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Cultural and Morphological Characteristics of Different *Xanthomonas axonopodis* pv. *punicae* Isolates on Nutrient Agar Media

A.G. Patil*, C.V. Ambadkar, K.M. Kanase and V.S. Kashid

Department of Plant Pathology, College of Agriculture, Latur Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India
**Corresponding author*

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A B S T R A C T

Cultural and morphological characters of different *Xap* isolates were studied on nutrient agar media. Five isolates collected from different locations differed in respect of size of colony, shape of colony and colour of bacterial colony. Isolates *Xap*-1 and *Xap*-4 developed medium to large bacterial colonies. The isolates *Xap*-1 and *Xap*-3 developed small to medium sized bacterial colonies, whereas, *Xap*-5 produced small bacterial colonies. The effect of varied temperature levels on the growth of *Xap* was studied. All the *Xap* isolates were tested at temperature 28^0C and 37^0C on NA agar medium. The temperature of 28^0C was found optimum for the growth of the pathogen as significantly maximum number of colonies was observed at this temperature. Maximum growth of *Xap* isolates was observed at pH 6.5 and 7, whereas, moderate growth of *Xap*-2, *Xap*-3, *Xap*-4, *Xap*-5 isolates was observed at pH 6.0 except *Xap*-1 which showed less growth at pH 6.0. All *Xap* isolates showed maximum growth at 1 per cent salt concentration, whereas, all these isolates failed to grow at 2 per cent and 3 per cent salt concentration.

Introduction

India is the leading pomegranate producer which contributes nearly 50 per cent of the world's production. Cultivation of high yielding varieties of pomegranate with intensive care and management in the recent past under irrigated condition with early stage exploitation of plants has lead to certain severe pest and disease problems. Among the major diseases leaf spot, fruit spot and wilt results in reduction of pomegranate fruit yield and put the growers in to hardship. Pomegranate grows very well on the moderately alkaline soils as well as slightly acidic soils. It is common to the tropics, sub-tropics and sub-temperate regions and is well adapted to areas with hot and dry summers. For the first time in India, leaf spot of pomegranate was reported Hingorani and Mehta (1952). Later on during 1959, Hingorani and Singh took a thorough investigation on the disease and causal organism and designated the pathogen as *Xanthomonas punicae.* Chand and Kishun (1991) had isolated the causal organism of bacterial blight of pomegranate and based on its pathological, cultural, biochemical and physiological features, identified as a *Xanthomonas campestris* pv. *punicae*. Further in year 1995, Vauterin *et al.*, (1995) had re-named this causal organism as *Xanthomonas*

axonopodis pv. *punicae* keeping in view of activity of the presence or absence of metabolic activity on different carbon substrates.

Materials and Methods

Morphological characters

The morphological characters such as shape, gram reaction and pigmentation characters were studied as described by Society of American Bacteriologists, Bradbury (1970) and Schaad and Stall (1992).

Cultural character

Different isolates of *Xanthomonas axonopodis* pv. *punicae* grown on nutrient agar media and stored at 28±2 ^{o}C temperature. The observations were recorded after 72 hours. The colour of colony was recorded following Ridway colour standard and colour nomenclature (Ridway, 1912).

Effect of physical factors on the growth and multiplication of *Xanthomonas axonopodis* pv. *punicae*

Temperature requirement

An experiment was conducted to know the optimum temperature required for the growth of *Xanthomonas axonopodis* pv. *punicae* isolates causing bacterial blight of pomegranate in different agroclimatic zones. The bacterial cultures were multiplied separately in nutrient broth. The cultures were prepared by inoculating a loopful of bacterial cultures from stock culture to 100 ml nutrient broth contained in 250 ml conical flask and incubated for 72 hours at 28°C. Fifty microlitre of the bacterial cultures diluted to a concentration of 5x10^{5}cfu/ml was poured on to the surface of nutrient agar medium taken in sterilized Petri dishes. The bacterial suspension was uniformly spread with the help of a sterilized glass spreader so as to obtain well distributed bacterial colonies. The inoculated plates were incubated at different temperatures *viz.*, 28 and 37°C for 48, 72, 96 and 120 hours. Observations were recorded on the number of colonies appearing after the incubation period at each temperature.

pH requirement

The effect of hydrogen ion concentration on the growth of the bacterial isolates was studied by adjusting the pH of the nutrient agar. The pH was adjusted to 5, 5.5, 6, 6.5 and 7 using appropriate buffers.

A loopful of 72 hours old bacterial culture was serially diluted to 9 ml sterilized water blanks. One ml of 10^{-5} dilution was plated separately on to the surface of the medium having varied pH levels. The suspension was spread uniformly over the medium with the help of sterilized spreader. Inoculated plates were incubated at 28^{o}C temperature for 72 hours. After the incubation period, observations were recorded for the development of colonies on the media having different pH. Colonies were counted and recorded. Data was analyzed statistically.

Salt concentration

The effect of salt concentration on the growth of bacterial isolates was studied by adjusting the salt concentration of Nutrient Agar. Salt sensitivity of different level such as 1%, 2% and 3% salt concentration was tested.

A loopful of 72 hours old bacterial culture was serially diluted to 9 ml sterilized water blank. One ml of dilution was plated separately on the surface of medium having varied salt concentration. The suspension was spread uniformly over the medium with the help of sterilized spreader. Inoculated plates

were incubated at 28°C temperature for 72 hours. After the incubation period, observations were recorded for the development of colonies on the media having different salt concentration. Colonies were counted and recorded.

Results and Discussion

Cultural and morphological characteristics of different *Xap* isolates on nutrient agar media

Cultural and morphological characters of different *Xap* isolates were studied on nutrient agar media (Table 1). The data revealed that the isolates differed in respect of size of colony as the isolates *Xap*-1 and *Xap*-4 developed medium to large bacterial colonies. The isolates *Xap*-1 and *Xap*-3 developed small to medium sized bacterial colonies, *Xap*-5 produced small bacterial colonies. The isolates of *Xanthomonas axonopodis* pv. *punicae* also differed greatly in shape of colony as the *Xap*-1 and *Xap*-3 produced small circular colonies, *Xap*-2 and *Xap*-4 produced circular to irregular bacterial colonies *Xap*-5 produced circular bacterial colonies.

The isolates also differed in respect to colour of bacterial colonies. *Xap*-1 and *Xap*-4 showed yellowish bacterial colonies, *Xap*-2 and Zap-5 showed light yellow coloured colonies, *Xap*-3 showed yellow coloured bacterial colonies. Five isolates showed significant difference in their appearance. *Xap*-3 and *Xap*-4 showed highly raised, glistering appearance, *Xap*-1, *Xap*-2 and *Xap*-5 showed slightly raised, glistering appearance. All the isolates showed single rod and convex elevation and slightly mucoid to highly mucoid texture. Results on morphological characters of *X. axonopodis* pv. *punicae* in present investigation are similar with the results of earlier workers Hingorani and Singh (1959), Kanwar (1976) and Jalaraddi (2006). Jalaraddi (2006) reported that the bacteria isolated from infected parts of pomegranate was found to be aerobic, Gram negative, capsulated, non-spore forming and monotrichously flagellated, whereas, Manjula (2002) observed that the repeated isolations made from the infected parts of pomegranate plant yielded yellow, mucoid, glistening, slimy, convex and odourless colonies on Nutrient Agar (Table 2).

Growth rate of different *Xap* isolates at 28^0C and 37^0C

The effect of varied temperature levels on the growth of *Xap* was studied and data so obtained is presented in Table 3. All the *Xap* isolates were tested at temperature 28^0C and 37 ^{0}C on NA agar medium. The data clearly indicated that the temperature of 28^0C was found optimum for the growth of the pathogen as significantly maximum number of colonies was observed at this temperature.

All isolates grew well at 28^0C but no growth was observed at temperature of 37 ^{0}C. Growth of *Xap* started 72 hrs after incubation. Maximum growth was observed after 120 hrs of incubation at 28^0C but no growth was observed after 48 hrs of incubation. At 37^0C no growth was observed upto 120 hrs.

Similar work on temperature requirement was carried out by Hingorani and Mehta (1952).They found that the pomegranate bacterium grows well at a cordial temperature of 30^0C and can tolerate a minimum and maximum temperature of 5 and 40^0C, respectively. Gour *et al.,* (2000) also got the similar results while working with *X. axonopodis* pv. *vignicola*, the causal agent of leaf blight of cowpea. They have recorded the maximum growth of the pathogen at a temperature level of 30^0C, whereas, Manjula (2002) recorded the highest number of colonies of *Xap* at a temperature of 27^0C.

Table.1 Isolates used for cultural, morphological and physiological test

Sr. No.	Name of isolate	Place of collection	District	Pathogen identified	Affected plant part
1	*Xap*-1	Naigaon	Osmanabad	*Xanthomonas axonopodis* pv. *punicae*	Leaf/Stem/Fruits
2	*Xap*-2	Murud	Latur	*Xanthomonas axonopodis* pv. *punicae*	Leaf/Fruits
3	*Xap*-3	Washi	Osmanabad	*Xanthomonas axonopodis* pv. *punicae*	Leaf
4	*Xap*-4	Ashta	Latur	*Xanthomonas axonopodis* pv. *Punicae*	Leaf/Fruits
5	*Xap*-5	Killari	Latur	*Xanthomonas axonopodis* pv. *punicae*	Leaf/Fruits

Table.2 Cultural and morphological characteristics of different *Xap* isolates on nutrient agar media

Colony characters	***Xap*-1**	***Xap*-2**	***Xap*-3**	***Xap*-4**	***Xap*-5**
Colour	Yellowish	Light yellow	Yellow	Yellowish	Light yellow
Size of colony	Small to medium	Medium to large	Small to medium	Medium to large	Small
Shape of colony	Small Circular colonies	Circular to irregular	Small Circular colonies	Circular to irregular	Circular
Cell shape	Single rod	Single rod	Single rod	Single rod	Single rod
Appearance	Slightly raised, glistening	Slightly raised, glistening	Highly raised, glistening	Highly raised, glistening	Slightly raised, glistening
Elevation	Convex	Convex	Convex	Convex	Convex
Margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin
Texture	Slightly mucoid	Highly mucoid	Slightly mucoid	Highly mucoid	Slightly mucoid

Table.3 Growth rate of different *Xap* isolates at 28^0C and 37^0C

Sr. No.	Isolates	Growth rate							
		28^0C				37^0C			
		48 hrs	72 hrs	96 hrs	120 hrs	48 hrs	72 hrs	96 hrs	120 hrs
1	*Xap*-1	-	+	++	+++	-	-	-	-
2	*Xap*-2	+	++	++	+++	-	-	-	-
3	*Xap*-3	+	++	++	+++	-	-	-	-
4	*Xap*-4	-	+	++	+++	-	-	-	-
5	*Xap*-5	-	+	++	+++	-	-	-	-

Colony growth: '-': no growth, '+': less growth, '++': moderate growth, '+++': maximum growth.

Table.4 Growth rate of different *Xap* isolates at different pH

Sr. no.	Isolates	Growth rate at pH				
		5.0	5.5	6.0	6.5	7.0
1	*Xap*-1	-	-	+	+++	+++
2	*Xap*-2	-	+	++	+++	+++
3	*Xap*-3	-	+	++	+++	+++
4	*Xap*-4	-	-	++	+++	+++
5	*Xap*-5	-	+	++	+++	+++

Colony growth: - '-' no growth, '+' less growth, '++' moderate growth, '+++' maximum growth.

Table.5 Salt sensitivity of different *Xap* isolates at different level

Sr. no	Isolates	Growth rate at salt concentration		
		1 %	2 %	3 %
1	*Xap*-1	++	-	-
2	*Xap*-2	++	-	-
3	*Xap*-3	++	-	-
4	*Xap*-4	++	-	-
5	*Xap*-5	+++	-	-

Colony growth: - '-' no growth, '+' less growth, '++' moderate growth, '+++' maximum growth.

Growth rate of different *Xap* isolates at different pH

The effect of varied pH levels on the growth of *Xap* was observed and the data so obtained is presented in Table 4. From the data it was clear that the maximum growth of *Xap* isolates was observed at pH 6.5 and 7, whereas, moderate growth of *Xap*-2, *Xap*-3, *Xap*-4, *Xap*-5 isolates was observe at pH 6.0 except *Xap*-1 which showed less growth at pH 6.0. Isolate *Xap*-2, *Xap*-3 and *Xap*-5 showed less growth at pH level 5.5, whereas, *Xap*-1 and *Xap*-4 showed no growth at pH 5.5. All the isolates failed to grow at pH 5.0.

The results of the present investigation correlates with results of earlier worker, where, Gour *et al.,* (2000) got the similar results while working with *X. axonopodis* pv. *vignicola*, the causal agent of leaf blight of cowpea. They have recorded the maximum growth of the pathogen at a pH of 7.0. Growth (number of colonies) declined considerably at pH values higher and lower than 7.0 being minimum at a pH of 5.0. Manjula (2002) recorded the highest number of colonies of *Xap* at pH of 7.2., whereas, Mondal and Kumar (2011) reported the growth of *Xap* at pH 6 and above and absence of growth at pH below 5.

Salt sensitivity of different *Xap* isolates at different level

The effect of varied salt concentrations on the growth of *Xap* was observed and the data so obtained is presented in Table 5. Five isolates were tested for their growth at different salt concentration level. All *Xap* isolates showed maximum growth at 1 per cent salt concentration, whereas, all these isolates failed to grow at 2 per cent and 3 per cent salt concentration. The results of the present study are in confirmation with the results of Mondal

and Kumar (2011), where they found that *Xap* tolerates upto 1% NaCl concentration, beyond that no growth of the pathogen was noticed. Basamma (2013) tested 18 isolates of *Xap*. Growth of all isolates at zero per cent NaCl concentration was significantly superior to (mean OD value 0.354) other treatments which was followed by one per cent (mean OD value 0.223) while growth at 3 and 4 per cent was zero. Among the isolates, *Xap*2 and *Xap*10 produced maximum OD value 0.231 and 0.230 which were on par with each other and was significantly superior to all other isolates followed by *Xap*14 (0.206). In general zero per cent NaCl concentration was found to be preferred by all isolates.

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