isolate 7 (0.20×10^7cfu / ml) in PSPB, isolate 3 (0.30×10^7cfu / ml) in NB and isolate 3 (0.76×10^7cfu / ml) in NDB. Rest all isolates exhibited intermediate range of growth in all the media under study.

Analysis of variance was also performed separately to compare different media with respect to cfu / ml of each isolate. The media in which individual isolate was found to show maximum growth (cfu / ml) was selected for the further studies of each respective isolate. Coefficients of variance calculated for different isolates exhibited that isolate 5

showed maximum variability (51.75%) in growth followed by isolate 9 (47.18%) whereas minimum variability was recorded in isolate 4 (6.31%) followed by isolate 6 (14.05%) with respect to different media used.

With the variability data recorded, isolates were placed in four groups (Fig 1). Group – I included isolates 5 and 9, group – II included isolates 1 and 2, group – III included isolates 3, 7 and 8, group – IV included isolates 4 and 6. The isolates in individual groups were found to be closely related with each other i.e. they were found to have less variability differences. All the groups were found to be distantly related with each other exhibiting large variability differences. This variability in isolates with respect to nutrient media may be attributed to their differential selection of nutrients available in different media.

Cultural characteristics of different bacterial isolates on various solid media

From the Table 3, it is clear that all the pathogen isolates tested were circular in shape on different media. Colour of the pathogen isolates varied from light yellow to dark yellow and elevation was measured as elevated, centre elevation, slight elevation, flat and suppressed.

Based on the colour of colonies produced on different nutrient media the isolates were grouped accordingly.

Nutrient sodium chloride agar

The isolates producing yellow colour were included in group – I (isolates 2, 3, 4, 6 and 7), isolates producing light yellow colour were placed in group – II (isolate 5) and isolates producing pale colour were placed in group – III (isolates 1, 8 and 9) (Fig 2).

Nutrient dextrose agar

The isolates producing yellow colour were placed in group – I (isolates 3, 7 and 8), isolates producing light yellow colour were included in group – II (isolates 2, 4 and 9), isolates producing pale colour were included in group – III (isolates 5 and 6) and isolates producing dark yellow colour were included in group – IV (isolate 1; Fig 3).

Potato sucrose peptone agar

The isolates producing yellow colour were included in group – I (isolates 5, 7 and 9), isolates producing light yellow colour were included in group – II (isolate 8), isolates producing pale colour were placed in group – III (isolates 2, 4 and 6) and isolates producing dark yellow colour were included in group – IV (isolates 1 and 3; Fig 4).

Yeast extract peptone dextrose agar

The isolates producing yellow colour (isolates 1, 4, 6 and 7), light yellow colour (isolates 8 and 9) and pale colour (isolates 2, 3 and 5) were included in group – I, II and III respectively (Fig 5).

Wakimoto agar

The isolates producing yellow colour (isolates 1, 2, 4, 5, 7, 8 and 9) and light yellow colour (isolates 3 and 6) were included in group – I and II respectively (Fig 6).

The observations recorded in terms of colony diameter of each isolate of *X. a.* pv. *Citri* grown on different solid media have been presented in Table 4.

From the data presented in the table, it is clear that irrespective of the isolates under study, significantly maximum mean colony diameter (3.30 mm) was observed on YEA medium

followed by colony diameter on PSPA (2.99 mm) and NA (2.33 mm). However, the mean colony diameter was recorded to be minimum on WA (1.87 mm) which was significantly followed by colony diameter on NDA (2.27 mm). Irrespective of the media under study, significantly maximum mean colony diameter was observed in isolate 2 (3.54 mm) which was followed by colony diameter in isolate 1 and 3 (3.04 mm). However, mean colony diameter was recorded to be minimum in isolate 8 (1.75 mm) which was significantly followed by mean colony diameter in isolate 6 (2.07 mm) which was statistically at par with isolate 7 (2.02 mm).

Body of the table revealed that significantly maximum colony diameter was observed in isolate 2 (6.00 mm) on YEA followed by colony diameter in isolate 9 (4.75 mm) on PSPA which was statistically at par with isolates 1 and 3 (4.50 mm) on YEA. However, minimum colony diameter was observed in isolate 6 (1.00 mm) on WA and isolate 8 (1.00 mm) on PSPA which was statistically at par with colony diameter of isolate 9 (1.12 mm) on YEA, isolate 4 (1.25 mm) on WA, isolate 6 and 9 (1.25 mm) on NDA and isolate 7 (1.25 mm) on YEA.

Variability analysis through coefficient of variation was also performed and it was found that isolate 9 exhibited maximum variability (53.23%) with respect to colony diameter on different media followed by isolate 6 (48.43%) and isolate 2 (41.11%). However, minimum variability was observed in isolate 5 (24.27%) which was followed by isolate 8 (28.57%) and isolate 1 (30.11%). Based on variability studies, the isolates were grouped into five groups (Fig. 7).

Group – I included isolates 1, 4 and 8, group – II included isolates 3 and 7, group – III included isolates 6 and 9, group IV included isolate 5 and group V included isolate 2. Isolates in individual groups were showing less variability differences compared with each other so, they were found to be closely related and groups were showing maximum variability differences compared with each other so, the groups were found to be distantly related.

Effect of different temperature regimes on the growth of *Xanthomonas axonopodis* pv. *citri*

It is clear from the Table 5 that irrespective of the different isolates under study, the average growth of all isolates was recorded to be significantly maximum (7.79×10^7cfu / ml) at 25˚C followed by 30˚C (4.74×10^7cfu / ml) which was statistically at par with growth at 20˚C (4.91×10^7cfu / ml). However, the average growth of all isolates was recorded to be minimum (1.99×10^7cfu / ml) at 35˚C followed by 15˚C (2.99×10^7cfu / ml). As far as the growth of individual isolate was concerned, isolate 6 exhibited significantly maximum average growth (13.73×10^7cfu / ml) after 48 h of incubation irrespective of the different temperature regimes followed by isolate 2 (7.88×10^7cfu / ml) which was statistically at par with isolate 4 (7.31×10^7cfu / ml) whereas, isolate 7 exhibited minimum average growth (0.21×10^7cfu / ml) which was statistically at par with isolate 3 (0.47×10^7cfu / ml) irrespective of the different temperature regimes under study.

Body of the table revealed that isolate 6 at 25˚C temperature exhibited maximum growth (23.20×10^7cfu / ml) after 48 h of incubation significantly followed by isolate 2 (18.20×10^7cfu / ml) at 20˚C and isolate 4 (14.06×10^7cfu / ml) at 30˚C. Also, at temperature 15 and 20˚C, no growth was observed in case of isolate 7. However, minimum growth (0.03×10^7cfu / ml) was recorded in isolate 3 at 15˚C which was statistically at par with same isolate (0.06×10^7cfu / ml) and isolate 7 (0.13

× 10^7cfu / ml) at 35˚C temperature. Rest all isolates exhibited intermediate range of growth at all the temperatures under study.

During present studies average growth of all isolates was found to be best at 25˚C followed by 20 and 30˚C, indicating that a range of 20 to 30˚C temperature to be optimum for the growth of all isolates. At 15 and 35˚C temperatures, the growth was significantly reduced.

Analysis of variance was also performed separately to compare different temperatures with respect to colony forming units (cfu) / ml of each isolate. The temperature at which individual isolate was found to show maximum growth (cfu / ml) was selected for further studies of respective isolate.

Coefficient of variation analysis was also carried out and it was found that isolate 8 exhibited maximum variability (46.22%) in growth followed by isolate 2 (32.43%) with respect to different temperatures. However, minimum variability in growth was observed in isolate 7 (9.63%) followed by isolate 9 (17.46%).

With this variability data, isolates were grouped into six groups (Fig 8). Group – I included isolates 3, 4 and 6, group – II included isolates 1 and 5, group – III included isolate 8, group – IV included isolate 7, group – V included isolate 9 and group VI included isolate 2. Isolates in individual groups were showing less variability differences compared with each other so, they were found to be closely related and groups were showing maximum variability differences compared with each other so, the groups were found to be distantly related.

During present studies average growth of all isolates was found to be best at 25˚C followed by 20 and 30˚C, indicating that a range of 20 to 30˚C temperature to be optimum for the growth of all isolates. At 15 and 35˚C temperatures, the growth was significantly reduced.

Effect of different pH levels on the growth of *Xanthomonas axonopodis* pv. *citri*

It is clear from the Table 6 that irrespective of the isolates under study, the average growth of all the isolates was recorded to be maximum (4.94 × 10^7cfu / ml) at pH 7 which was significantly followed by the average growth of isolates at pH 8 (2.96 × 10^7cfu / ml) and pH 6 (2.26 × 10^7cfu / ml). However, the average growth of all the isolates was recorded to be minimum at pH 5 (0.31 × 10^7cfu / ml) which was significantly followed by average growth at pH 9 (1.52 × 10^7cfu / ml). As far as the growth of individual isolate was concerned, irrespective of the different pH levels, isolate 5 exhibited maximum average growth (5.68 × 10^7cfu / ml) after 48 h of incubation which was significantly followed by isolate 2 (4.33 × 10^7cfu / ml) and isolate 8 (3.88 × 10^7cfu / ml). However, isolate 3 exhibited minimum average growth (0.49 × 10^7cfu / ml) which was significantly followed by growth of isolate 1 (0.79 × 10^7cfu / ml) and isolate 7 (1.34 × 10^7cfu / ml).

Body of the table revealed that significantly maximum growth was recorded in isolate 5 (10.70 × 10^7cfu / ml) grown at pH 7 after 48 h of incubation followed by growth of same isolate at pH 6 (8.40 × 10^7cfu / ml) which was statistically at par with growth of isolate 2 (7.80 × 10^7cfu / ml) at pH 7. It was interesting to note that at pH 5 isolates 4, 6, 7, 8 and 9, at pH 6 isolate 9 and at pH 9 isolates 3 and 7 did not show any growth at all after 48 h of incubation. However, minimum growth (0.03 × 10^7cfu / ml) was recorded in isolates 1 and 3 each grown at pH 5 which was statistically at par with, isolate 1 (0.10 × 10^7cfu / ml) at pH 6, isolate 5 (0.26 × 10^7cfu / ml) at pH 5,

isolate 6 (0.36 × 10^7cfu / ml) and isolate 3 (0.43 × 10^7cfu / ml) at pH 6, isolate 3 (0.58 × 10^7cfu / ml) at pH 8 and isolate 1 (0.26 × 10^7cfu / ml) and isolate 6 (0.73 × 10^7cfu / ml) at pH 9 after 48 h of incubation. Rest all isolates exhibited intermediate range of growth at all levels of pH under study.

Analysis of variance was also performed separately to compare different levels of pH with respect to colony forming units (cfu) / ml of each isolate. The pH at which individual isolate was found to show maximum growth (cfu / ml) was selected for further studies of respective isolate.

Table.1 Details of various isolates collected from different areas

District	Area	*Citrus* spp.	Isolate name
Sirmour	Behrabala	*Citrus reticulata* (Kinnow)	Isolate – 1
	Kolar	*C. sinensis* (Mosambi)	Isolate – 2
	Dhaulakuan	*C. jambhiri* (Jattikhatti)	Isolate – 3
Kangra	Basabzain	*C. reticulata* (Kinnow)	Isolate – 4
	Panjarda	*C. sinensis* (Mosambi)	Isolate – 5
	Jachh	*C. jambhiri* (Jattikhatti)	Isolate – 6
Hamirpur	Neri	*C. aurantifolia* (K. lime)	Isolate – 7
	Dhanwan	*C. aurantifolia* (K. lime)	Isolate – 8
	Lambloo	*C. jambhiri* (Jattikhatti)	Isolate – 9

Table.1 Growth and variability in various isolates of *Xanthomonas axonopodis* pv. *citrias* affected by different liquid media

Media	Isolate									Mean
	1	2	3	4	5	6	7	8	9	
	Growth (cfu/ ml ×10^7)									
NB	9.36 (3.20)	14.43 (3.92)	0.30 (1.13)	10.60 (3.39)	16.03 (4.12)	4.96 (2.44)	1.40 (1.54)	0.60 (1.26)	1.46 (1.56)	6.57 (2.51)
PSPB	5.60 (2.53)	7.66 (2.92)	2.70 (1.92)	13.50 (3.80)	12.40 (3.65)	12.93 (3.71)	0.20 (1.09)	7.98 (2.99)	8.80 (3.12)	7.97 (2.86)
WB	6.53 (2.74)	12.40 (3.65)	4.63 (2.37)	9.03 (3.16)	6.30 (2.65)	11.06 (3.47)	2.73 (1.91)	3.70 (2.16)	19.86 (4.55)	8.47 (2.96)
NDB	0.00 (1.00)	4.30 (2.27)	0.76 (1.32)	9.83 (3.28)	0.10 (1.04)	7.63 (2.93)	0.00 (1.00)	9.86 (3.29)	10.10 (3.32)	4.73 (2.16)
YEPB	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	10.96 (3.45)	0.00 (1.00)	9.33 (3.21)	0.00 (1.00)	5.20 (2.48)	0.00 (1.00)	2.83 (1.68)
MEAN	4.30 (2.09)	7.76 (2.75)	1.68 (1.55)	10.78 (3.41)	6.96 (2.49)	9.18 (3.15)	0.86 (1.31)	5.47 (2.44)	8.04 (2.71)	
***$CD_{0.05}$**	0.51	0.54	0.20	NS	0.55	0.46	0.26	0.29	0.38	
CV (%)	43.97	38.10	33.40	6.31	51.75	14.05	27.78	28.98	47.18	
****$CD_{0.05}$**	Media 0.12 Isolate 0.17 Media × isolate 0.38									

NB – Nutrient sodium chloride broth, NDB – Nutrient dextrose broth, PSPB – Potato sucrose peptone broth, YEPB – Yeast extract peptone dextrose broth, WB – Wakimoto broth.

Figures in parentheses showing square root transformed values

* For one factor, ** For two factors

Table.2 Colony characteristics of various isolates of *Xanthomonas axonopodis pv. citri* on different solid media

Isolates	Characters	Media				
		NA	NDA	PSPA	YEA	WA
1	Colour	Pale	Dark yellow	Dark yellow	Yellow	Yellow
	Elevation	Flat	Elevated	Elevated	Elevated	Flat
	Shape	Circular	Circular	Circular	Circular	Circular
2	Colour	Yellow	Light yellow	Pale	Pale	Yellow
	Elevation	Elevated	Suppressed	Elevated	Elevated	Flat
	Shape	Circular	Circular	Circular	Circular	Circular
3	Colour	Yellow	Yellow	Dark yellow	Pale	Light yellow
	Elevation	Slight elevation	Elevated	Flat	Elevated	Elevated
	Shape	Circular	Circular	Circular	Circular	Circular
4	Colour	Yellow	Light yellow	Pale	Yellow	Yellow
	Elevation	Flat	Centre elevation	Centre elevation	Slight elevation	Elevated
	Shape	Circular	Circular	Circular	Circular	Circular
5	Colour	Light yellow	Pale	Yellow	Pale	Yellow
	Elevation	Flat	Flat	Flat	Slight elevation	Flat
	Shape	Circular	Circular	Circular	Circular	Circular
6	Colour	Yellow	Pale	Pale	Yellow	Light yellow
	Elevation	Flat	Centre elevation	Centre elevation	Slight elevation	Elevated
	Shape	Circular	Circular	Circular	Circular	Circular
7	Colour	Yellow	Yellow	Yellow	Yellow	Yellow
	Elevation	Flat	Flat	Flat	Flat	Flat
	Shape	Circular	Circular	Circular	Circular	Circular
8	Colour	Pale	Yellow	Light yellow	Light yellow	Yellow
	Elevation	Flat	Flat	Elevated	Elevated	Flat
	Shape	Circular	Circular	Circular	Circular	Circular
9	Colour	Pale	Light yellow	Yellow	Light yellow	Yellow
	Elevation	Flat	Flat	Flat	Elevated	Flat
	Shape	Circular	Circular	Circular	Circular	Circular

Table.4 Variability in colony diameter of various isolates of *Xanthomonas axonopodis* pv. *citri* on different solid media

Media	Isolate									Mean
	1	2	3	4	5	6	7	8	9	
	Colony diameter (mm)									
NA	3.25	2.50	2.12	2.62	2.62	1.75	1.50	2.00	2.62	2.33
NDA	2.12	4.37	2.50	1.50	3.75	1.25	2.00	1.75	1.25	2.27
PSPA	3.37	2.87	4.12	2.87	2.12	2.62	3.25	1.00	4.75	2.99
YEA	4.50	6.00	4.50	2.62	3.50	3.75	1.25	2.50	1.12	3.30
WA	2.00	2.00	2.00	1.25	2.12	1.00	2.12	1.50	2.87	1.87
Mean	3.04	3.54	3.04	2.17	2.82	2.07	2.02	1.75	2.52	
CV (%)	30.11	41.11	34.46	30.47	24.27	48.43	34.13	28.57	52.23	
$CD_{0.05}$	Media 0.14 Isolate 0.19 Media × isolate 0.42									

NA – Nutrient sodium chloride agar, NDA – Nutrient dextrose agar, PSPA – Potato sucrose peptone agar, YEA – Yeast extract peptone dextrose agar, WA – Wakimoto agar.

Table.3 Growth and variability in various isolates of *Xanthomonas axonopodis* pv.*citri* as influenced by different temperature regimes

Temperature (˚C)	Isolate									Mean
	1	2	3	4	5	6	7	8	9	
	Growth (cfu/ ml ×10^7)									
15	1.71 (1.64)	5.43 (2.53)	0.03 (1.01)	3.85 (2.19)	0.38 (1.17)	13.76 (3.84)	0.00 (1.00)	0.23 (1.10)	1.51 (1.58)	2.99 (1.78)
20	3.70 (2.12)	18.20 (4.37)	0.16 (1.07)	4.56 (2.35)	1.37 (1.53)	13.86 (3.85)	0.00 (1.00)	0.66 (1.28)	1.68 (1.62)	4.91 (2.13)
25	7.30 (2.85)	9.50 (3.22)	1.93 (1.69)	7.53 (2.91)	4.40 (2.32)	23.20 (4.91)	0.65 (1.28)	12.93 (3.73)	2.66 (1.89)	7.79 (2.75)
30	3.80 (2.15)	4.51 (2.34)	0.16 (1.08)	14.06 (3.88)	0.98 (1.40)	12.83 (3.71)	0.26 (1.12)	3.40 (2.09)	2.68 (1.90)	4.74 (2.19)
35	1.06 (1.43)	1.78 (1.66)	0.06 (1.03)	6.53 (2.73)	0.70 (1.30)	5.00 (2.43)	0.13 (1.06)	2.41 (1.84)	0.26 (1.12)	1.99 (1.62)
Mean	3.51 (2.04)	7.88 (2.82)	0.47 (1.18)	7.31 (2.81)	1.56 (1.54)	13.73 (3.75)	0.21 (1.09)	3.93 (2.01)	1.76 (1.62)	
***$CD_{0.05}$**	0.69	0.46	0.25	0.40	0.24	0.37	0.05	0.26	0.45	
CV (%)	24.06	32.42	21.89	20.99	26.15	21.00	9.63	46.22	17.46	
****$CD_{0.05}$**	Temperature 0.11 Isolate 0.15 Temperature × isolate 0.34									

Figures in parentheses showing square root transformed values; * For one factor; ** For two factors

Table.4 Growth and variability in various isolates of *Xanthomonas axonopodis* pv.*citri* as influenced by different pH levels

pH	Isolate									Mean
	1	2	3	4	5	6	7	8	9	
	Growth (cfu/ ml ×10^7)									
5	0.03 (1.01)	2.43 (1.85)	0.03 (1.01)	0.00 (1.00)	0.26 (1.12)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.31 (1.11)
6	0.10 (1.04)	4.60 (2.36)	0.43 (1.19)	2.20 (1.78)	8.40 (3.06)	0.36 (1.16)	2.01 (1.73)	2.26 (1.80)	0.00 (1.00)	2.26 (1.68)
7	2.43 (1.85)	7.80 (2.96)	1.43 (1.55)	3.16 (2.03)	10.70 (3.40)	4.70 (2.38)	4.68 (2.36)	7.13 (2.85)	2.43 (1.85)	4.94 (2.36)
8	1.13 (1.46)	4.70 (2.38)	0.58 (1.25)	2.86 (1.96)	5.81 (2.61)	2.30 (1.81)	0.00 (1.00)	6.86 (2.80)	2.38 (1.83)	2.96 (1.90)
9	0.26 (1.12)	2.13 (1.76)	0.00 (1.00)	2.15 (1.77)	3.21 (2.05)	0.73 (1.31)	0.00 (1.00)	3.16 (2.03)	2.01 (1.73)	1.52 (1.53)
Mean	0.79 (1.30)	4.33 (2.26)	0.49 (1.20)	2.07 (1.71)	5.68 (2.45)	1.62 (1.57)	1.34 (1.42)	3.88 (2.09)	1.36 (1.48)	
***$CD_{0.05}$**	0.10	0.13	0.12	0.26	0.35	0.14	0.30	0.26	0.14	
CV (%)	24.44	19.05	16.78	21.61	32.80	32.84	38.91	32.71	26.80	
****$CD_{0.05}$**	pH 0.06 Isolate 0.08 pH × isolate 0.19									

Fig.1 Grouping of isolates of *Xanthomonas axonopodis* pv.*citri* on the basis of variability observed on various nutrient liquid media

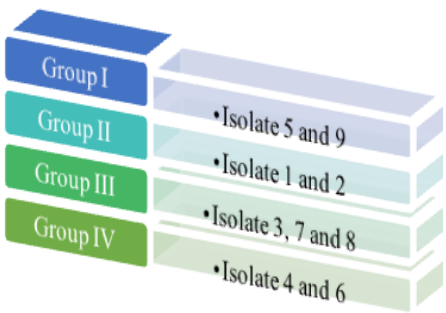


Fig.2 Grouping of isolates of *Xanthomonas axonopodis* pv.*citri* on the basis of colony colour produced on nutrient sodium chloride agar

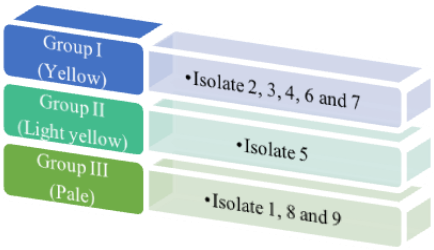


Fig.3 Grouping of isolates of *Xanthomonas axonopodis* pv. *Citri* on the basis of colony colour produced on nutrient dextrose agar

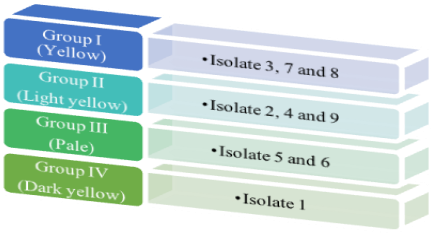


Fig.4 Grouping of isolates of *Xanthomonas axonopodis* pv. *citri* on the basis of colony colour produced on potato sucrose peptone dextrose agar

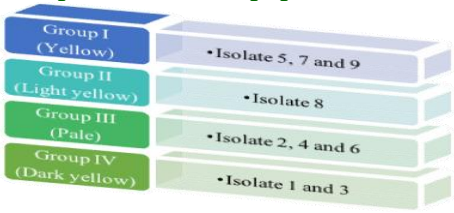


Fig.5 Grouping of isolates of *Xanthomonas axonopodis* pv. *citri* on the basis of colony colour produced on yeast extract peptone dextrose agar

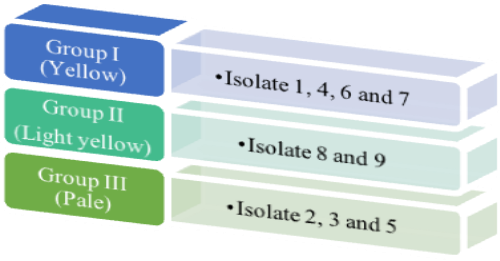
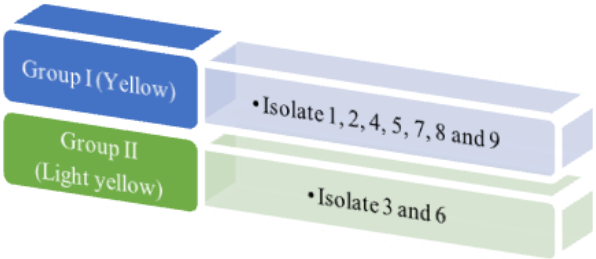


Fig.6 Grouping of isolates of *Xanthomonas axonopodis* pv. *citri* on the basis of colony colour produced on Wakimoto agar



Variability analysis was also performed by calculating coefficient of variation and it was found that isolate 7 exhibited maximum variability (38.91%) with respect to different pH levels followed by isolate 6 (32.84%) and isolate 5 (32.80%) whereas, minimum variability was recorded in isolate 3 (16.78%) which was followed by isolate 2 (19.05%) and isolate 4 (21.61%). With the variability data recorded, the isolates were grouped into four groups (Fig 9). Group – I included isolates 5, 6 and 8, group – II included isolates 1 and 9, group – III included isolates 2, 3 and 4 and group – IV included isolate 7.

Isolates in individual groups were showing less variability differences compared with each other so, they were found to be closely related and groups were showing maximum variability differences compared with each other so, the groups were found to be distantly related. Growth of any pathogen at a particular pH level gives an idea about its occurrence at acidic, basic and neutral soil conditions in nature. During present studies all isolates were able to grow at neutral pH i.e. pH 7.0 while at pH 6.0 and 8.0 two different isolates could not grow at all. This indicated that all isolates can survive and cause disease at neutral pH and most can survive at slightly acidic or alkaline pH levels. The isolates exhibited variable reaction towards different pH levels.

The results of present findings of Wakimoto broth to be the best supportive medium for the growth of all isolates were in accordance with Singh and Thind (2014) and Falico de Alcaraz (1980). The variation in colony colour of different isolates when grow on five different media can be attributed to variable amount of xanthomonadin produced by each isolate on respective nutrient medium. The results are in accordance with Srinivasan *et al.,* (1959) who reported strain variability with respect to cultural studies in *Xanthomonas* spp. The results are further supported by findings of Jadhav *et al.,* (2018), who reported that, from nine isolates, colonies of seven isolates of *X. a.* pv. *citri* to be yellow in colour while two isolates to be light yellow in colour. The literature pertaining to colony diameter of *X. axonopodis* pv. *citri* is not available. However, Suresh *et al.,* (2013) reported thatthe colony diameter of *X. oryzae* pv. *oryzae* to be in the range of 1.0 to 5.0 mm on different solid media.

For cultural studies of *X. axonopodis* pv. *Citri* 25˚C and pH 7 was found to be the best. These results are supported by the findings of Lakpale *et al.,* (2006), Das (2003) and Muduli (2017). However, variability studies are in accordance with the findings of Negi (2015).

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