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ISOLATION AND CHARACTERIZATION OF BACTERIA ISOLATED FROM THE RICE CROP IN LOWER SINDH

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ABSTRACT

Bacterial leaf Blight (BLB) considered as the most important disease among various potential diseases of rice in Pakistan. The first and most important step for its management is the characterization and identification of associated pathogen. For this purpose, survey of three districts were made to collect disease samples. BLB were found to prevail in all the served area with varying severity. Bacterial strains were isolated from these samples by direct plating method. Isolated culture was purified and characterized. Their colonies were found to be small, medium and large; their shape was irregular, circular and filamentous on Nutrient Agar (N.A) media. The elevation of bacterial colonies was found to be raised and convex and their edges were undulate and entire. The color of most of the colonies was pale yellow and yellow and some were found off white, reddish and creamy and surface of most of the colonies was smooth.

Key Words: Rice diseases, Bacterial pathogens, Purification and characterization

INTRODUCTION

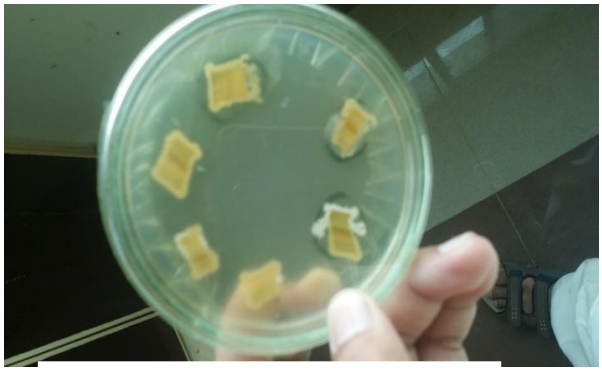
Rice is one of the most important staple food crop of the world and the chief source of food security to about half of its inhabitants. With the total production of 741.5 it ranks third highest agricultural commodity after sugarcane and maize (FAOSTAT, 2017). Rice as the third largest crop of Pakistan covers about 10% cultivated area and contributes about 17% towards total cereal grains production of the country (Ahmad *et al.,* 2005). However, per unit production of rice in Pakistan i.e. 2.4 tons/hectare is far below the world average of 3.1 tones/hectare as well as from Australia and China, that's average yield is 9.1 and 4.7 tons/hectare, respectively (OECD, 2017). Besides other factors contributing in low yield, infectious plant pathogens that caused destructive diseases in our rice crop and ultimately caused huge economical losses i.e. Rice crop is attacked by 50 different diseases, i.e. fungal, bacterial, parasitic (nematodes) and viral diseases (Mew and Gonzales, 2002). The three most important pathogens of rice are *Pyricularia oryzae, Helminthosporium oryzae* and *Xanthomonas oryzae,* which causes blast, brown spot and bacterial blight correspondingly (Singh *et al.,* 2013; Jatoi *et al.,* 2016). Bacterial leaf blight (BLB) is considered as the most devastating disease prevailed in most rice growing regions of the world (Swings *et al.,* 1990). It became endemic and caused heavy losses in some rice growing countries under favorable conditions (Ou, 1985; Mew *et al.,* 1993). If untreated seeds grown in the field then seed-borne pathogen reduce the crop yield up to15-90% (Zafar *et al.* 2014). In Pakistan, after first occurrence in 1977, BLB incidence has been gradually spread throughout the country and become a threat for successful rice cultivation (Mew & Majid, 1977; Akhtar *et al*., 2003; Ali *et al*., 2009; Bashir *et al*., 2010; Ahmed and Majid, 1980; Jabeen *et al*., 2012). BLB is a vascular disease and manifesting itself either at seedling stage caused severe wilting or as leaf blight that eventually results in leaves drying. Under acute BLB infection, plant failed to produced panicles or if do, they were either sterile or contained immature grains of no value (Ou, 1985; Akhtar *et al*., 2008). Since last few years, the incidence and severity of BLB in Sindh province has been increasing (Akhtar *et al.,* 2003). Therefore, present studies were initiated to isolate, identify and characterize the BLB pathogen from different rice growing areas of Sindh province. The accurate identification of BLB causing pathogen could help in the development of an effective and durable disease management strategy.

Materials and methods

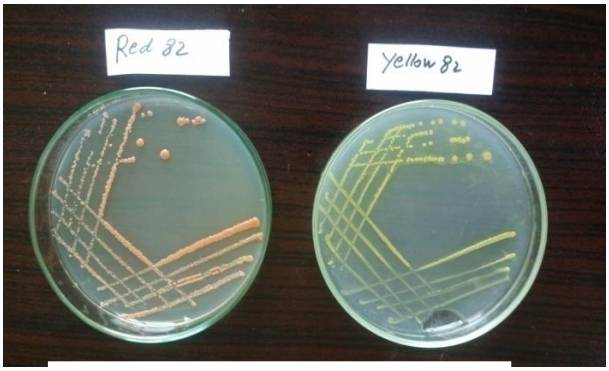
Sample collection: Leaves from rice plants showing characteristics symptoms of BLB were collected from different rice field of district Badin, Tando Muhammad Khan (T.M.K) and Hyderabad. The samples were placed in a paper bags, properly labeled and put in refrigerator (4°C) at Plant Pathology Laboratory, Nuclear

Institute of Agriculture, (NIA) Tandojam for further study.

Isolation and purification of bacteria: The collected leaves were washed with tap water to remove dust or soil particles, then cut into small pieces of 1-2 cm. They were surface sterilized in 1% Sodium hypochlorite solution for 3 minutes and then washed twice in distilled sterilized water. After drying on sterilized blotting paper, they were placed (6 pieces/plate) on to Petri plates containing nutrient agar medium and incubated at 25–28°Cfor 72 hours. The appearing colonies were purified on fresh agar plates. For this purpose a small loop from growing colony was streak on fresh plates of nutrient agar medium and incubate at 28 ±2°C for a period 24 hours.



Isolation of bacterial samples



Purification of Bacterial cultures

Gram Staining: Bacterial cultures were grown in nutrient broth for overnight. Smear was prepared by putting 1-2 loops of broth culture on a clean glass slide and was heat fixed. Then smear was treated with 1-2 drops of crystal violet for I minute. Then was washed with water. Gram's iodine solution was then applied on the smear for 1 minute followed by washing with 95% alcohol and again stained with Safranin for 30 seconds. The smear was air dried and examined under light microscope by using magnification of 10-100X. The Gram positive bacterial cells appeared violet while gram negative bacteria turned pink to red (Vincent, 1970; Gerhardt 1981).

Characterization of bacterial isolates: Rhizo-bacterial isolates were morphologically characterized on the basis of colony morphology cell morphology and Gram staining (Pandy, 2003, Jatoi *et al.,* 2016). Purified single bacterial colonies and their respective bacterial cells were characterized and identified on the basis of shape, color, size and orientation using light microscope. The bacterial cells were also differentiated on the basis of their gram reactions as described by Schaad (1988).

RESULTS AND DISCUSSION

Isolation and characterization of bacteria associated with rice crop: Several colonies appeared on Nutrient Agar (N.A) medium. Among them, 14 colonies were selected from samples of three locations i.e. Hyderabad, Tando Muhammad Khan and Badin. The selected bacterial colonies were purified. Colonies were found to be small, medium and large in size. Their shapes were raised, round, spherical bearing green, white, off-white, pale and reddish colors (Table-1). All the bacterial cells isolated from Badin, were found to be non-motile. Moreover, three were found to be gram positive and four-gram negative on staining. Out of seven bacterial strains isolated from Hyderabad and T.M.K two were found to be motile and five non-motile. Moreover, two were found to be gram positive and five- gram negative (Tables-2). Jabeen, 2012, Arshad *et al.,* 2015, Kala, 2015 characterized some bacteria isolated from rice crop and identified as *Xanthomonas oryzae*. Some of our bacteria are suspected to be *Xanthomonas oryzae* pv. *oryzae,* the causal agent of bacterial blight of rice. This needs further confirmation by some other biochemical test or molecular studies using PCR. *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922; Swing *et al*., 1990) is one of the most destructive pathogen of rice crop (Mew, 1987) in all the rice growing regions of the world. The disease was first reported by Japan's farmers in 1884-85 while later its prevalence was reported in different rice growing regions of the world, including Australia, Bangaldesh, India, Sri Lanka, Thailand, The Philippines, USA, West Africa and Vietnam (Ezaku and Kuka, 2006). The incidence of this disease in Pakistan was reported by Mew and Majid (1977) while its occurrence on a wider scale in the entire country was observed in subsequent studies (Akhtar and Akram, 1987; Akhtar *et al*., 2003). Recently, there has been an increased incidence of bacterial leaf blight in all rice growing zones of the country, including

"Kaller" belt which is famous for superior basmati rice cultivation (Khan *et al*., 2000; Akhtar *et al*., 2003). This disease reduces the crop yield up to 30% in case of mild 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 available commercial rice germplasm of the country is lacking resistance against this disease (Akhtar, 2005; Shah *et al*., 2009). Thus, there is an urgent need to develop bacterial leaf blight resistant rice cultivars and to identify the emerging pathotypes.

Table-1Colony characteristics of bacteria isolated from different districts.

Location	Strain	Size	Shape	Elevation	Edges	Color	Surface
Badin	S.H.B-1	Medium	Irregular	Raised	Undulate	Yellowish	Smooth
	S.H.B-2	Small	Circular	Convex	Entire	Yellowish	Smooth
	S.H.B-3	Medium	Filamentous	Raised	Undulate	Creamy	Smooth
	S.H.B-4	Medium	Circular	Raised	Entire	Yellow	Smooth
	S.H.B-5	Small	Irregular	Convex	Entire	Off White	Smooth
	S.H.B-6	Medium	Circular	Raised	Entire	Orange	Smooth
	S.H.B-7	Large	Circular	Raised	Entire	Red	Smooth
Hyderabad	S.H.B-8	Large	Filamentous	Raised	Undulate	Pale Yellow	Smooth
	S.H.B-9	Large	Circular	Raised	Entire	Off White	Smooth
	S.H.B-10	Small	Circular	Convex	Entire	Yellowish	Smooth
T.M.K	S.H.B-11	Medium	Circular	Convex	Undulate	Pale Yellow	Smooth
	S.H.B-12	Large	Circular	Raised	Entire	Reddish	Smooth
	S.H.B-13	Large	Irregular	Convex	Undulate	Creamy	Smooth
	S.H.B-14	Small	Irregular	Raised	Undulate	Pale Yellow	Smooth

Table-2Cell characteristics of bacteria isolated from different districts.

Location	Strain	Shape	Motility	Gram Reaction
Badin	S.H.B-1	Short Rod	N	-
	S.H.B-2	Short Rod	N	+
	S.H.B-3	Short Rod	N	-
	S.H.B-4	Short Rod	N	-
	S.H.B-5	Short Rod	N	+
	S.H.B-6	Short Rod	N	-
	S.H.B-7	Short Rod	N	+
Hyderabad	S.H.B-8	Short Rod	N	-
	S.H.B-9	Short Rod	M	+
	S.H.B-10	Medium Rod	N	-
T.M.K	S.H.B-11	Short Rod	N	-
	S.H.B-12	Small Rod	M	-
	S.H.B-13	Short Rod	N	-
	S.H.B-14	Short Rod	N	-

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