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BIOCHEMICAL CHARACTERIZATION OF SOME STRAINS OF *XANTHOMONAS* AND THEIR RESPONSE AGAINST GENTICIN-AN ANTIBIOTIC

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ABSTRACT

In present research investigations, enzyme (catalase) secretion, salt tolerance and Geneticin response against few strains of *Xanthomonas oryzae pv oryzae* was checked under laboratory conditions. Consequences regarding enzyme efficacy (catalase) varied among the isolates. The findings regarding temperature tolerance unveiled that all the strains were able to grow at 1%, 3%, 5% NaCl concentration. Moreover, Geneticin against tested bacteria exhibited development of maximum inhibition zone i.e. 3, 4, 5 milimeter in eleven days of incubation period against 6 isolates; ASNNBXI, ASNNB-XII, ASNNB-XVIII, ASNNB-XIV, ASNNB-XV, ASNNB-X. Moreover, strains ASNNB-XIII, ASNNB-XII exhibited minimum inhibition zone i.e. 0 millimeters against12 isolates

Keywords: *Xanthomonas*, biochemical tests, rice *(Oryza sativa)*, disease, Sindh

INTRODUCTION

Rice (*Oryza sativa* L.) is extensively being grown in all rice growing areas of the world (Ezuka & Kaku, 2000). It has high nutritional values and is consumed by 2.7 billion people at world level and mostly 85% of the world rice is cultivated in the Asian countries (Salim *et al*., 2003). Pakistan is the 11th largest country for the global rice production with the cultivated area of 2.3 million hectares (Anonymous, 2013). In-spite of the fact that rice is an imperative crop, but mean production is extremely low in Pakistan than other countries of world. i.e top rice producing countries in general and in particular very below from many neigh boring countries china and India. This crop is being hampered by biotic and abiotic disorders (Khan *et al*., 2009). Among those factors, Bacterial leaf blight (BLB) disease of rice is most dangerous one. This disease was observed by Japanese grower during 1884- 85. Afterward, its presence was noted in many other paddy cultivating areas i.e., Thailand, India, Philippines, Australia, Bangladesh, West Africa, Vietnam, USA and Sri Lanka, (Ezaku and Kuka, 2000). The occurrence of this malady in our country (Pakistan) for the first time was reported by two research scientists; Mew and Majid (1977), its spread over large scale across the world recorded in consequent later research investigations (Akhtar *et al*., 2003, Akhtar & Akram, 1987). In current years, intensity of bacterial leaf blight (BLB) is being increased especially in Keller belt of Pakistan where high quality basmati rice is grown. (Khan *et al*., 2000; Akhtar et al., 2003). Almost all basmati rice growing area is prone to BLB (Cheema *et al*., 1998; Khan *et al*., 2000; Akhtar *et al*., 2008; Ali *et al*., 2009). Under normal disease situations, crop losses up to 25-30% have been recorded (Shahjehan et al., 1991) and under conducive conditions crop losses can be reached up to 80-100% (Ghose et al., 1970). The associated agent can damage rice from seedling stage to final crop maturity and it can be identified by either leaf blight or kresek symptoms. In the leaf blight phase bacteria enters the plant through injuries, wounds or through water pores present on the marginal spaces of upper part of the leaf blades. The objectives of our studies were to develop a sound and successful integrated management strategy by describing *X. oryzae*pv. *oryzae.* Strains on biochemical basis. Furthermore, response of bacterial strains against antibiotic was also checked.

MATERIAL AND METHODS

Samples collection, isolation and purification of BLB pathogen: Leaves infected with Bacterial leaf blight (BLB) disease were obtained from Plant Pathology laboratory, NIA, Tandojam. Diseased samples were cut into small pieces of about (28×7 mm. After cutting, these pieces were surface sterilized with 75% ethanol for three to five minutes and then rinse in autoclaved sterilized distilled water (SDW) to remove harmful effects of ethanol. Then these were air dried. Four to five pieces were placed on nutrient media amended plates and Plates were kept at 27±2°C for three days. When bacterial growth appeared, It was streaked further on NA plates for purification. Purified cultures were then identified on basis of cell morphology, colony morphology and gram staining, afterwards purified and identified cultures were kept in SDW in speci-

fic preservative as illustrated in the findings of (Wilson *et al*., 1993; Nawaz *et al*., 2020).

Characterization of *Xanthomonas oryzae* on Biochemical basis: All isolates were characterized on biochemical basis as per protocol practiced by Maheshwari and Dubey (2006). Methodology of procedures is illustrated below.

Gram staining: Gram staining technique was used to separate bacteria on the basis of gram reaction i.e. gram negative and gram positive. Fresh culture of *Xanthomonas oryzae* was inoculated in nutrient broth. Two days old culture was used for gram stain-ing. Bacterial smear was made from 1-2 drops of culture on glass slide. Then it was heat fixed. 1-2 drops of crystal violet solution were poured on smear for 1 minute and then washed with sterilized distilled water. Then Gram's iodine solution was poured on bacterial smear and then washed with 95% ethanol. Safranin was poured for half minuet and slide was washed by SDW. Then smear was kept for drying and was examined under light micr-oscope. Gram positive bacteria were found violet and gram negative bacteria were seen pink to red as documented by Vincent 1970

Catalase production: In catalase analysis, bacte-rial smears were placed on microscope slide. A drop of hydrogen peroxide was mixed with smear by inoculating loop. If homogeneous mixture that forms air bubbles or froth, the strains was consi-dered as catalase-positive while the mixture that does not make bubbles or froth, the organism was scored as catalase negative.

Genticin response test: Genticin response test done by cup method. In this assay agar medium contain-ing Beef extract 10 g, Peptone 10 g, Sodium chlo-ride 5 g, Glucose 1 g, Agar 20 g per liter of distilled water, pH-7.5 was made for bacterial culture. Bac-terial suspension (culture) was spread on petri plates containing nutrient agar (NA) media. In each media amended plate, 6 holes were made by auto-claved cup borer. Antibiotic (Genticin) @ 100 ppm concentration was put in holes of pre-inoculated plates. Media plates were then kept in an incubator at 27±2°C for 11 days. Inhibitory regions formed by antibiotic were measured in millimeters with measuring scale. Media amended plates without genticin were considered as control plate.

RESULTS

Biochemical characterization

Gram staining: Gram staining studies reviled that 11 isolates (61%) i.e. ASNNB-1, ASNNB-II, AS-NNB-IV, ASNNB-VIII, ASNNB-IX, ASNNB-X-III, ASNNB-IV, ASNNB-XV, ASNNB-XVI, ASN-NB-XVII, ASNNB-XVIII, were found gram nega-tive while 7 strains (39%) I .e. ASNNB-III, ASN-NB-IV, ASNNB-VI, ASNNB-X, ASNNB-XI, AS-NNB-XII were found to be Gram positive (Table-1).

Table-1: Biochemical based characterization of *Xanthomonas oryzae pv oryzae strains*

Strain No.	Isolates Name	Gram staining response	Catalase Production	Salt Tolerance		
				1%	3%	5%
1	ASNNB-I	-	+	+	+	+
2	ASNNB-11	-	+	+	+	+
3	ASNNB-III	+	+	+	+	+
4	ASNNB-IV	+	+	+	+	+
5	ASNNB-V	-	+	+	+	+
6	ASNNB-VI	+	+	+	+	+
7	ASNNB-VII	+	+	+	+	+
8	ASNNB-VIII	-	+	+	+	+
9	ASNNB-IX	-	+	+	+	+
10	ASNNB-X	+	+	+	+	+
11	ASNNB-X1	+	-	+	+	+
12	ASNNB-XII	+	-	+	+	+
13	ASNNB-XIII	-	+	+	+	+
14	ASNNB-XV	-	+	+	+	+
15	ASNNB-XVI	-	+	+	+	+
16	ASNNB-XVII	-	+	+	+	+
17	ASNNB-XVII	-	+	+	+	+
18	ASNNB-XVIII	-	+	+	+	+

Catalase production test: The finding of catalase test resulted that 88% i.e. 16 bacterial isolates were found to be catalase positive and 2 (12 %) i.e. 2 isolates were declared as catalase negative.

Genticin response: Bacterial isolates displayed varying level of response against Genticin. More-over, Genticin sensitivity against tested isolates responses production of maximum inhibitory reg-ion i.e. 6,7,9 mm in eleven days of incubation agai-nst the isolate ASNNB-XV ASNNB-III, ASNNB-X, ASNNBVI (**Table-2**).

Table-2: Efficacy of Bacterial strains against anti-biotic (Genticin)

Isolates	Zone Diameter produced by Genticin					
	5 DAYS		8 DAYS		11 DAYS	
ASNNB-I	0 mm	0±	0 mm	0.0±	0 mm	0.0±
ASNNB-11	0 mm	0±	0 mm	0.0±	5 mm	0.0±
ASNNB-III	4 mm	1±	4 mm	1.0±	7 mm	0.0±
ASNNB-IV	0 mm	0±	0 mm	0.0±	0 mm	0.0±
ASNNB-V	0 mm	0±	0 mm	0.0±	3 mm	0.0±
ASNNB-VI	0 mm	0±	0 mm	0.0±	9 mm	0.0±
ASNNB-VII	1 mm	1±	1 mm	0.0±	3 mm	0.6±
ASNNB-VIII	0 mm	0±	0 mm	0.0±	2 mm	0.0±
ASNNB-IX	0 mm	0±	0 mm	0.0±	2 mm	0.0±
ASNNB-X	4 mm	0±	4 mm	1.0±	7 mm	0.0±
ASNNB-X1	3 mm	0±	3 mm	2.0±	2 mm	0.0±
ASNNB-XII	2 mm	1±	3 mm	2.0±	3 mm	1.0±
ASNNB-XIII	5 mm	0±	5 mm	1.73±	1 mm	0.00±
ASNNB-XV	3 mm	1±	3 mm	1.15±	6 mm	0.00±

ASNNB-XVI	2 mm	0±	3 mm	1.15±	5 mm	1.00±
ASNNB-XVII	2 mm	0±	2 mm	1.15±	0 mm	2.00±
ASNNB-XVII	1 mm	1±	4 mm	1.15±	3 mm	0.00±
ASNNB-XVIII	3 mm	0±	3 mm	2.31±	4 mm	0.00±

DISCUSSION

Identification of Bacterial isolates: BLB isolates were identified as *Xanthomonas oryzae pv oryzae* on tentative basis. Research finding regarding identification are accordance with (Muneer *et al*., 2007; Rafi *et al*., 2013; Joint, 2016; Jatoi *et al*., 2016b). Who describe yellow colored, mucous type, dome, smooth, circular shaped colonies on media amended with Nutrient Agar. *Xanthomonas* produces extracellular polysaccharides (EPS) in sugar containing medium which resulted yellow colored and mucoid colonies and it is good detection characteristics of *Xanthomonas*. Moreover, they highlighted that *Xanlhomonas* were found to produce colonies that were yellow, convex, mucoid and shiny in texture. Such colonies were consistently observed and tentatively identified as *Xanthomonas oryzae pv oryzae.*

Biochemical characterization: Biochemical procedures are used for the identification of bacterial strains to check their behavior in response to different chemicals. Bacterial strains in our present studies were detected as positive as well as negative in gram staining studies. Majority were observed gram negative and few gram positive. Both gram negative and positive bacterial strains were subjected to other biochemical tests. It is found that all strains except one was catalase positive. It is also an indication of detection of (Xoo) in a study Joint, 2016 identified his all catalase positive strains as *Xoo* and considered this bacterial behavior as a confirmatory tool and dispose of catalase negative from his studies. The results regarding salt tolerance behavior of bacteria showed that all the strains were able to grow at 1%, 3%,5% NaCl concentration. This needs further studies to check response of *Xanthomonads* below 1 and above 5% NaCl concentration.

Antibiotic resistance: Genticin an antibiotic at100 ppm was used in the study to check the response of different bacteria up to 11 days of incubation. Inhibitory clean zone for bacterial growth were formed in the form of circle in the region of the antibiotic poured holes. These transparent zones were measured by measuring scale. All strains respond different reaction in response to Gentic in, not a single strain was able to display complete resistance against Genticin at 100 ppm in 11 days period. Furthermore, antibiotic (Genticin efficacy against tested isolate resulted configuration of maximum clear zone of 3,4,5 mm after eleven days of incubation against strainsASNNB-11, ASNNB-12, ASNNB-18, ASNNB-14, ASNNB-15, ASNNB-10, ASNNB-13, ASNNB-17. Disease management by chemicals is efficient by killing all kinds of pathogens. Bacterial microflora is one of the most dangerous micro flora that causes rapid and high death rate in animals and plants due to its faster multiplication. Bacterial blight (BLB) can be managed using antibiotics like copper– mercury fungicides, copper–soap, Bordeaux mixture. However, effective and cheap control needs to be developed against this disquieting disease because; *Xanthomonas* population is very sensitive to the antibiotics. The production and formulation of drug-resistant strains also cause serious difficulty in production of proper control agents. (Gnanamanickam *et al*., 1999; Jatoi *et al*., 2016a; Bhutto *et a*l., 2018; Jatoi *et al*., 2018).

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