Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [1] January 2023 : 155-157.

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

DISTINCTIVE COLONY MORPHOLOGY- A TOOL FOR DIFFERENTIATION OF *XANTHOMONAS AXONOPODIS* PV *PUNICAE* FROM OTHER MEMBERS OF GENUS *XANTHOMONAS*

M. M. Bendigeri*[1], Y.C. Attar[2], S.G. Chonde[1] and G.R. Pathade[3]
[1]Assistant professor, Krishna Institute of Allied Sciences, Krishna Institute of Medical Sciences [Deemed to be university], Karad, 415539.
[2] Principal, Rajaram College, Kolhapur.
[3] Dean, Krishna Institute of Allied Sciences, Krishna Institute of Medical Sciences [Deemed to be university], Karad.
**email- bendigerimrudula@yahoo.in*

ABSTRACT
Xanthomonas axonopodis PV punicae [Xap] is the causative agent of bacterial blight of pomegranate. The disease is severe and can cause upto 90% decrease in the yield of pomegranate. A variety of approaches are being tried by various researchers for control of the disease. The first step in such study is isolation of bacterial blight pathogen Xap from infected pomegranate fruits or leaves. Normally, Xanthomonas develops lemon yellow, moist colonies on Nutrient Glucose Agar [NGA] while Xap develops colourless to faint yellow, highly mucoid colonies on NGA. Many researchers get confused with the colonies of Xap and other Xanthomonas species. In current research work also, correct isolation of the pathogen was essential for further work. Here, the suspected pathogen was isolated from the infected plant parts by standard isolation procedure. The suspected pathogen was then streaked on NGA plate. The plate was incubated at 28^{0}C for 72 hrs. After incubation, the critical point of study was the selection of Xap colonies. These colonies were selected as suspected Xap isolates. When these isolates were further characterised by morphological, biochemical, physiological and genetic characters, these isolates were confirmed to be proper Xap.
***Keywords**: Xanthomonas axonopodis PV punicae [Xap], NGA, mucoid colonies*

Received 12.09.2022 Revised 23.11.2022 Accepted 18.12.2022

INTRODUCTION

Pomegranate (*Punica granatum* L), belonging to family Lythraceae, is an ancient favourite fruit of tropical and subtropical countries of the world. It is called as 'fruit of paradise' due to its multiple uses [1]. According to National Horticulture Board of India, India is the largest producer of pomegranate in the world, about 36 per cent of the world's production and about 30 per cent of the international pomegranate trade [2]. Pomegranate plant is adversely affected by various diseases. These diseases considerably decrease the yield of pomegranate and cause economic loss. Among these various diseases, the highly hazardous is bacterial blight [3]. This disease causes considerable pomegranate losses. The causative agent of the disease is *Xanthomonasaxonopodis* PV. *Punicae [Xap]*. Various researchers are working throughout the world for control of the disease. Current research was also performed to develop an effective control method. The first step in this studywas isolation of *Xap from* infected fruits and leaves. The infected materials wereprocessed by routine microbiological technique. When the processed material was streaked on Nutrient Glucose Agar [NGA] plate, typical faint yellow, highly mucoid colonies were developed on the medium [4]. These colonies were selected as suspected *Xap* colonies and processed for further characterisation. Generally, members of genus *Xanthomonas* produce lemon yellow colonies while *Xap* produces colourless to faint yellow glue drop like colonies. Many times, the researchers get confused with the colonies of *Xanthomonas* withthe colonies of *Xap*. The lemon yellow colonies are selected as *Xap* by mistake. These organisms are used for further morphological, cultural, biochemical, physiological and genetic analysis. At the end, the organisms are finally confirmed as different organisms and the entire work needs to be repeated. Hence, cultural characterisation of *Xap*must be done very perfectly to avoid further misinterpretation.

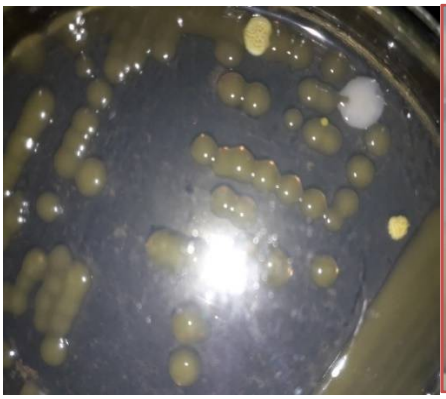
MATERIALS AND METHODS

Infected fruits and leaves were collected from pomegranate fields at Chikmahud, Sangola district. Infected tissues from diseased leaves and fruits were cut by using sterile surgical blade. The cut pieces were

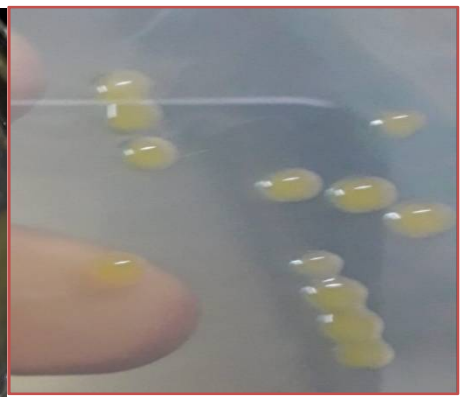
suspended in 70% ethanol solution for 30 seconds for surface sterilization.Then they were washed with sterile distilled water for 10-20 seconds to remove the traces of ethanol. These pieces were then transferred to sterile Petri plate containing 1 ml of sterile distilled water and gently crushed with sterile surgical blade. Due to crushing, oozing of bacterial cells occurred and the water became slightly turbid. This suspension was streaked aseptically on the surface of sterile nutrient glucose agar plate. The streaked plates were then incubated at 28^0C for 72 hours and observed for development of suspected bacterial colonies with typical characters. The suspected colonies were again streaked on sterile NGA plate for further purification. Finally, pure *Xap* colonies were selected and preserved at 4^0C for further work. The pure cultures were characterised by morphological, biochemical, physiological and genetic characters.

RESULTS AND DISCUSSION

Pomegranate fields are present at Chikmahud, Sangola district. From these fields, infected fruits and leaves were collected. Leaves with water-soaked lesions and brown black irregular spots on their surface were selected [[5]. Fruits with water-soaked lesions on their surface and cracked fruits were collected. The suspension prepared from diseased leaf and fruit samples was streaked on NGA plates & incubated for 72 hours. Various colonies were developed on NGA plate (Photoplate 1). Typical yellow, circular, entire, mucoid, high convex, glistening colonies were selected for purification. These colonies initially appeared like a minute glue drop. The colonies were initially colourless. Then the colour was slowly changed to faint yellow. When these suspected bacterial colonies were restreaked on sterile NGA plate and incubated for 72 hours, pure culture of pathogen was obtained (Photoplate 2). For characterisation of the pathogen, its cultural, morphological, biochemical, physiological and genetic characters were studied. In addition, the pathogenicity of the isolate was also studied. After genetic characterisation and pathogenicity studies, the isolate was confirmed as *Xanthomonasaxonopodis* PV *Punicae*.



Photoplate 1 Mixed Colonies on NGA



Photoplate 2 Pure culture of Xap on NGA

ACKNOWLEDGEMENT

The entire work has been performed in the laboratory of Rajaram College, Kolhapur.

CONFLICT OF INTEREST

There is no any conflict of interest between the authors. Each author has a contribution in this research and publication work.

REFERENCES

1. Asgary S., Javanmard S., Zarfeshany A., (2014). Potent health effects of pomegranate. *Advanced Biomedical Research*, 3(1), 100. https://doi.org/10.4103/2277-9175.129371
2. S. Ayyappan, I. (2015). Executive summery of vision 2015,(pp 1-15),*Vision 2050*.
3. Doddaraju P., Kumar P., Gunnaiah R., Gowda A.A., Lokesh V., Pujer P., Anjunatha G., (2019). Reliable and early diagnosis of bacterial blight in pomegranate caused by *Xanthomonas axonopodis*pv. *Punicae* using sensitive PCR techniques. *Scientific Reports* , 9 (1). https://doi.org/10.1038/s41598-019-46588-9
4. Sharma, J., Sharma, K. K., Kumar, A., Mondal, K. K., Thalor, S., Maity, A., Gharate, R., Chinchure, S., & Jadhav, V. T. (2017). Pomegranate bacterial blight: Symptomatology and rapid inoculation technique for xanthomonas axonopodis pv. punicae. *Journal of Plant Pathology*, *99*(1), 109–119.

https://doi.org/10.4454/jpp.v99i1.3825
5. Ashish, & Arora, A. (2016). An overview of bacterial blight disease: A serious threat to pomegranate production. *International Journal of Agriculture, Environment and Biotechnology*, *9*(4), 629. https://doi.org/10.5958/2230-732x.2016.00082.6

CITATION OF THIS ARTICLE

M. M. Bendigeri , Y.C. Attar , S.G. Chonde and G.R. Pathade : Distinctive Colony Morphology- A Tool For Differentiation Of *Xanthomonas Axonopodis* PV *Punicae* From Other Members Of Genus *Xanthomonas,* . Bull. Env.Pharmacol. Life Sci., Spl Issue [1]: 2023:155-157.