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Bio-chemical characterization of *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight of rice from Telangana region

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Abstract
Bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases in rice which limits the annual rice production in both tropical and temperate regions of the world. The present study was conducted to isolate, characterize and identify the *Xanthomonas oryzae* pv. *oryzae* obtained from infected rice foliar samples. Infected leaf samples were collected from one of the major agro-ecological rice zones in Telangana. Six *Xoo* isolates collected from different rice growing regions of Telangana were characterized for their reaction to different biochemical tests. The *Xoo* isolates were gram negative, showed positive reaction for catalase production, oxidase production, Voges Prausker's test, methyl red test and gelatin liquefaction. The *Xoo* isolates showed variable reactions with respect to utilization of various carbon sources like maltose, fructose, galactose, mannose, sorbitol, mannitol, adonitol, cellobiose and arabinose. With respect to utilization of various carbon sources the results indicate that there exists variation in physiological characters among the *Xoo* isolates and this may have impact on their virulence and survival.

Keywords: Bio-chemical, characterization, bacterial, *Xanthomonas oryzae* Pv. *Oryzae*

Introduction
Rice (*Oryza sativa* L.) is the second most important cereal crop and the staple food for more than half of the world's population. It is cultivated in all the continents except Antarctica, over an area of more than 150 M ha, but maximum rice production takes place in Asia. Rice varieties like BPT 5204, MTU 1010, JGL 1798 and Tellahamsa are widely grown in Telangana and are very popular among the farmers because of their high yield, desirable grain and cooking quality.

Despite their popularity, they are susceptible to many diseases like blast, bacterial blight, sheath blight, brown spot, tungro virus and others. Bacterial blight (BB) is a major production constraint in India especially in irrigated and rain fed lowland ecosystem (Laha *et al.*, 2009) [2]. The disease appeared in epidemic form in north western India during 1979 and 1980, in Pallakad region of Kerala during 1998 (Priyadarishini and Gnanamanickam, 1998) [6] and several parts of Andhra Pradesh during 2010 and 2013 (Yugander *et al.*, 2014) [14].

BLB is a typical vascular disease and has three distinct phases of symptoms. Leaf blight phase is the most common and the symptom starts as water-soaked lesions on the tip of the leaves and increases in length downwards. Initially, the lesions are pale green in colour and later turn into yellow to straw-coloured stripes with wavy margins. The most destructive phase of the disease in the tropics is 'Kresek' or wilt phase resulting from early systemic infection in the nursery or from seed infection. The leaves roll completely, droop, turn yellow or grey and ultimately the tillers wither away.

In severe cases, the affected hills may be completely killed. Pale yellow leaf phase of the disease has been reported from Philippines. Some of the youngest leaves in a clump may become pale yellow or whitish. The diseased leaves later wither, turn yellowish brown and dry up. This symptom is not usually found in other countries Bacterial blight caused by *Xoo* has become a serious constraint for rice production especially after introducing high yielding rice varieties.

Crop loss assessment studies revealed that this disease reduces grain yield to varying levels, depending on the stage of the crop when the disease appears, climatic conditions, season and extent of nitrogen fertilizers. In India, the extent of yield losses can be as high as 50% during

severe infection (Rao and Kauffman, 1971) [7]. *Xoo* is highly dynamic in nature and several studies have reported existence of pathogenic variation in the pathogen populations (Lore *et al*., 2011; Mishra *et al*., 2013; Yugander *et al*., 2017) [3, 4, 8]. However, information regarding biochemical variation among *Xoo* isolates is lacking. Therefore, an attempt was made to study the profile of different biochemical characters among the *Xoo* isolates from Telangana.

Materials and Methods

The diseased leaves of rice showing typical bacterial blight (BB) symptoms were collected in brown paper bags from fields of Khammam, Nalgonda, Warangal and Rangareddy districts of Telangana state. A total of six isolates were collected from the heavily infected areas of the districts and the details of each isolate is represented in the (Table: 1)

Table 1: List of isolates of *Xoo* used in the present study

Isolate	Cultivar	Location	Season
NB-1	BPT -5204	Rangareddy	Kharif
NB-2	BPT -5204	Rangareddy	Rabi
NB-3	BPT -5204	Khammam	Rabi
NB-4	BPT -5204	Warangal	Rabi
NB-5	BPT -5204	Nalgonda	Kharif
NB-6	BPT -5204	Nalgonda	Kharif

The isolation of pathogen was carried out from the infected leaves of rice plant collected from fields. The samples which have showed typical leaf blighting and exuded bacterial ooze from the cut section during microscopy were used for isolation. The diseased portions along with healthy tissues were cut into 1.5-2 cm pieces and were surface sterilized for 30 seconds in 0.1% sodium hypochlorite solution. Further, they were washed thrice with sterilized MilliQ water in aseptic condition to remove the traces of sodium hypochlorite. The pieces were kept on microscopic slide and further cut with the help of sterilized blade.

The bacterial suspension was prepared by dropping sterilized MilliQ water on the surface of cut pieces placed on the glass slide. The suspension was streaked on nutrient agar medium with the help of sterilized wire loop. The inoculated plates were incubated at (27 ± 2 ºC) in a bacteriological incubator for 48 h. Typical pin head sized, mucoid, convex colonies of *Xoo* grown on nutrient agar were further streaked on the NA. Thus, the obtained pure cultures were preserved in glycerol at -40 ºC for further investigations.

Biochemical characterization of *Xoo*

Different biochemical tests *viz*., catalase test, oxidase test, Voges Prausker's test, methyl red test and gelatin liquefaction were conducted using standard protocols (Biyyani *et al.*, 2018) [1].

Catalase test

Catalase test was performed by taking a drop of 3% hydrogen peroxide and adding to 48 h old bacterial colony on a clean glass slide. The effervescence indicates catalase activity.

Oxidase test

The bacterial isolates were grown in nutrient agar slants. Oxidase paper discs of were kept on fully grown cultures for 48 h. A colour change to purple indicates positive result.

Voges Prausker's test

The test was performed by adding alpha-naphthol and potassium hydroxide to the Voges Prausker's broth. A cherry red colour indicates a positive result, while a yellow- brown colour indicates a negative result.

Methyl red test

Sterilized glucose phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2 ºC for 48 h. After incubation, five drops of methyl red indicator was added to each tube and gently shaken. Red color production was taken as negative for the test.

Gelatin liquefaction

The overnight cultures of the test isolates were inoculated in sterilized nutrient gelatin deep tubes and incubated for 24 h at 28 ± 2 ºC. Then, the tubes were kept in the refrigerator for 30 min at 4 ºC. The isolates showing liquefied gelatin was taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative.

Carbohydrate utilization test

The organism use carbohydrate differently depending upon their enzyme complement. The pattern of fermentation is characteristics of certain species, genera or groups of organisms and this property has been extensively used as a method for biochemical differentiation of microbes. To carry out this test various carbon sources were used. Firstly, a litre of nutrient broth was prepared and a pinch of phenol red indicator was added which is responsible for the colour change during the growth of bacteria in the nutrient broth. The nutrient broth of about 10 ml was poured in to the test tubes and durham tubes were placed in each test tube and autoclaved. After autoclaving the test tubes were added with different carbon sources like maltose, fructose, galactose, mannose, sorbitol, mannitol, adonitol, cellobiose and arabinose. To this test tubes 1ml of 48 hr old cultures is added and observed for culture change in the test tubes.

Results and Discussions

Six *Xoo* isolates collected from different rice growing regions of Telangana were characterized with different biochemical tests, *viz*., catalase test, oxidase test, Voges-Proskauer, methyl red test and gelatin liquefaction. All the six isolates (NB-1, NB-2, NB-3, NB-4, NB-5 and NB-6) were tested positive for catalase and were aerobic. They were also positive for oxidase test and revealing the presence of cytochrome oxidase enzyme. The production of acetoin by all the isolates was also confirmed from Voges Prausker's test**.** All the bacterial isolates showed negative results towards methyl red test and gelatin liquefaction (Plate 1). These results were in agreement with the findings of Padmaja *et al.* (2017) [5].

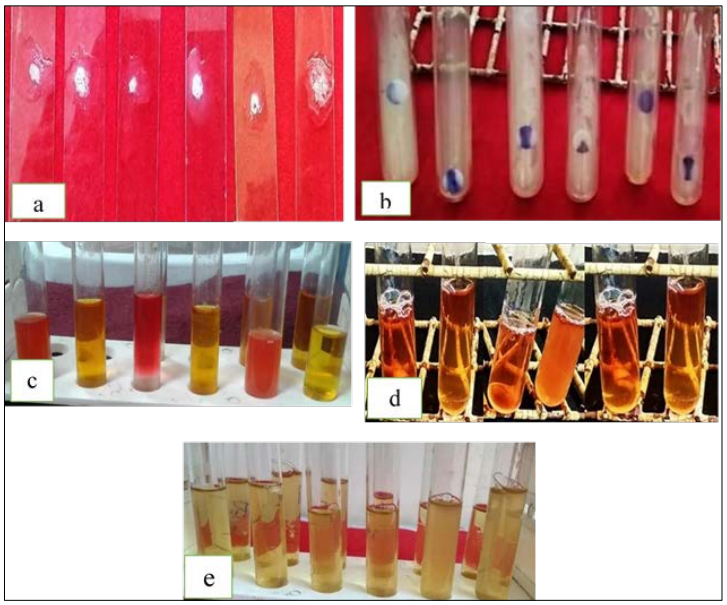

Plate 1: Biochemical characterization of different isolates of *Xanthomonas oryzae* pv. *Oryzae* (a) Catalase test (b) oxidase test (c) Methyl red test (d) Voges proskauer test (e) Gelatin test

The carbohydrate fermentation test is used to determine whether or not bacterial isolates can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. The basal medium containing a single carbohydrate source such as maltose, fructose, galactose, mannose, sorbitol, mannitol, adonitol, cellobiose and arabinose is used for this purpose. When microorganisms ferment carbohydrate an acid or acid with gas are produced. Depending up on the organisms involved and the substrate being fermented, the end products may varies.

Common end-products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, carbon dioxide and hydrogen. The production of the acid lower the pH of the test medium, which is detected by the color change of the pH indicator. Colour of the medium changes from red colour to yellow colour if the test is positive, while medium remains red in colour if the test is negative (Table: 2).

Table 2: Utilization of carbon source by different isolates of *Xoo*

Tests	NB-1	NB-2	NB-3	NB-4	NB-5	NB-6
Maltose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	-	-	-	-	-	+
Mannose	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-
Adonitol	-	-	-	-	+	+
Cellobiose	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-

Positive reaction = +, Negative reaction = -

The carbon source utilization profiles of these all eight bacterial isolates were quite different. There were 9 types of different carbon sources (maltose, fructose, galactose, mannose, sorbitol, mannitol, adonitol, cellobiose and arabinose) utilized by different isolates which were collected. Maltose, fructose and mannose are utilized by all the *Xoo* isolates. Sorbitol, mannitol, cellobiose and arabinose were not utilized by all the isolates where as galactose and adonitol showed variable reaction to the *Xoo* isolates.

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