



**ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGEN
PSEUDOMONAS AERUGINOSA CAUSING BROWN SOFT ROT DISEASE IN ONION
FROM ALWAR DISTRICT OF RAJASTHAN, INDIA**

Laxmi Meena*, Laxmikant Sharma and Ashwani Kumar Verma

Dept. of Botany, Raj Rishi Govt. College, Alwar (Raj.)

Corresponding Author: Laxmi Meena

Dept. of Botany, Raj Rishi Govt. College, Alwar (Raj.)

Article Received on 30/06/2022

Article Revised on 21/07/2022

Article Accepted on 11/08/2022

ABSTRACT

Onions are an important daily vegetable also suffers from various microbial and nematode diseases. Alwar red onions are in huge demand in domestic and international markets. In the present study onion samples suffers from brown soft rot disease were collected and tested for the presence of disease causing bacterial pathogen. A total of 19 onion samples out of 206 collected samples showing 0-22.22% disease incidence in seven blocks of Alwar district were isolated. These isolated bacterial colonies were subjected to morphological, cultural and biochemical tests for characterization and identification. On the basis of various experimental tests performed the isolated bacterial colonies were identified as *Pseudomonas aeruginosa*. The study revealed significant incidence of bacterial pathogen in seven blocks of Alwar district of Rajasthan, India which can be effectively managed by evaluating economical and eco-friendly measures in further studies.

KEYWORDS: Brown soft rot, bacteria, characterization, Incidence, Onion.

INTRODUCTION

Onion (*Allium cepa* L.) is one of the important bulbous vegetable grown worldwide for its culinary and medicinal values. It is one of the commercially grown few vegetables which can be stored for long time. Vegetables belong to approximately thirteen plant families and some of them are native of India. Onions are largely used as vegetable and as spice in its both immature and mature bulb stages. Worldwide onion cultivated on over 3 MH with annual production apprx. 55MT. Alwar red onions are always in huge demand in both domestic and international markets. Alwar exports onions to neighboring states Delhi, Gujarat and Punjab while internationally to Afghanistan and Pakistan. The other main crops are traded in Alwar district are Mustard, Wheat, Bajra, Arhar, Barley, Gram and cotton. Onion is a cool season crop but can be grown under wide range of climatic conditions. It grows well under mild climate without excessive rainfall or extreme hot or extreme cold. Onions are very good source of biotin, dietary fibres, vitamin A, vit C, vit E and vit B6 and folic acid. Onions are also rich in minerals such as magnesium, potassium, chromium, manganese and sodium. Onion bulbs are high in sulphur containing compounds such as diallyl sulphides and their derivatives e.g. diallyl monosulphides (DMS), diallyl trisulphides (DTS) and diallyl tetrasulphides (DTTS) (Teshika et al., 2019, Kochhar, 2016). Onion leaves and bulbs are eaten

raw as salad and extensively used in a variety of savoury dishes, flavouring soups, ketchups, stews, sausages, meat products and in curries. Dehydrated onion products are also used as convenient flavouring (Kochhar, 2016, Thamburaj and Singh, 2005). Onion crop suffers from large number of diseases in the field which are mainly caused by bacteria, fungi, virus and nematodes. The present research work was undertaken to isolate and characterize the bacterial pathogen associated with onion seeds and plants collected from Alwar district of Rajasthan, India.

MATERIALS AND METHODS

Collection of onion seed and plant samples

During year 2018-2022 field visits to study different aspects of crop practices and management, onion plant samples were collected to study. The seeds, plants and bulb sample of onion were collected from all 16 subdivisions of Alwar district viz. Kishangarhbas, Bansur, Ramgarh, Laxmangarh, Malakhera, Thanagazi, Rajgarh, Raini, Kathumar, Neemrana, Tijara, Behror, Mundawar, Kotkasim, Govindgarh and Alwar.

Morphological, cultural and biochemical characterization of bacterial pathogens

Collected symptomatic onion seeds and plant samples were incubated on nutrient agar media (NA) to isolate the bacterial pathogens associated with the onion

samples. Different developed bacterial colonies on NA plates were subjected to re-isolation to obtain pure cultures. These pure cultures re-streaked on different culture media to determine their morphological characters such as Gram's staining, KoH solubility test and catalase activity test and cultural characters such as development of colonies on KmB agar media to observe fluorescence under UV, and on sucrose nutrient agar (SNA) medium to observe formation of leavn. Biochemical characterization of isolated bacterial pathogens were done using LOPAT (Levan formation, Kovac's oxidase test, Potato soft rot test, Arginine dihydrolase test and Tobacco hypersensitivity reaction) (Lelliot and Stead, 1987, Mortensen, 1997, Kovac's, 1956, Schaad, 1975), Gelatin liquefaction, Indole production test, Growth on cetrimide agar, Citrate utilization test and pathogenicity test (Brown and Lowbury, 1965, King et al. 1954).

RESULTS AND DISCUSSIONS

Two hundred six onion samples (seeds, seedlings and plant samples) were collected from fields subjected to direct plating on nutrient agar to isolate the bacterial colonies. Nineteen samples were found to be associated with presence of bacterial pathogen with an incidence range of 0-22.22% from seven sub-divisions viz. Neemrana, Behror, Mundawar, Kishangarhbas, Kathumar, Govindgarh and Alwar of Alwar district of Rajasthan. The highest incidence was recorded from Mundawar (22.22%) onion samples. In morphological characterization isolates showed Gram's negative, positive KoH solubility test and positive catalase activity

test. The bacterial colonies isolated from plant tissues can be differentiated on the basis of KoH test as a rapid and accurate supplement to Gram's staining (Suslow et al. 1981). The catalase enzyme based test facilitates the identification of bacterial pathogens. Catalase concentration in bacterial pathogens has been correlated with their pathogenicity (Reiner et al. 2016). The bacterial colonies developed on nutrient agar media appeared as round, smooth, translucent with flat edges and elevated centers which give a fried egg appearance. On KmB agar plates, convex, smooth, white yellowish colonies were appeared. When these plates examined under UV light (360 nm), a typical blue green fluorescence was observed. Wahba and Darrell (1965) observed smooth, smooth-rough, rough, gelatinous and mucoid type of colonies on nutrient agar which were circular to irregular in shape, convex raised to effuse elevation and variable blue-green colonies. In various biochemical tests our study observed +-+- type of LOPAT test. Bacterial colonies showed positive gelatin liquefaction and growth on cetrimide agar while negative indole production test and citrate utilization test (Fig. 1 and Table 1). Ewing et al. (1970) used various biochemical tests based on their reactions to differentiate species and bioserotypes of salmonella bacteria.

In the present study, on the basis of above morphological, cultural and biochemical characterizations, bacterial colonies isolated from onion samples were identified as *Pseudomonas aeruginosa* causing brown soft rot disease in onion.

Table 1: Identification of bacterial isolates (*Pseudomonas aeruginosa*) isolated from onion seeds and plant samples.

S. No.	Characteristics	<i>Pseudomonas aeruginosa</i> (Isolate nos. Paos-2112)	
1.	Gram stain reaction	Negative	
2.	KOH test	Positive	
3.	Catalase test	Positive	
4.	Colonies on NA	Round, smooth, translucent, fried egg appearance	
5.	Colonies on KmB agar	Blue-green fluorescence under UV	
3.	LOPAT	Levan formation on sucrose nutrient agar (SNA)	Negative
		Oxidase test	Positive
		Potato soft rot test	Negative
		Arginine dihydrolase test	Positive
		Tobacco hypersensitivity response	Negative
5.	Gelatin liquefaction	Positive	
6.	Indole production test	Negative	
7.	Growth on cetrimide agar	Positive	
8.	Citrate utilization test	Negative	
9.	Pathogenicity test	Yellow lesions and water soaked spots	



Fig 1: Identification of *P. aeruginosa* from onion samples. Bacterial isolate on NA, A positive catalase test, Positive Gelatin liquefaction test, positive growth on cetrinide agar and positive arginine dihydrolase test (Left to right).

ACKNOWLEDGMENT

The authors are highly thankful to the Principal, Raj Rishi Govt. College, Alwar for providing facilities and infrastructure and moreover his support to conduct the study. We also extend our thanks to the faculty members and staff of Department of Botany for their support in many ways.

REFERENCES

1. Teshika, J.D., Zakariyyah, A.M., Zaynab, T., Gokhan Zengin, Kannan RR Rengasamy, Shunmugiah Karutha Pandian & Mahomoodally M. Fawzi. 2019. Traditional and modern uses of onion bulb (*Allium cepa* L.): a systematic review, Critical Reviews in Food Science and Nutrition, 59: sup1, S39-S70, DOI: 10.1080/10408398.2018.1499074.
2. Kochhar, S. L., 2016. Economic botany fifth edition. Cambridge University Press. ISBN: 978131663822.pp 680.
3. Thamburaj, S., Singh, N. (Eds.), 2005. Text book of vegetables, Tuber crops and spices. Indian Council of Agricultural Research, New Delhi, pp 469.
4. Lelliott, R. A. and Stead, D. E., 1987. Methods for the diagnosis of bacterial diseases of plants. In Methods in Plant Pathology, Vol. 2. Blackwell Scientific Publication, Oxford, London, pp 216.
5. Mortensen, C. N., 1997. Seed-borne bacterial diseases. Danish Govt. Institute of Seed Pathology for developing Countries (DGISP), Copenhagen, Denmark, pp 68.
6. Kovac's, N., 1956. Identification of *Pseudomonas pyocyanea*: The oxidase reaction. Nature, London. 178: 703.
7. Schaad, N. D. and Kendrick, R., 1975. A qualitative method for detecting *Xanthomonas campestris* In crucifer seeds. Phytopath, 65: 1035- 1036.
8. Brown, V.I. and Lowbury, E.J.L. (1965). Use of an improved cetrinide agar medium and other culture method for *Pseudomonas aeruginosa*. J. Clin. Path, 18: 752.
9. King, E.O., Ward, M.k. and raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Medicine, 44: 301.
10. Suslow, T.V., Schroth, M.N. and Isaka, M. (1981). Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology, 72: 917-918.
11. Reiner, K. (2016). Catalase test protocol. America Society for Microbiology. Barlett Publishers, Sudbury, MA.
12. Wahba, A.H. and Darrell, J.H. (1965). The identification of atypical strains of *Pseudomonas aeruginosa* J. Gen. Microbiol, 38(3): 329-342.
13. Ewing, W.H., Ball, M.M., Bartes, S.F. and Mcwhorter, A.C. (1970). The biochemical reactions of certain species and bioserotypes of Salmonella. The Journal of Infectious diseases, 121(3): 288-294.