J. appl. Res in Plant Sci. Vol. 1(1), 30-35 https://doi.org/10.38211/joarps.2020.1.1.5
www.joarps.org.

STUDIES ON CHARACTERISTICS OF *XANTHOMONAS ORYZAE* ISOLATES ASSOCIATED WITH RICE CROP

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Article Received 19-04-2020, Revised 29-04-2020, Accepted 09-05-2020

Abstract

Rice Bacterial Leaf Blight (BLB) is considered the most imperative disease among various dangerous maladies of rice in Pakistan. There is no any reliable source of resistance against this disease. Moreover, pathogen has a vast diversity in its population. So, first and most important step for its control is pathogen identification and characterization. Therefore, present studies were carried out for surveillance of disease and to collect disease specimens from Hyderabad and Tando-Muhammad Khan (TMK) Districts. Associated pathogen was isolated from collected samples by direct plating method. Fourteen cultures were purified by streaking method and were characterized on the basis of colony morphology, cell morphology and gram staining. The colonies were found to be large, medium and small. Their shapes were filamentous, irregular and circular. They were found to be raised, undulate, entire and convex showing pale yellow, yellow, off white, reddish and creamy color and surface of most of the colonies was observed to be smooth.

Key Words: Rice, Bacteria, Characterization, Disease, Sindh

Introduction

Bacterial Leaf blight (BLB) a very devastating disease which is caused by the *Xanthomonas oryzae*pv. *Oryzae* (*Xoo*), is an important factor, reducing rice production and yield at global level because of its great injurious effect (Sharma *et al.*, 2017). Bacterial blight infection (the incidence and severity) in Pakistan is increasing in current decade especially in rice growing areas of Punjab that are famous for production of high quality rice (Akhtar *et al*., 2003; Ali *et al*., 2009; Bashir *et al*., 2010; Sadam *et al* 2018). There are two different phases of *Xanthomonas oryzae* pv. *oryzae* i.e. kresek phase and leaf blight phase (Ou, 1985; Akhtar *et al.*, 2008). The first phase i.e. leaf blight phase occurs and infects leaf blade of rice plant. Usually, plants are attacked at the maximum tillering stage. Crop yield decreases up to 20–30%. Infection at the tillering stage can cause 99% yield losses (Mew *et al.*, 1993; Busungu, 2017). The prominent symptoms and signs of this phase are observed on most susceptible and prune genotypes when they are fertilized with excessive doses of fertilizers. The above half of the leaf or whole leaf turn pale then wilted and finally die. The kresek symptom is differentiated by systematic type of infection. The sign of this phase commonly develops and appears seven to fourteen days after transplantation; the leaves turns light green, gradually wither and twisted. In conducive conditions and high pressure of inoculums density, leaves of rice crop became blighted, severely infected leaves tend to dry rapidly, the chlorophylls became non-synthetic, result in gin low yield up to 70-80%. (Thanh, 1975). The topographic and crucial environmental conditions. also influence disease, severity and development. This phase frequently appears in areas receiving high rainfall i.e above 200 mm. Temperature 25°C-30°C supports disease progress. In initial stages of pathogenic infections, acidic soils are thought-out to be an important factor (Maruyama, 1909). Excessive amount of fertilizers application is also one of the important agronomic practices that affect the expansion of bacterial leaf

blight of rice. Among NPK, nitrogenous chemicals are mostly responsible for increasing disease progress and development (Cha, 1982; Sania *et al*., 2015). Dense population of rice plants also effect intensity and severity of bacterial blight if rice nursery is transplanted in wider space in the field. In these miserable conditions of disease severity and considerable losses caused by this dangerous disease (BLB), the researchers/scientists diverted their priority and concentration towards its control and management by the use of resistant varieties. For this purpose, first step is the isolation morphological characterization and authenticated identification of casual pathogen. In these studies our focal point is the isolation and characterization of *Xanthomonas oryzae* pv. *oryzae*. Hence, these studies will be useful in identification of rice associated bacteria and its control to manage bacterial blight for protection of rice crop.

Materials and methods

Sample collection: Samples were taken from Plant Pathology Laboratory, Nuclear Institute of Agriculture, (NIA) Tandjam already collected from Distt. Hyderabad and Tando-Muhammad Khan. (TMK)

Isolation of bacteria: Infected leaf pieces of rice (28× 7 mm) were excised with a sterile scalpel. The leaf surface was sterilized with 1% Clorox for three minutes and then washed with sterile distilled water (SDW). Leaf pieces (6-7) of rice leaves after drying on sterile blotting paper were transferred to nutrient agar (N.A) medium and incubated at 25°C–28°C for 72 hours. (Tariq *et al*., 2015). Bacterial growth appeared around infected pieces after incubation period.

Purification of Bacterial Pathogens: Bacterial growth was streaked on new N.A plates by inoculating loop for separation of bacterial cells. Individual colonies appeared on plates after incubation period of 24-48 hours. The cultures were suspended in sterilized distilled water (SDW), for short term preservation and in silica gel for long term preservation (Wilson *et al*., 1993 and Bhutto *et al*., 2018).

Gram staining: Bacteria were grown overnight in nutrient broth the smear was prepared from 1-2 drops of culture on clean slide and heat fixed. 1-2 drops of crystal violet solution were applied on the fixed smear for 1 min and then washed with sterile distilled water. Gram's iodine solution was applied for 1 min and then washed with 95% alcohol. Finally, safranin was applied for 30 seconds again washed with sterile distilled water. The smear was air dried and examined under light microscope by using immersion oil. The Gram-positive bacterial cells appeared violet while gram negative bacteria turned pink to red (Vincent, 1970; Jatoi *et al*., 2016a; Jatoi *et al.,* 2019).

Characterization of rhizobacterial isolates: Purified single bacterial colonies and their respective bacterial cells were morphologically characterized on the basis of shape, color, size and orientation. The bacterial cells were also differentiated on the basis of their reactions. i.e. positive or negative by gram staining, according to the method described by Schaad (1988; Jatoi *et al*., 2016b).

Results and Discussion

Isolation and characterization of bacteria associated with rice crop: Plant diseases have capacity to damage all kind of field crops i,e cereals, vegetables, fruits and ornamentals. Normal losses due to plant diseases have been reported up to 20-30%. Under favorable weathering conditions responsible for epidemics, they can cause 100% yield losses. Bacterial diseases are more dangerous due to faster multiplication and adoptability of pathogen. Similar case is with bacterial leaf blight if rice. Due to devastating behavior of *Xanthomonas oryzae*, 90-100% losses have been reported in previous studies and very little work has been done on bacterial blight survey, pathogen characterization, identification, pathotyping and control in Sindh province. Moreover, nominal information is available about BLB work in Sindh in literature, Therefore, these preliminary studies were initiated to characterize and identify *Xanthomonas oryzae*. Our results regarding

morphological studies of bacterial colonies and cells revealed that:

Colonies appeared on media plates amended with Nutrient Agar were found to be large, medium and small in size; they were round, spherical, raised, bearing green, white, off-white, pale and reddish colors. Among seven (7) bacterial cells selected from Hyderabad location (district) all were visualized to be non-motile. Moreover, gram staining results unveiled that among studied bacterial isolates four (4) were seen as gram positive and three gram negative. Similarly, morphological studies of bacteria taken from TMK district samples, revealed that out of seven (7) cells, all were seen to be non-motile. Moreover, gram staining studies of these cells resulted one gram positive and six-gram negative bacteria (Tables1-4). Similar results were found by Jabeen, 2012; Arshad *et al* 2013; Kala A, 2015; Tariq *et al*., 2019). when they characterized and identified some bacteria isolated from rice crop. These tentatively identified bacterial strains needs further confirmation which can be done with some other biochemical test or molecular tools. i.e. DNA based primers, markers etc.

Currently, there has been found a progressive trend of rice bacterial leaf blight in all rice paddy growing areas of Pakistan especially in rice belt comprising of Lahore, Gujranwala, Sheikhopura, Sialkot and Hafiz abad. (Khan *et al*., 2000; Akhtar *et al*., 2003). This disease has ability to reduces the crop yield up to 30% in case of normal infection (Shahjehan *et al*., 1991) while under severe infection, the yield of rice crop could be reduced even up to 90-100% (Ghose *et al*., 1970) and Personal observations in the rice fields).

Unfortunately, the existing commercial rice germplasm of the country is lacking resistance against this devastating malady (Akhtar, 2005; Shah *et al*., 2009). Thus, there is an imperative need to deploy bacterial leaf blight resistant rice material and to identify the emerging pathotypes.

Table-1. Colony characteristics of bacteria isolated from District Hyderabad

Location	**Strain**	**Size**	**Shape**	**Elevation**	**Edges**	**Color**	**Surface**
Hyderabad	**ASNN-1**	Small	Somewhat circular	Raised	Entire	Light yellow	Smooth shiny
	ASNN-2	Medium	Filamentous	Raised	Undulate	White	Rough
	ASNN-3	Small	Irregular	Raised	Undulate	Off white	Rough
	ASNN-4	Large	Circular	Raised	Round	Yellow/ Agar color	Rough
	ASNN-5	Large	Minute circular	Raised	Round	Light yellow	Smooth shiny
	ASNN-6	Medium	Circular	Raised	Round	Light Yellow	Smooth shiny
	ASNN-7	Small	Raised	Raised	Round	Light Yellow	Smooth shiny

Table-2. Colony characteristics of bacteria isolated from District T.M.K

	Strain	Size	Shape	Elevation	Edges	Color	Surface
T.M. K	**ASNN-12**	Medium	Circular	Raised	Entire	Light yellow	Smooth shiny
	ASNN-13	Small	Circular	Raised	Undulate	White	Rough
	ASNN-14	Large	Circular	Raised	Undulate	Off white	Rough
	ASNN-15	Large	Circular	Raised	Round	Yellow/ Agar color	Rough
	ASNN-16	Medium	Somewhat circular	Raised	Round	Light yellow	Smooth shiny
	ASNN-17	Small	Somewhat circular	Raised	Round	Light Yellow	Smooth shiny
	ASNN-18	Small	Somewhat circular	Raised	Round	Light Yellow	Smooth shiny

Table-3. Cell characteristics of bacteria isolated from District Hyderabad

Location	Strain	Shape	Motility	Gram Reaction	Tentative identification
Hyderabad	**ASNN-1**	Circular	Non motile	Negative	*Xanthomonas*
	ASNN-2	Small rods	Non motile	Negative	*Xyllella*
	ASNN-3	Circular	Non motile	positive	*Micrococcus*
	ASNN-4	Small rods	Non motile	Positive	*Bacillus*
	ASNN-5	Medium rods	Non motile	Negative	*Xanthomonas*
	ASNN-6	Medium rods	Non motile	Positive	*Bacillus*
	ASNN-7	Short rod	Non motile	Positive	*Not determined*

Table-4. Cell characteristics of bacteria isolated from District T.M.K

Location	Strain	Shape	Motility	Gram Reaction	Tentative identification
T.M.K	**ASNN-12**	Round	Non motile	Positive	*Enterococci*
	ASNN-13	Small rods	Non motile	Negative	*Xanthomonas*
	ASNN-14	Rods	Non motile	Negative	*Xanthomonas*
	ASNN-15	Rods	Non motile	Negative	*Xanthomonas*
	ASNN-16	Rods	Non motile	Negative	*Xanthomonas*
	ASNN-17	Rods	Non motile	Negative	*Xanthomonas*
	ASNN-18	Small round	Non motile	Negative	*Xanthomonas*

Conclusion

As bacterial leaf blight of rice is very injurious disease and it is spreading in all rice growing areas of the country. So, there is dire need to have resistance source because existing rice germplasm lackes resistance. Hence, these studies will be helpful to use identified bacterial strains in screening programme to explore resistant genotypes against the disease to protect rice crop from BLB.

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