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MORPHOLOGICAL CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL BLIGHT PATHOGENS ASSOCIATED WITH RICE CROPS OF SINDH

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ABSTRACT

Rice bacterial leaf blight is an injurious disease among all disquieting problems of rice in Pakistan. The key and main procedure for its control is identification and characterization of its causal agent. To achieve the goals, a comprehensive surveillance was made to collect infected samples. Associated bacteria were isolated from these diseased specimens by direct plating method. Then cultures were subjected for purification and morphological characterization. Their colonies were small, medium and large. The shapes of colonies were filamentous, circular and irregular on Nutrient Agar (N.A) media. Their elevation was convex, raised and their edges were entire and undulate. The color of colonies was yellow, pale yellow, light orange, off white, creamy and reddish. Most of the colonies were smooth.

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

INTRODUCTION

Xanthomonas oryzae pv. *oryzae*, the causal agent of BLB is an important restrictive factor in rice yield globally due to its devastating and disquieting prospective (Sharma *et al.,* 2017). In current years appearance and prevalence of bacterial leaf blight has increased in our country especially in rice growing areas that are renowned for production of high-quality rice. This syndrome has become menace for good and better rice cultivation (Akhtar *et al*., 2003; Ali *et al*., 2009; Bashir *et al*., 2010, Sadam *et al* 2018). There are two specific phases of this dangerous disease i.e. leaf blight phase and kresek phase (Ou, 1985; Akhtar *et al*., 2008). Kresek phase can be distinguished with systemic blight development. The signs of this phase appear ten to fifteen days after transplanting of rice nursery. The leaves of crop appear grayish green, roll up and wither suddenly. In conducive environmental conditions and abundant pressure of bacterial pathogen, the leaves became severely infected, blighted and started to wilt rapidly and became chlorotic. These become unable to produce food also. In this way production of rice limits up to 80%. (Thanh, 2018). Leaf blight phase occurs and prevails on leaf blade of rice plant. Usually, at the maximum tillering stage crop is affected. Yield of crop is abridged by 20–30%. Heavy attack at this stage can provoke total crop losses (Mew *et al.,* 1993; Busungu, 2017). The diagnostic signs of the disease appeared on most prone material when they are given high doses of nitrogenous fertilizers and other macronutrients. The top portion of leaf and entire leaf seems to be chlorotic and wilted. The fluctuation in weather i.e. temperature, humidity and rainfall also affect disease development and severity. This phase mostly outbreaks in region where rainfall occur approximately 200 mm on monthly basis. 25-30°C temperature favors development of bacterial lesions in the early days of disease development, acidic soils are a key factor (Maruyama, 1909). High doses of fertilizer also effect appearance and prevalence of *Xanthomonas oryzae* pv. *oryzae*. Among NPK, nitrogen fertilizer is generally accountable for enhancing disease expansion (Cha, 1982, Sania *et al*., 2015). Dense population of paddy plants also influenced the incidence of BLB. It became more severe if transplanting is done on wider space at constant nitrogen level. In these alarming situations of considerable and major losses due to bacterial leaf blight, the researchers diverted their interest toward its remedy and control using highly tolerant material. To achieve this goal, first step is to isolate, characterize and identify bacterial blight associated pathogen. In these current studies, we focused on the identification, and morphological characterization of associated bacteria i.e. Xoo. Hence these studies will be helpful in identification of its associated patho types and its management to direct BLB for protection of rice crop in Sindh Province.

MATERIALS AND METHODS

Sample collection: Two hundred (200) samples were taken from Plant Pathology Laboratory, Nuclear Institute of Agriculture, (NIA) Tando jam already collected from upper and lower Sindh.

Isolation of bacteria: Diseased leaf samples of paddy crop were cut into small pieces with razor. Then these small pieces were treated with 1-2%

sodium hypochlorite for three minutes and were dipped in autoclaved water. Lastly these pieces were shifted to Nutrient Agar (NA) medium plates and were kept at 25°C –28°C for incubation of three days. Bacterial cultures were inoculated in sterilized distilled water (SDW), for short term storage and in nutrient broth for long term presservation (Wilson and Lindow1993).

Purification of bacterial Isolates: Purification of bacterial cultures was done by streaking method in which bacterial cultures were spreaded on agar plate and incubated at a 26-28 ^{0}C for 24 h.

Gram staining: Bacteria were grown in nutrient broth for 24-48 hours. Bacterial smear was made from 1-2 drops of broth on microscopic slide and was heat fixed. Then solutions of reagents were prepared. First of all, one or two droplets of crystal violet were poured on fixed coat for 1 minute and then cleaned with autoclaved distilled H_2O. Then gram's iodine was applied for 1 minute and then drained by 95% ethanol. The last step was pouring of safranine for thirty seconds and again washing with autoclaved/ sterilized water. Then smear could be dry and viewed using compound microscope with immersion oil. The gram-negative bacteria were seen pink to red while gram positive bacterial cells were found violet (Vincent, 1970).

Characterization of bacterial isolates: Colonies as well as bacterial cells were morphologically characterized based on size, orientation, shape and color. The bacterial cells were also characterized based on their reactions. i.e. negative or positive by gram staining according to the method described by Schaad and talk (1988).

RESULTS AND DISCUSSION

Isolation, purification and morphological characterization of rice associated bacteria: Several bacterial colonies appeared on nutrient agar (N.A) amended plates. Among them, fourteen (14) colonies were chosen from infected specimens of both the localities i.e. T.M.K and Hyderabad. Then selected bacterial colonies were again streaked on NA medium for purification. These were examined to be large, medium and small, their shapes were spherical, round, raised bearing green, reddish, white, off-white and pale colors. Among seven bacterial cells isolated from T.M.K, all were seen to be non-motile while out of seven bacterial cells selected from Hyderabad, all were also seen to be non-motile. Moreover, three (3) were examined to be gram positive and three (3) gram negative on gram staining. Moreover, one (1) was found to be gram positive and six gram negative from Distt. TMK bacterial isolates (Tables1-4).

Jabeen (2012), Arshad *et al.,* (2015), Kala (2015), in their studies, identified and characterized few of their bacteria isolated from paddy crop and documented as *X. oryzae.* pv. *oryzae.* Few of our bacterial isolates were seemed to be *X. oryzae pv oryzae,* the pathogens of rice bacterial blight. This desires more studies of confirmation using some other pathological techniques of diagnostic i.e. biochemical methods or molecular procedures. *X. oryzae* pv. *oryzae* (Ishiyama, 1922; Swing *et al*., 1990) is one of important bacterial pathogen of paddy (Mew, 1987) in several paddy growing belts at global level and it is most disquieting and devastating bacteria having historical back-ground as it was first reported in 1884-85 by Japanese grower/farmers. Later-on its occurrence was recorded in many rice/paddies growing areas of the world i.e. Thailand, Philippines, Australia, Bangla desh, West Africa and Vietnam. India, Sri Lanka and USA (Ezaku and Kuka, 2006). In Lahore (Pakistan), at Rice Research Institute Kala Shah Kaku (KSK), this disease appeared and was reported by Mew and Majid (1977) while its prevalence on a vast area, in the whole country was recorded in successive studies (Akhtar and Akram, 1987; Akhtar *et al*., 2003). Currently, there is high incidence of bacterial leaf blight (BLB) in all rice growing areas of Pakistan including "Kaller" belt which is well-known for cultivation of superior basmati rice (Khan *et al*., 2000; Akhtar *et al*., 2003). Bacterial blight has ability to limit the yield up to thirty (30%) in development of light infections (Shahjehan *et al*., 1991). In conducive environmental conditions and high pressure of inoculum, rice yield could be drop down ninety to hundred (90-100%) as reported by (Ghose *et al*., 1970) and my own studies and personal observations. Unluckily the accessible commercial rice genotypes/germplasm/material of Pakistan is lacking resistance against rice bacterial blight (Akhtar, 2005; Shah *et al*., 2009). Hence, it is a vital and urgent necessity to made BLB resistant rice material / lines / genotypes to characterize and identify the existing and emerging pathotypes.

Table-1 Characterization of colonies of bacterial isolates of District Hyderabad

Area	Strain	Size	Shape	Elevation	Edges	Color	Surface
Upper	**ASNN-1**	Small	Somewhat circular	Raised	Entire	Light yellow	Smooth shiny
	ASNN-2	Large	Filamentous	Raised	Undulate	White	Rough

Sindh	**ASNN-3**	Small	Irregular	Raised	Undulate	Off white	Rough
	ASNN-4	Medium	Circular	Raised	Round	Yellow/ Agar color	Rough
	ASNN-5	Small	Minute circular	Raised	Round	Light yellow	Smooth shiny
	ASNN-6	Medium	Circular	Raised	Round	Light Yellow	Smooth shiny
	ASNN-7	Large	Raised	Raised	Round	Light Yellow	Smooth shiny

Table-2 Characterization of colonies of bacterial isolates of District T.M.K

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

Table-3 Cell characteristics of bacteria isolated from District Hyderabad

Area	**Strain**	**Shape**	**Motility**	**Gram Reaction**	**Tentative identification**
Upper Sindh	**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 bacterial isolates from District T.M.K

Area	**Strain**	**Shape**	**Motility**	**Gram Reaction**	**Tentative identification**
Lower Sindh	**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*

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