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Biochemical characterization of *Xanthomonas axonopodis* pv. *punicae* for identification of bacterial blight of pomegranate

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Abstract
Bacterial blight is one of the most destructive diseases of pomegranate, caused by *Xanthomonas axonopodis* pv. *punicae*. Infected samples were collected from Rahata, and the associated bacterium was isolated and purified on Nutrient Sucrose Agar (NSA) medium. The isolates were maintained on NSA slants (coded as Xap) and subjected to morphological and biochemical characterization. Colony morphology, Gram staining, KOH solubility, and catalase activity were examined. The isolates produced yellow, smooth colonies, were Gram-negative rods, catalase-positive, and KOH-positive, confirming their identity as *X. axonopodis* pv. *punicae*.

Keywords: Bacterial blight, Pomegranate, KOH test, Catalase, *Xanthomonas axonopodis* pv. *punicae*

Introduction
Bacterial blight of pomegranate, caused by *Xanthomonas axonopodis* pv. *punicae*, was first reported in India from Delhi in 1952 (Hingorani and Mehta, 1952) [5] and subsequently from Bangalore, Karnataka, in 1959 (Hingorani and Singh, 1959) [6]. Since then, the disease has become a major constraint in pomegranate production, causing heavy losses in yield and fruit quality. Outbreaks have been reported in several states, with severe incidences in Western Maharashtra, particularly Solapur (Dhandar *et al*., 2004) [2].

The pathogen infects stems, leaves, and fruits. On stems, lesions appear as dark brown to black spots around nodes. Leaf symptoms begin as small, irregular, water-soaked spots (2–5 mm) with necrotic centers, later turning brown with a distinct yellow halo. Fruits exhibit brown to black lesions with characteristic L- or Y-shaped cracks on the pericarp.

Xanthomonas belongs to the family Xanthomonadaceae. Cells are Gram-negative, rod-shaped, aerobic, and motile with a single polar flagellum. Colonies are yellow, smooth, and mucoid. The optimum temperature for growth ranges between 25–30°C.

The present study was undertaken to isolate, purify, and characterize *X. axonopodis* pv. *punicae* based on morphological and biochemical features for its identification.

Materials and Methods
Collection, Isolation, and Maintenance of Strains
Pomegranate leaves showing typical bacterial blight symptoms were collected from fields at Rahata. The bacterium was isolated using the tissue isolation method on Nutrient Sucrose Agar (NSA) medium. Pure cultures were maintained on NSA slants coded as Xap, and sub-cultured periodically. Three-day-old cultures were used for further studies.

Identification of the Pathogen
The isolates were identified through morphological and biochemical characterization following standard microbiological protocols.

Morphological Studies
Pure cultures were streaked on NSA medium, and colony morphology (shape and colour) was observed.

Biochemical Tests

The following tests were performed for biochemical confirmation:

- Gram's reaction
- KOH solubility test
- Catalase activity

Fig 1: Disease symptoms

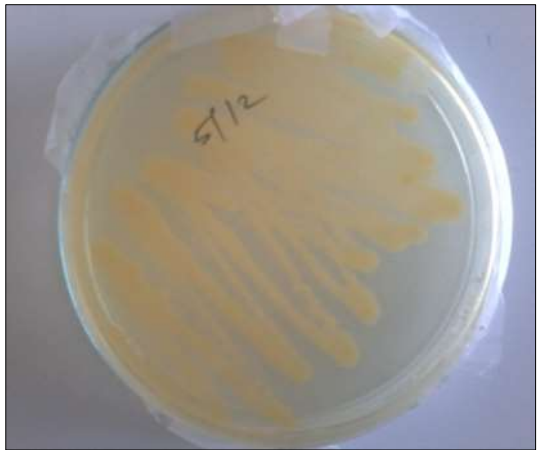
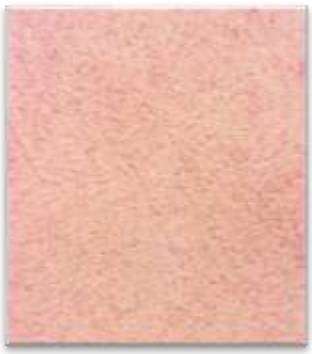


Fig 2: Isolate of *Xanthomonas axonopodis* pv. *punicae*

Table 1: Biochemical characterization

Gram's Staning	KOH Test	Catalase Test
		

Results and Discussion

Morphological and Biochemical Characteristics

The isolates produced yellow, smooth colonies on NSA medium. Microscopic examination revealed Gram-negative short rods. Biochemical characterization showed positive reactions for both catalase and KOH solubility tests (Table 1).

Table 2: Morphological and Biochemical Characteristics of *Xanthomonas axonopodis* pv. *Punicae*

Sr. No.	Character	Xap Isolate
1.	Shape	Rod
2.	Colony color	Yellow
3.	Gram reaction	–ve
4.	Catalase	+++
5.	KOH test	+++

Note: + weak, ++ moderate, +++ strong reaction

- **Gram's reaction:** The isolates were Gram-negative.
- **Catalase test:** Bubbles of oxygen were released within one minute after adding H_2O_2, confirming positive catalase activity.
- **KOH test:** The formation of mucoid threads indicated a positive reaction.

These results are in agreement with earlier reports (Manjula, 2002; Gottwald *et al*., 2000) [8, 4], confirming the identity of the bacterium as *X. axonopodis* pv. *punicae*.

Conclusion

The morphological and biochemical analyses confirmed the isolates as *Xanthomonas axonopodis* pv. *punicae*, the causal agent of bacterial blight of pomegranate. Colony morphology, Gram-negative reaction, catalase positivity, and KOH solubility were the key diagnostic features.

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