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Cultural, morphological and biochemical studies of *Xanthomonas axonopodis* pv. *punicae*: The causative agent of oily spot in pomegranate

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Abstract

Bacterial blight disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is one of the most destructive disease of pomegranate (*Punica granatum*). Study was undertaken to conduct collection of samples, isolation, identification, pathogenicity, study of different characters of pathogen, morphological variability, cultural variability and biochemical variability of selected isolates and *in vitro* evaluation. The isolations were made from the symptomatic samples collected from different districts of Maharashtra (India). The studies takes for all eight isolates on gram staining was showed gram negative reaction. The studies on biochemical characteristics of *Xanthomonas axonopodis* pv. *punicae* showed their positive reactions for potassium hydroxide (KOH) solubility test, starch hydrolysis test, Gelatin liquification, H₂S Production, catalase test, oxidase test, Tolerance to 1% NaCl whereas, negative response for indole production, urease test, Arginine test Methyl red test.

Keywords: *Xanthomonas axonopodis* pv. *punicae*, Biochemical test, *in vitro* sensitivity, H₂S production, catalase test, oxidase test

Introduction

Pomegranate suffers from many diseases caused by fungi, bacteria and viruses. Among the bacterial diseases, oily spot (Bacterial blight) caused by *Xanthomonas axonopodis* pv. *punicae*. This disease have been reported as a threat to pomegranate production in India. It has become a chief contributing factor in the production of fruit. Different strain of *Xanthomonas axonopodis* pv. *punicae* shows variability on the basis of morphological, cultural and biochemical properties. It is considered that distinct variability existed among the *Xanthomonas axonopodis* pv. *punicae* isolates collected from different locations/Districts of the Maharashtra state. The use of effective chemicals for control of oily spot is the need for management of the disease. The morphological, cultural and biochemical characterization of *Xanthomonas axonopodis* pv. *punicae* is necessary for detection, diagnosis and study the variability among its isolates.

Material and Methods

Disease sample were collected from different districts of Maharashtra and Isolation and purification of *Xanthomonas axonopodis* pv. *punicae* was carried. The Pathogenicity test was conducted.

Morphological characters

Morphological characters such as colour of colony, shape of colony, cell shape, appearance, elevation, margin and texture of colony were examined on different media.

Cultural characters

The observations viz. colony shape, colony growth rate, margin, elevations and pigmentation of different 8 isolates were recorded after 72 hrs of incubation. The colour of colony was recorded following Ridway colour standard and colour nomenclature (Ridway, 1912).

Biochemical characters

Biochemical characters of *Xanthomonas axonopodis* pv. *punicae* pathogen were studied by subjecting the bacterial isolates to various biochemical tests, viz. Gram staining, Potassium hydroxide (KOH) solubility test, Starch hydrolysis test, H₂S production test, gelatin liquefaction, Indole production test, Catalase test, Oxidase test, Urease test, Methyl red test, arginine test. These all test procedure obtained from a safrinet manual for phytobacteriology named as Introduction to practical phytobacteriology compiled by T. Goszczynska, J.J serfontein and S. serfontein Bacterial Diseases unit, ARC-PRRI, South Africa

Result and Discussion

Pathogenic variability among different isolates of *Xanthomonas axonopodis* pv. *punicae*

The data presented in Table 1 showed that amongst eight isolates Xap-3 isolate had more lesion size i.e. 3.5 mm after 18 days of inoculation which showed water soaked circular to irregular dark brown spots with yellow halo symptoms followed by Xap-7 which showed 1.5 mm lesion size after 18 days after inoculation showing water soaked circular to irregular, light brown spots.

Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis* pv. *punicae* on NA media

The data presented in Table 2, Fig 2 showed that On NA medium the isolates Xap-4, Xap-5 and Xap-8 produced dark yellow colonies whereas Xap-1, Xap-2, Xap3, Xap-6, Xap-7 produced Light yellow colonies. Variation in size and shape on the similar medium revealed that all the colonies of Xap-1 to Xap-8 produced small to medium (1.0 to 2.0 mm) colonies. The all colonies of Xap-1 to Xap-8 isolates were circular in shape. The isolates Xap-4 and Xap-8 showed highly raised colonies and and Xap-1, Xap-2, Xap3, Xap-5, Xap-6, Xap-7 showed slightly raised colonies. The elevation of colonies in all isolates are convex. Cell shape was observed as single rod with entire margin. Xap-4 and Xap-8 showed highly mucoid Texture and remaining other isolates showed slightly mucoid texture. Patil *et al.* (2017) [3] reported that 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.

Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis* pv. *punicae* on GYCA media

The data presented in Table 3, Fig 3 showed that on GYCA media the isolates Xap-1 to Xap-4 Showed dark yellow colour colonies, Xap-5 Showed light yellow colonies and Xap-6 showed whitish yellow colonies. Xap-7 and Xap-8 did not grow on this particular media. Variation in size and shape on the similar medium revealed that all the colonies of Xap-1 to Xap-6 produced small to medium (1.0 to 2.0 mm) colonies. Isolate Xap-7 and Xap-8 didn't produced colonies on this particular media. The all colonies of Xap-1 to Xap-6 isolates were circular in shape. The isolates Xap-1 to Xap-5 showed highly raised colonies whereas Xap-6 Showed slightly raised colonies. The elevation of colonies in all isolates are convex except Xap-7 and Xap-8. Cell shape was observed as single rod with entire margin. Xap1, Xap- 2, Xap-3 showed highly mucoid Texture and Xap-4, Xap-5 showed slightly mucoid texture.

Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis* pv. *punicae* on NSA media

The data presented in Table 4, Fig 4 showed that on NSA media The isolates Xap-1, Xap3, Xap-4 produced dark yellow colonies whereas Xap-2, Xap-5, Xap-6, Xap-7 and Xap-8 produced light yellow colonies on this particular media. Variation in size and shape on the similar medium revealed that all the colonies of Xap-1 to Xap-8 produced small to medium (1.0 to 2.0 mm) colonies. The all colonies of Xap-1 to Xap-8 isolates were circular in shape. The isolates Xap-1 and Xap3 showed highly raised colonies and other remaining isolates Showed Slightly raised Colonies. The elevation of colonies in all isolates are convex. Cell shape was observed as single rod with entire margin. Xap-1 and Xap-3 showed highly mucoid Texture and other isolates showed Slightly mucoid texture.

Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis* spv. *punicae* on YDCA media

The data presented in Table 5, Fig 5 showed that on YDCA media, the isolates differed greatly in respect of colony colour. The isolates Xap-2, Xap-3, Xap-7 exhibited dark yellow colonies. Xap-5 and Xap-6 exhibited light yellow coloured colonies whereas Xap-1, Xap-4 and Xap-8 produced whitish colony on YDCA media. Variation in size and shape on the similar medium revealed that all the colonies of Xap-1 to Xap-8 produced small to medium (1.0 to 2.0 mm) colonies. The all colonies of Xap-1 to Xap-8 isolates were circular in shape.

Manjula (2002) [4] reported that, seven isolates of *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vauterin *et al.*, (1995) obtained from different pomegranate gardens in Karnataka and Andhra Pradesh states, yielded yellow, slimy, glistening, mucoid, convex, small round to irregular colonies on nutrient agar medium and pale yellow to dark yellow colonies, convex with copious slime on YDCA medium. SX and BSCAA media supported luxuriant growth of all the seven isolates.

Biochemical characteristic of different *Xanthomonas axonopodis* pv. *punicae* isolates

The data presented in Table 6, Fig 6 and 7 showed that on Biochemical characteristics of *Xanthomonas axonopodis* pv. *punicae* showed their positive reactions for potassium hydroxide (KOH) solubility test, starch hydrolysis test, Gelatin liquification, H₂s Production, catalase test, oxidase test, Tolerance to 1% NaCl whereas, negative response for Gram staining, indole production, urease test, Arginine test Methyl red test.

Table 1: Pathogenic variability among different isolates of *Xanthomonas axonopodis* pv. *punicae* on Pomegranate 45-60 day old seedlings

Sr. No	Isolates	Lesion size(mm)
		After 18 days
1	Xap1	3
2	Xap2	3
3	Xap3	3.5
4	Xap4	3
5	Xap5	2
6	Xap6	2
7	Xap7	1.5
8	Xap8	2

Table 2: Cultural and morphological characteristics of different isolates of *Xanthomonas axonopodis* pv. *punicae* on Nutrient Agar media

Sr. no.	Colony character	Xap-1	Xap-2	Xap-3	Xap-4	Xap-5	Xap-6	Xap-7	Xap-8
1	Colour	Light Yellow	Light Yellow	Light yellow	Dark yellow	Dark Yellow	Light yellow	Light Yellow	Dark yellow
2	Size	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium
3	Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
4	Cell shape	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod
5	Appearance	Slightly raised,	Slightly raised,	Slightly raised,	Highly raised, glistening	Slightly raised, glistening	Slightly raised, glistening	Slightly raised, glistening	Highly raised, glistening
6	Elevation	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
7	Margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin
8	Texture	Slightly mucoid	Slightly mucoid	Slightly mucoid	Highly mucoid	Slightly mucoid	Slightly mucoid	Slightly mucoid	Highly mucoid

Table 3: Cultural and morphological characteristics of different Xap isolates on Glucose Yeast Chalk Agar media

Sr. no.	Colony character	Xap-1	Xap-2	Xap-3	Xap-4	Xap-5	Xap-6	Xap-7	Xap-8
1	Colour	Dark yellow	Dark yellow	Dark yellow	Dark yellow	Light yellow	Whitish yellow	-----	-----
2	Size	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	-----	-----
3	Shape	Circular	Circular	Circular	Circular	Circular	Circular	-----	-----
4	Cell shape	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod	-----	-----
5	Appearance	Highly raised, glistening	Highly raised, glistening	Highly raised, glistening	Highly raised, glistening	Highly raised, glistening	Slightly raised, glistening	-----	-----
6	Elevation	Convex	Convex	Convex	Convex	Convex	Convex	-----	-----
7	Margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	-----	-----
8	Texture	Highly mucoid	Highly mucoid	Highly mucoid	Slightly mucoid	Slightly mucoid	Slightly mucoid	-----	-----

----- = 'No growth'

Table 4: Cultural and morphological characteristics of different Xap isolates on Nutrient Sucrose Agar media

Sr. no.	Colony character	Xap-1	Xap-2	Xap-3	Xap-4	Xap-5	Xap-6	Xap-7	Xap-8
1	Colour	Dark Yellow	Yellow	Dark yellow	Dark yellow	Light Yellow	Light yellow	Light yellow	Light yellow
2	Size	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium
3	Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
4	Cell shape	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod
5	Appearance	Highly raised, glistening	Slightly raised	Highly raised, glistening	Slightly raised, glistening	Slightly raised	Slightly raised	Slightly raised	Slightly raised,
6	Elevation	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
7	Margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin
8	Texture	Highly mucoid	Slightly mucoid	Highly mucoid	Slightly mucoid	Slightly mucoid	Slightly mucoid	Slightly mucoid	Slightly mucoid

Table 5: Cultural and morphological characteristics of different Xap isolates on Yeast Extract Dextrose Calcium Carbonate Agar media

Sr. no.	Colony character	Xap-1	Xap-2	Xap-3	Xap-4	Xap-5	Xap-6	Xap-7	Xap-8
1	Colour	Whitish Yellow	Dark Yellow	Dark yellow	White	Light Yellow	Light yellow	Dark yellow	White
2	Size	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium	Small to medium
3	Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
4	Cell shape	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod	Single rod
5	Appearance	Slightly raised	Highly raised, glistening	Highly raised, glistening	Slightly raised	Highly raised	Highly raised, glistening	Highly raised, glistening	Slightly raised,
6	Elevation	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
7	Margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin	Entire margin
8	Texture	mucoid	Highly mucoid	Slightly mucoid	mucoid	mucoid	Slightly mucoid	Highly mucoid	mucoid

Table 6: Biochemical characteristics of different *Xanthomonas axonopodis* pv. *punicae* isolates from Maharashtra state

Sr. no.	Biochemical tests	Isolates (Reaction)							
		Xap1	Xap2	Xap3	Xap4	Xap5	Xa6	Xap7	Xap8
1	Gram staining	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
2	KOH Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	H ₂ S production	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
4	Starch hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	Gelatine Liquefaction	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6	Catalase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7	Oxidase Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
8	Indole Production Test	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
9	Tolerance to 1% NaCl	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
10	Urease test	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
11	Arginine test	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
12	Methyl red test	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve



Fig 1: Different Isolates of *Xanthomonas axonopodis* pv. *punicae* on NA media

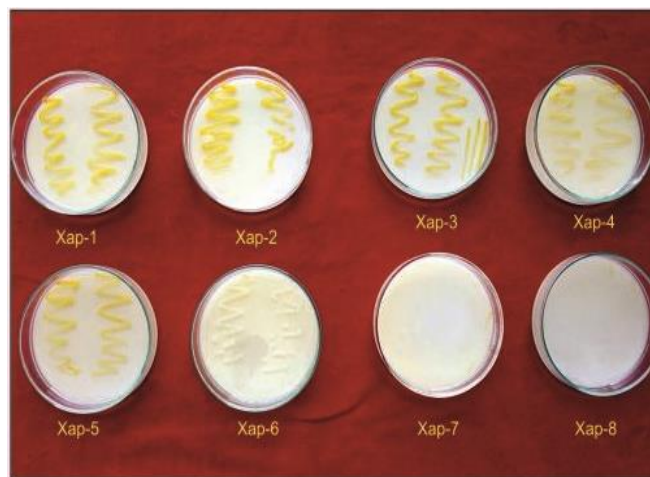


Fig 3: Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis* pv. *Punicae* on GYCA media



Fig 2: Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis* pv. *Punicae* on NA medi



Fig 4: Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis* pv. *Punicae* on NSA media



Fig 5: Cultural and Morphological variations of different isolates of *Xanthomonas axonopodis*pv. *Punicae* on YDCA media

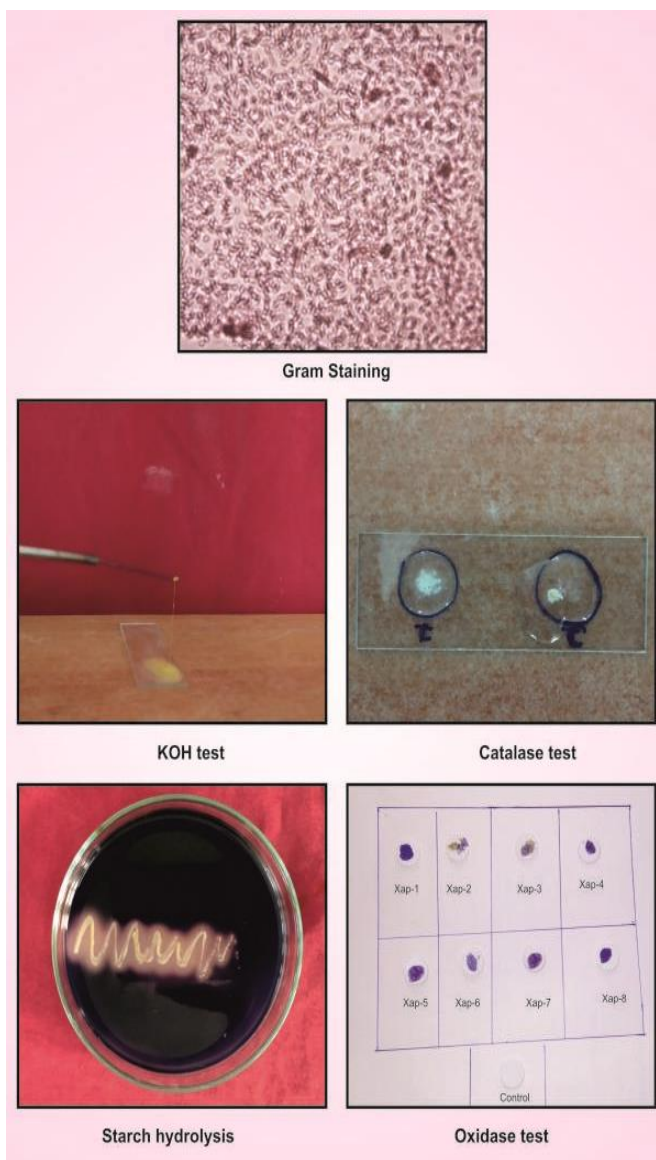


Fig 6(a): Biochemical characteristics of different *Xanthomonas axonopodis* pv. *punicae* isolates from Maharashtra state

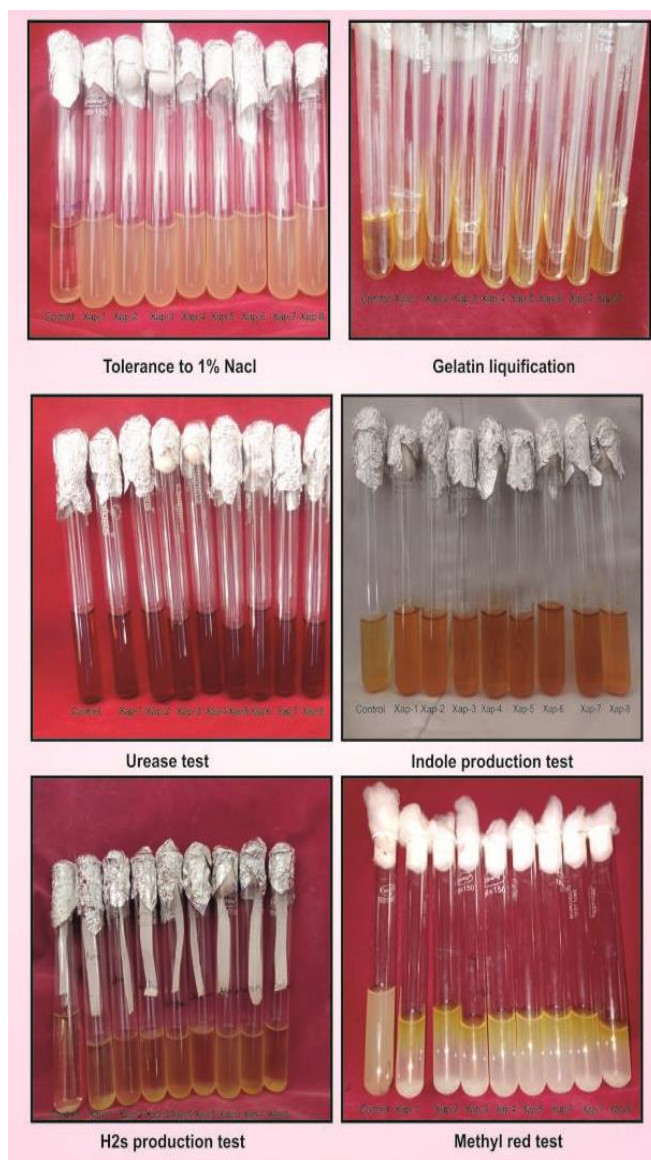


Fig 6(b): Biochemical characteristics of different *Xanthomonas axonopodis* pv. *punicae* isolates from Maharashtra state

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