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Characterization of *Xanthomonas oryzae* pv. *oryzae* Isolates from Rice Growing Regions of Southern India

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ABSTRACT

Bacterial leaf blight (BLB) of rice caused by Xanthomonas oryzae pv. oryzae (Xoo) is a major biotic constraint in rice cultivation and is wide spread in Asia, including India. A total of 54 isolates were collected from different rice growing zones of southern India. All the isolates were obtained and subjected to pathogenicity test on BPT-5204 that varied significantly in terms of disease severity among each other and further confirmed with colony morphological features and biochemical tests. Colony size of the isolates ranged from 1.2 mm to 4.0 mm with light yellow to yellow and creamy yellow colour with circular to irregular margin. Most of the isolates produced raised, slimy colonies but few isolates produced flattened and slimy colonies. All the isolates were subjected to different biochemical tests and were found to be positive for 3 per cent KOH test, gelatin liquefaction, catalase test, while, negative for starch hydrolysis and oxidase tests. Five isolates namely, Xoo7, Xoo16, Xoo22, Xoo27 and Xoo50 found negative for H_2S production test.

Key words: *Bacterial leaf blight, Biochemical characterization, BLB, Rice, Morphology, Xanthomonas oryzae pv. oryzae*

INTRODUCTION

The bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the causal pathogen of the bacterial leaf blight disease (BLB) of rice, widely distributed in all the major irrigated low land rice growing regions of Asia[8]. Bacterial leaf blight disease appears widespread every year in varying degrees in both irrigated and rainfed rice growing areas of the world and can cause 30-35% yield loss[1,13]. *Xoo* has the potential to reduce yield upto 50% or more depending on the variety, stage of the crop and climatic conditions.

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In India, the first report of BLB was made by Bhapkar *et al*.[2], and it is one of the most devastating diseases during monsoon season and a major production constraint in rice cultivation particularly in irrigated and rainfed lowland ecosystems of rice growing states of India[10].

BLB is a major problem in *Kharif* season crop in rice growing regions of Punjab, Haryana, Uttaranchal, Bihar, West Bengal, Tripura, Assam, Tamil Nadu, Karnataka, coastal areas of Andhra Pradesh, Eastern Uttar Pradesh and Andaman and Nicobar Islands, Kerala, parts of Maharashtra, Chhattisgarh, Gujarat and Himachal Pradesh. In the present study, isolates were collected from all rice growing zones of southern Indian states and subjected them for pathogenicity test, morphological and biochemical characterization.

MATERIALS AND METHODS

The studies on isolation, morphological and biochemical characterization were carried out at Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences Raichur, Karnataka (India).

Collection of diseased leaves

Diseased rice leaves showing typical bacterial leaf blight symptom were collected during *Kharif*-2013 from various rice fields over 18 agroclimatic zones of southern (Table 1). Samples were collected based on the random sampling method, on rice plant at heading to approaching maturity stages, as the disease usually develops well in these plant growth stages. Disease leaves were detached and put into the paper envelope. These envelopes were labeled explaining variety, location, sampling date, rice ecosystem, and the samples were taken into the laboratory and kept in the refrigerator for further process.

Isolation of *Xanthomonas oryzae* pv. *oryzae*

Diseased leaves were cleaned with tap water, and air dried. These leaves were cut into small pieces about 5 to 7 cm and sterilized with 1% sodium hypochloride solution, then washed in sterilized distilled water. These pieces were cut into smaller pieces about 5 x 5 mm in size and put into the test tube containing sterilized distilled water for about 10 to 15 min, to allow the bacteria to ooze out from the leaf tissue. Using the sterilized loop needle with bacterial suspension streak onto Petri dishes containing modified Wakimoto's medium (WF-P)[12]. The plates were incubated in room temperature (27 ± 1 ^{0}C) for 2 to 3 days. The single yellow, round and smooth margin, non flat, mucous colonies were selected and transferred into slant WF-P medium as pure culture. The single colony was selected as a representative strain and maintained at 4 ^{0}C for further studies.

Pathogenicity test

Paddy variety 'BPT-5204' seeds were soaked in 500 ppm solution of streptocycline for 8 hour to avoid the seed transmission. Disinfected seeds were sown in earthen pots (20 cm dia) and then seeded pots were placed in the glasshouse. The bacterial inoculum was prepared by dissolving one loopful of the pure bacterial culture in 10 ml of sterilized distilled water, so as to get the 10^7 CFU/ml. Seedlings of 45 days old, grown in glass house condition were artificially clip inoculated with the bacterial suspension. The inoculated plants were observed for the development of symptoms. After the symptom development, the bacterium was re-isolated from the artificially inoculated seedlings to prove the Koch's postulates and compared with the original culture.

Morphological characterization

The study of colonial morphology of 54 *Xoo* isolates was studied using the standard procedure described by Bradbury[3] and Schaad[17] with special consideration to the colour, size of colonies, and their outline – whether circular and entire or indented or wavy or rhizoid. Their elevations were recorded as convex, flat, plate-like or nodular and their appearance. The loopfull of culture was taken from 24 hour old culture and shaken in a sterile water column (10 ml sterile water in a test tube). From this, 6 dilutions (10^{-1} - 10^{-6}) were made by transferring 1 ml of the suspension to successive water columns. From last two series of the dilution, 0.1 ml was taken and poured into the petri dishes which contains nutrient agar medium. The plates were then incubated at columns 27 ± 1^{0}C for 48 hours after that they were examined for appearance of the colonies.

Biochemical characterization

Gram staining

Gram staining procedure was performed as described by Gerhardt[6]. Bacteria were heat

fixed on a glass slide treated with (0.5%) crystal violet for 30 seconds then washed with tap water. After that, Iodine was added for 1 min, washed again and decolorized with (95%) ethanol for 30 seconds, washed again and counter-stained with safranin. Magnifications of 10X and 40X was used microscopic observation. G -ve bacteria stained red whereas G +ve retained the color of crystal violet.

Potassium Hydroxide Test

Potassium hydroxide (KOH 3%) test is an excellent validation assay for Gram staining[20]. The bacterial culture taken tooth pick was vigorously stirred in drop of 3% KOH solution. Thread-like slime formation when picked the toothpick indicated the presence of G -ve bacterium. But no slime or thread formation was the indication of G +v bacterium[15,16].

Starch hydrolysis

The starch agar medium is being used to carry out the starch hydrolysis. For each hydrolysis test 20g Nutrient Agar (NA) was added to 80 ml of water and dissolved by successive heating and stirring similarly two gram starch was then thoroughly dissolved in 10 ml distilled water separately and added to hot molten agar with through stirring. Amount of 100 ml of this basal medium was then transferred to conical flask (250 ml) and autoclaved at 115 ^{0}C for duration of 10 minutes. The medium was then poured into Petri plates. The plates were then inoculated with individual isolate aseptically, labeled and sealed to avoid chances of contamination. These plates were then incubated in upside down position at 27 ^{0}C for 7 days. After scraping bacterial growth to each plate Lugol's iodine was added which was prepared by mixing 1 g iodine and 2 g potassium iodide in 300 ml distilled water, stirred for until dissolved completely. The appearance of cleared zones around the colonies was indicative of presence or absence of starch hydrolysis as described by Cowan[4].

Gelatin liquefaction

Fifteen ml of freshly prepared and autoclaved nutrient agar added with 0.4 per cent (4 g/1000 ml) gelatin was poured into the sterilized petriplates (six plates were poured with the medium). After the medium gets solidified, spot inoculation using a tooth prick on the surface of the medium was done. Plates were incubated at 27 ± 1^{o}C for three days. After the incubation period is over, plates were flooded with 10 ml of acid mercuric chloride solution ($HgCl_2$, 12 g; Distilled water, 80 ml; Concentrated HCl, 16 ml). Observations were made for the formation of clear zone around the growth of the bacterium.

H_2S Production

The peptone broth was prepared and sterilized. A loopful culture of 48 hr. old test bacterium is inoculated in to the slants containing the peptone broth. Filter paper discs (Whatman No. 42) impregnated with 10 per cent solution of neutral lead acetate was taken and air dried and then inoculated. The sterilized stripes were placed in to the inoculated test tubes, in such a manner that one end of the strip held by the cotton plug and other end left free and hanged inside the tube. The inoculated tubes were incubated at 27 ± 1^{o}C for 72 hr. Observations were drawn for the H_2S production. Blackening of the stripes indicated the positive reaction.

Catalase

A loopful of 48 hr. slant growth of the test bacterium was smeared on a slide and was covered with few drops of hydrogen peroxide (20 volumes). The reaction will be positive if gas bubbles are produced.

Oxidase activity

One day old bacterial colony, grown on nutrient agar as described previously, supplemented with 1% glucose was used in this assay. A loopfull of the inoculum was rubbed onto a filter paper impregnated with 1% (w/v) freshly prepared aqueous solution of tertramethyl-p-phenylene diamine dihydrochloride. The isolate was rated oxidase-positive if a purple colour developed within 10 seconds, delayed positive if coloration developed within 10-60 seconds; and negative if no colour developed after 60 seconds.

RESULTS AND DISCUSSION

Isolation

The causal organism was isolated from the infected leaf, showing the typical symptoms of bacterial leaf blight. Isolation was done by employing the streak plate method using modified Wakimoto's medium. Repeated

isolation from the infected leaf yielded typical well separated, yellow, mucoid colonies of the bacterium on medium after 48 hours of incubation at 27 ± 1°C (Fig. 1). The colonies were purified by streaking the isolated colony on nutrient agar and pure colonies so obtained were further streaked on to the nutrient agar slants and kept for incubation at 27 ± 1 °C for 48 h. Cultures, so obtained were stored in the refrigerator at 4 °C, which served as a stock culture for further studies. Based on the morphological and cultural characters, the bacteria was identified as *Xanthomonas oryzae* pv. *oryzae*.

Pathogenicity test

Artificial inoculation of the pathogenic bacterium was carried out to prove the pathogenicity using leaf clip inoculation technique under glass house condition (Fig. 2). Bacterial suspension was clip inoculated to 45 days old plant of paddy variety BPT-5204. After 15 days of inoculation, symptoms were produced with the formation of water soaked lesions appeared from the margin and moved all along the leaf blade in wavy pattern and lesions were extended in length and width. The region adjoining the healthy part showed water soaked blighted lesions extended rapidly to cover large areas of the leaf blade, turned white and later became greyish. The bacteria was re-isolated, which resembled the original culture of *Xanthomonas oryzae* pv. *oryzae*. These results well correlate with the study of Ghasemie *et al*.[7], where they characterized large number of *X*. *oryzae* pv. *oryzae*, isolates by pathogenicity test.

Morphological characterization

Morphological characterization of *Xoo* isolates revealed that, colonies of the bacterium appeared as circular, convex, and yellow to creamy yellow coloured with smooth surface on the nutrient agar medium (Table 2). The maximum colony size (4.0 mm) was observed in Xoo2 and Xoo19 isolates and minimum colony size of 1.2 mm was observed in isolate Xoo15. All isolates exhibited slightly varied colour colonies, the colony colour of most of the isolates were light yellow to yellow, whereas, isolates Xoo8 from northern dry zone of Karnataka, Xoo14 and Xoo19 produced creamy yellow coloured colonies on nutrient agar medium. The colony shape of most of the isolates were circular whereas five isolates Xoo2, Xoo4, Xoo6, Xoo14 and Xoo19 were found circular to irregular in shape. Most of the isolates exhibited raised, slimy colonies whereas some isolates produced flattened and slimy colonies. Present results are supported with similar results obtained by Han *et al*.[9], where *Xoo* colonies were slightly convex, smooth, with regular to irregular diffused edges. Colonies of the bacterium appeared as circular, convex, yellow to straw yellow coloured with smooth surface on the nutrient agar medium and were opaque against the transmitted light on 48 h old culture[19].

Biochemical characterization

A total of seven biochemical tests were conducted to characterize the pathogen isolated from BLB infected samples collected from different regions of southern India. The tests included Gram staining, potassium hydroxide test, starch hydrolysis test, gelatin liquefaction, H_2S production, catalase activity and oxidase test (Table 3).

All isolates exhibited gram negative reactions with red colour when studied under light microscope (Fig. 3a). Positive results were obtained for potassium hydroxide (3%) solubility test (Fig. 3b), gelatin liquefaction and catalase activity (Fig. 3c) tests but, negative for starch hydrolysis (Fig. 3d) and oxidase tests, same result has been reported by Sreeramulu and Nayudu[18]. Whereas, differential result recorded for H_2S production test (Fig. 3e), out of 54 isolates 49 showed positive and remaining 5 isolates (Xoo7, Xoo16, Xoo22, Xoo27 and Xoo50) did not produce H_2S. On the contrary, Swings *et al*. described the bacterium as catalase positive and negative for oxidase test. All isolates were negative for oxidase and gram reaction[11], but Elham *et al*.[5] did not find these findings in their isolates except gram negative reaction. Thimmegowda *et al*.[21], reported similar results of biochemical studies showing positive reaction for gelatin liquefaction and hydrogen sulphide production, but the few isolates were found negative for starch hydrolysis. Rukshana *et al*.[14], reported the bacterium was gram negative, rod shaped and producing red colour when counter stained with safranin. In the present study, based on differential reaction in some biochemical tests, it was established that genetic variability was detected in *Xanthomonas oryzae* pv. *oryzae* isolates.

Table 1: List of *Xanthomonas oryzae* pv. *oryzae* isolates collected from different zones of southern India

Isolate name	Origin	Cultivar	Agro-climatic zones	Isolate name	Origin	Cultivar	Agro-climatic zones
Xoo1	Gudeballur	BPT-5204	Southern Telangana zone (TS)	Xoo28	Sreepuram Junction	Kaveri Sona	Northern dry zone (KA)
Xoo2	Balanagar	Tella Hamsa	Southern Telangana zone (TS)	Xoo29	Kumbalur	Sriram gold	Central dry zone (KA)
Xoo3	Telkapalli	MTU1010	Southern Telangana zone (TS)	Xoo30	Arsapura	Ankur Sona	Central dry zone (KA)
Xoo4	Chilkur	BPT-5204	Southern Telangana zone (TS)	Xoo31	Mudugere	IR-64	Eastern dry zone (KA)
Xoo5	Pedda Mungal	Tella Hamsa	Southern Telangana zone (TS)	Xoo32	Mugur	GJL7854	Southern dry zone (KA)
Xoo6	Nagaladinne	BPT-5204	Scarce rainfall zone (AP)	Xoo33	Goluru	Mini Long	Southern dry zone (KA)
Xoo7	Gopalapuram	Swarna	North coastal zone (AP)	Xoo34	Yelandur	Mini Long	Southern dry zone (KA)
Xoo8	Pithapuram	Swarna	Godavari zone (AP)	Xoo35	Mullur	Mini Long	Southern dry zone (KA)
Xoo9	Samarlakota	Swarna	Godavari zone (AP)	Xoo36	Narahalli	IR-64	Southern dry zone (KA)
Xoo10	Sitharampuram	Swarna	Godavari zone (AP)	Xoo37	Kudaligere	MTU1001	Southern transition zone (KA)
Xoo11	Kakaramilli	Swarna	Godavari zone (AP)	Xoo38	Honnali	Sriram gold	Southern transition zone (KA)
Xoo12	Palakollu	BPT-5204	Godavari zone (AP)	Xoo39	Harakere	Jaya	Sothern transition zone (KA)
Xoo13	Anakoderu	BPT-5204	Godavari zone (AP)	Xoo40	Mugad	Mugad Siri	Northern transition zone (KA)
Xoo14	Maruteru	Swarna	Godavari zone (AP)	Xoo41	Brahmavara	Jyothi	Coastal zone (KA)
Xoo15	Chamarru	BPT-5204	Krishna zone (AP)	Xoo42	Nallepilly	Matta Thriveni	Central zone (KL)
Xoo16	Kanakavalli	BPT-5204	Krishna zone (AP)	Xoo43	Parali	Jyothi	Central zone (KL)
Xoo17	Velpuru	MTU1010	Krishna zone (AP)	Xoo44	Paruthoor	Jyothi	Central zone (KL)
Xoo18	Naikal	BPT-5204	North eastern dry zone (KA)	Xoo45	Puvanikuppam	BPT-5204	North eastern zone (TN)
Xoo19	Gugi	BPT-5204	North eastern dry zone (KA)	Xoo46	Thiruppur	BPT-5204	North eastern zone (TN)
Xoo20	Gabburu	BPT-5204	North eastern dry zone (KA)	Xoo47	Mannur	ADT-43	Western zone (TN)
Xoo21	Kalmala	BPT-5204	North eastern dry zone (KA)	Xoo48	Maiyalapuram	BPT-5204	Cauvery delta zone (TN)
Xoo22	Kapgal	BPT-5204	North eastern dry zone (KA)	Xoo49	Mela Ulur	ADT-38	Cauvery delta zone (TN)
Xoo23	Devapur	BPT-5204	North eastern dry zone (KA)	Xoo50	Aduthurai	CR-1009	Cauvery delta zone (TN)
Xoo24	Kampli	BPT-5204	Northern dry zone (KA)	Xoo51	Tholur	BPT-5204	Cauvery delta zone (TN)
Xoo25	Dhadesugur	BPT-5204	Northern dry zone (KA)	Xoo52	Koothanallur	ADT-36	Cauvery delta zone (TN)
Xoo26	Gangavathi	BPT-5204	Northern dry zone (KA)	Xoo53	Amathur	ASD-16	Southern zone (TN)
Xoo27	Muneerabad	BPT-5204	Northern dry zone (KA)	Xoo54	Sepparai	Laxmi	Southern zone (TN)

TS: Telangana state; AP: Andhra Pradesh; KA: Karnataka; KL: Kerala; TN: Tamil Nadu

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Table 2: Morphological characteristics of the *Xanthomonas oryzae* pv. *oryzae* isolates of southern India

Isolate	Colony size range (mm)	Avg. colony size (mm)	Colony colour	Shape	Appearance
Xoo1	1 - 2	1.5	Light yellow to Yellow	Circular	Raised, slimy
Xoo2	2-6	4	Yellow	Circular	Raised, slimy
Xoo3	1-2	1.5	Light yellow	Circular	Raised, slimy
Xoo4	2-4	3	Light yellow	Circular	Raised, slimy
Xoo5	1-5	3	Yellow	Circular	Raised, slimy
Xoo6	2 - 6	4.0	Light Yellow	Circular to irregular	Flattened, slimy
Xoo7	2 - 4	3.0	Yellow	Circular	Raised, slimy
Xoo8	2-5	3.5	Light yellow	Circular	Raised, slimy
Xoo9	1-2	1.5	Yellow	Circular	Raised, slimy
Xoo10	1-4	2.5	Light yellow	Circular	Raised, slimy
Xoo11	1-2	1.5	Yellow	Circular	Raised, slimy
Xoo12	1 - 5	3.0	Yellow	Circular to irregular	Flattened, slimy
Xoo13	1-3	2	yellow	Circular	Raised, slimy
Xoo14	1-2	1.5	Light yellow	Circular	Flattened
Xoo15	1-3	2	yellow	Irregular	Flattened
Xoo16	2 – 3	2.5	Light Yellow	Circular	Raised, slimy
Xoo17	0.5-2	1.2	Light yellow	Circular	Raised, slimy
Xoo18	1-4	2.5	Yellow	Circular	Flattened
Xoo19	1-3	2	Light yellow	Circular	Flattened
Xoo20	1-4	2.5	Yellow	Circular	Raised, slimy
Xoo21	0.5-2	1.2	Yellow	Irregular	Flattened
Xoo22	1 - 6	3.5	Yellow	Circular to irregular	Flattened, slimy
Xoo23	0.5-2	1.2	Yellow	Circular to irregular	Raised, slimy
Xoo24	1-5	3	Light yellow	Circular	Raised, slimy
Xoo25	1-5	3	Light yellow	Irregular	Flattened
Xoo26	1 - 3	2.0	Yellow	Circular	Raised, slimy
Xoo27	1 – 3	2.0	Creamy Yellow	Circular	Flattened, slimy
Xoo28	0.5-5	2.7	Yellow	Circular	Raised, slimy
Xoo29	0.5-3	1.7	Light yellow	Circular	Raised, slimy
Xoo30	1 – 2	1.5	Light Yellow	Circular	Raised, slimy
Xoo31	1 – 3	2.0	Light Yellow	Circular	Flattened, slimy
Xoo32	1-2	1.5	Yellow	Circular	Raised, slimy
Xoo33	1-2	1.5	Light yellow	Circular	Raised, slimy
Xoo34	0.5-2	1.2	Light yellow	Circular	Raised, slimy
Xoo35	1 – 3	2.0	Yellow	Circular to irregular	Flattened, slimy
Xoo36	1-2	1.5	Light yellow	Circular	Raised, slimy
Xoo37	0.5-3	1.7	Yellow	Circular	Raised, slimy
Xoo38	1 – 4	2.5	Light Yellow	Circular	Flattened, slimy
Xoo39	1-4	2.5	Yellow	Circular	Raised, slimy
Xoo40	1 – 4	2.5	Light Yellow	Circular	Raised, slimy
Xoo41	2 – 4	3.0	Creamy Yellow	Circular to irregular	Flattened, slimy
Xoo42	0.5 - 2	1.2	Light Yellow	Circular	Raised, slimy
Xoo43	1 – 4	2.5	Yellow	Circular	Flattened, slimy
Xoo44	1-3	2	Yellow	Circular	Flattened
Xoo45	0.5 - 4	2.7	Yellow	Circular	Raised, slimy
Xoo46	0.5-2	1.2	Yellow	Circular	Raised, slimy
Xoo47	1 – 3	2.0	Light Yellow to Yellow	Circular to irregular	Flattened, slimy
Xoo48	1-2	1.5	Yellow	Irregular	Flattened
Xoo49	1-3	2.0	Yellow	Circular	Raised, slimy
Xoo50	1 – 7	4.0	Creamy Yellow	Irregular	Flattened, slimy
Xoo51	0.5-3	1.7	Yellow	Circular	Raised, slimy
Xoo52	1-4	2.5	Yellow	Circular	Flattened
Xoo53	1-2	1.5	Light Yellow	Circular to irregular	Raised, slimy
Xoo54	1 - 4	2.5	Yellow	Circular	Flattened, slimy

Table 3: Biochemical characterization of various *Xoo* isolates collected from different rice growing zones of southern India

Isolates	Gram reaction	KOH Test (3%)	Starch Hydrolysis	Gelatin Liquefaction	Catalase Test	H_2S Production	Oxidase Test
Xoo1	-	+	-	+	+	+	-
Xoo2	-	+	-	+	+	+	-
Xoo3	-	+	-	+	+	+	-
Xoo4	-	+	-	+	+	+	-
Xoo5	-	+	-	+	+	+	-
Xoo6	-	+	-	+	+	+	-
Xoo7	-	+	-	+	+	-	-
Xoo8	-	+	-	+	+	+	-
Xoo9	-	+	-	+	+	+	-
Xoo10	-	+	-	+	+	+	-
Xoo11	-	+	-	+	+	+	-
Xoo12	-	+	-	+	+	+	-
Xoo13	-	+	-	+	+	+	-
Xoo14	-	+	-	+	+	+	-
Xoo15	-	+	-	+	+	+	-
Xoo16	-	+	-	+	+	-	-
Xoo17	-	+	-	+	+	+	-
Xoo18	-	+	-	+	+	+	-
Xoo19	-	+	-	+	+	+	-
Xoo20	-	+	-	+	+	+	-
Xoo21	-	+	-	+	+	+	-
Xoo22	-	+	-	+	+	-	-
Xoo23	-	+	-	+	+	+	-
Xoo24	-	+	-	+	+	+	-
Xoo25	-	+	-	+	+	+	-
Xoo26	-	+	-	+	+	+	-
Xoo27	-	+	-	+	+	-	-
Xoo28	-	+	-	+	+	+	-
Xoo29	-	+	-	+	+	+	-
Xoo30	-	+	-	+	+	+	-
Xoo31	-	+	-	+	+	+	-
Xoo32	-	+	-	+	+	+	-
Xoo33	-	+	-	+	+	+	-
Xoo34	-	+	-	+	+	+	-
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Xoo36	-	+	-	+	+	+	-
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Xoo38	-	+	-	+	+	+	-
Xoo39	-	+	-	+	+	+	-
Xoo40	-	+	-	+	+	+	-
Xoo41	-	+	-	+	+	+	-
Xoo42	-	+	-	+	+	+	-
Xoo43	-	+	-	+	+	+	-
Xoo44	-	+	-	+	+	+	-
Xoo45	-	+	-	+	+	+	-
Xoo46	-	+	-	+	+	+	-
Xoo47	-	+	-	+	+	+	-
Xoo48	-	+	-	+	+	+	-
Xoo49	-	+	-	+	+	+	-
Xoo50	-	+	-	+	+	-	-
Xoo51	-	+	-	+	+	+	-
Xoo52	-	+	-	+	+	+	-
Xoo53	-	+	-	+	+	+	-
Xoo54	-	+	-	+	+	+	-

+ = Positive, - = Negative

Fig. 1: Colonies of Xanthomonas oryzae pv oryzae

Fig. 2: Pathogenicity test on BPT-5204

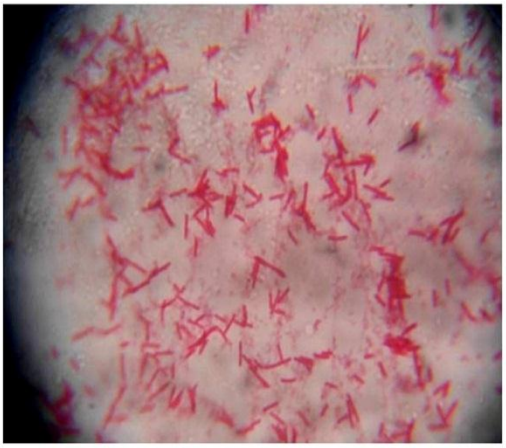


Fig. 3:a. Gram stained cells of *Xoo*

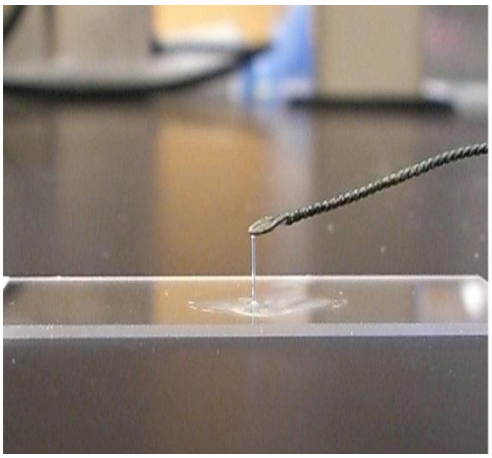


Fig. 3:b. KOH (3%) test

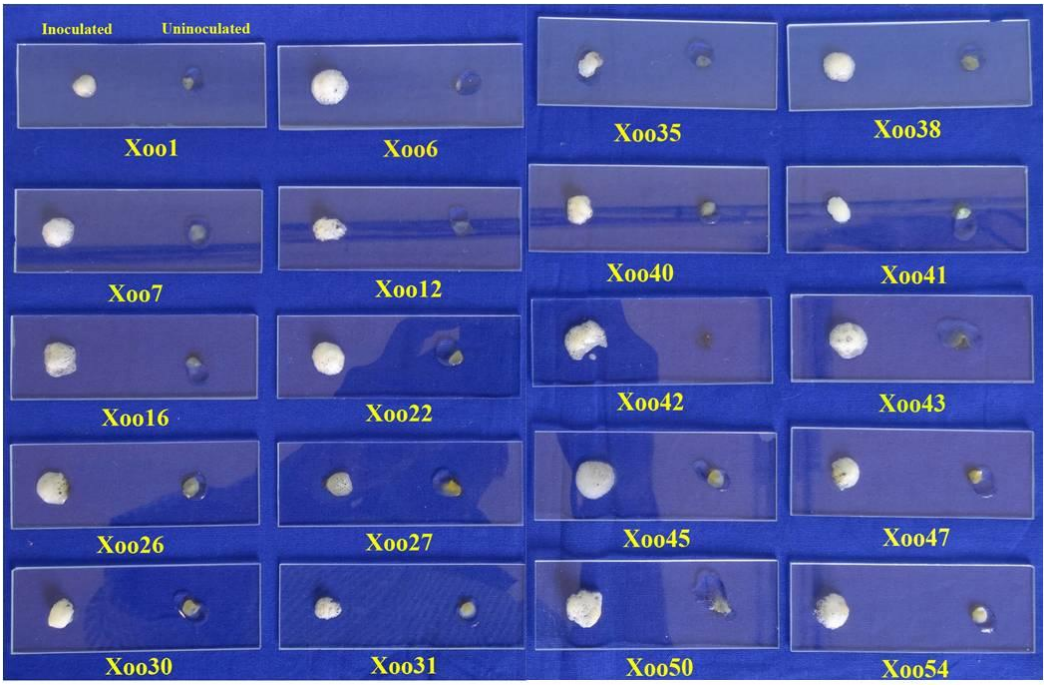
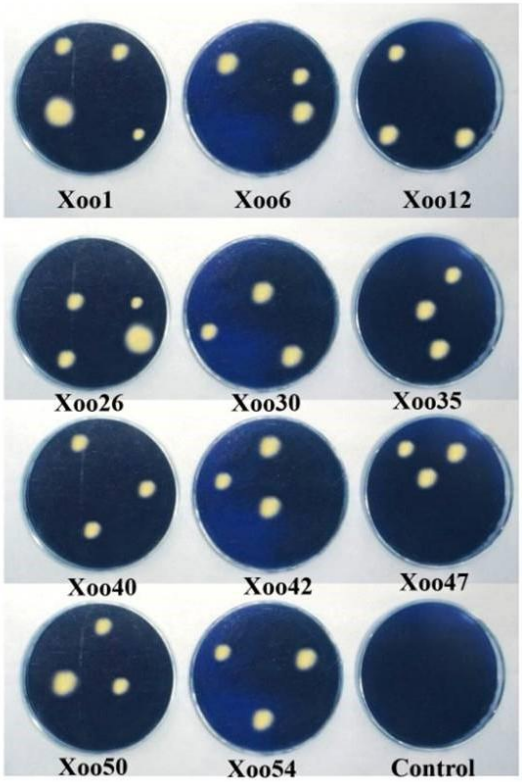


Fig. 3:c. Catalase test



A

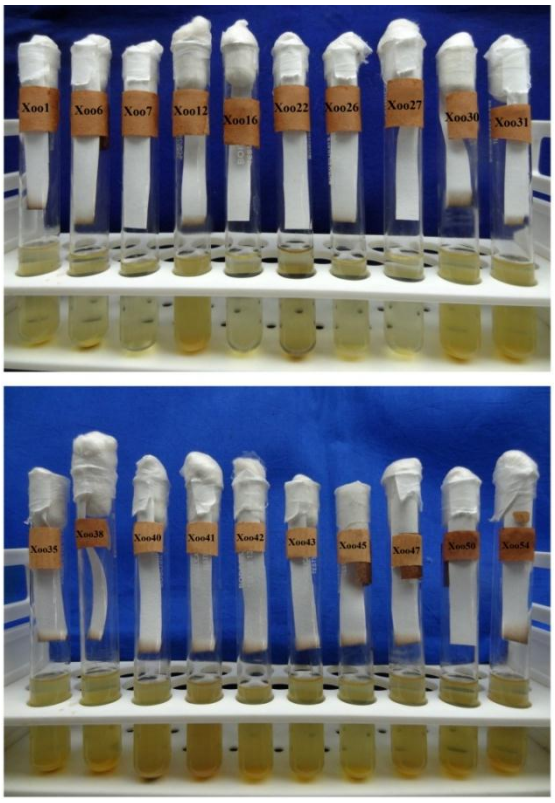


Fig. 3:d. Starch hydrolysis

Fig. 3:e. H2S production test

CONCLUSION

Bacterial blight of rice is an important disease of rice. Currently there are very few resistant rice cultivars are available to combat the disease. Current studies show that, inoculum build up in rice growing zones of southern India has created alarming situation. Present study showed 100 percent recovery of the causal bacterium from samples collected from different agro-ecological zones of Southern Indian states, clearly confirming previous findings. Close monitoring of inoculum build up in rice growing areas and search for sustainable management in search of resistant cultivars is recommended to minimize yield losses that occurred due to BLB.

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REFERENCES

1. Adhikari, T. B., Vera Cruz, C. M., Zhang, Q, Nelson, R. J., Skinner, D. Z., Mew, T. W. and Leach, J. E., Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Applied Environment and Microbiol*ogy **61:** 966-971 (1995).
2. Bhapkar, D. G., Kulkarni, N. B. and Chavan, V. M., Bacterial blight of paddy., *Poona Agricultural College Magazine*, **51:** 36-46 (1960).
3. Bradbury, T. F., Isolation and preliminary study of bacteria from plants. *Review of Plant Pathology* **45(15):** 213-218 (1970).
4. Cowan, S. T., *Manual for the identification of medical bacteria*. Cambridge Univ. Press, Great Britain. p. 238 (1974).
5. Elham, G., Mostafa, N. K. and Ferydon, P., Isolation and identification of *Xanthomonas oryzae* pv. *oryzae* causal agent of bacterial blight of rice in Iran. *Journal Plant Protection Research* **48(1):** 53-61 (2008).

6. Gerhardt, P., *Manual of Methods of General Bacteriology*. Amer. Soc. of Microbiol Washington, D.C. p. 65 (1981).
7. Ghasemie, E., Kazempour, M. N. and Ferydon, P., Isolation and identification of *Xanthomonas oryzae* pv. *oryzae* the causal agent of bacterial blight of rice in Iran. *Journal Plant Protection Res*earch **48(1):** 53-62 (2008).
8. Gnanamanickam, S. S, Priyadarshini, V. B., Narayanan, N. N., Vasudevan, P. and Kavitha, S., An overview of bacterial blight disease of rice and strategies for its management. *Current Science* **77:** 1435-1444 (1999).
9. Han, J., Lei, S., Xiuzhu, D., Zhengqiu, C., Xiaolu, S., Hailian, Y., Yunshan, W. and Wei, S., Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. *Systems of Applied Microbiology* **28:** 66-76 (2005).
10. Laha, G. S., Reddy, C. S., Krishnaveni, D., Sundaram, R. M., Srinivas Prasad, M., Ram, T., Muralidharan, K. and Viraktamath, B. C., Bacterial blight of rice and its management. *Bulletin*., DRR, Rajendranagar, Hyderabad, India. pp. 1-15 (2009).
11. Najeeya, M., Abdul, R. and Muhammad, A. A., Isolation and characterization of *Xanthomonas oryzae pv. oryzae* isolates from north west frontier province, Pakistan. *Sarhad Journal of Agriculture* **23(3):** 743-751 (2007).
12. Ou, S. H., *Rice diseases*, 2nd edition. Commonwealth mycological institute, Kew, Surrey (England). pp. 61-69 (1985).
13. Reddy, A. P. K., Bacterial blight: crop loss assessment and disease management. *Proceeding of the International workshop on Bacterial Blight of Rice*. pp 79-88 (1989). International Rice Research Institute, Manila, Philippines.
14. Rukhsana, J., Tehreema, I. and Huma, B., Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. *Pakistan Journal of Botany* **44(1):** 261-265 (2012).
15. Ryu, E., A simple method of differentiation between Gram-positive and Gram-negative organisms without staining. *Medicine* **17:** 58-63 (1940).
16. Schaad, N. W., Laboratory guide for identification of plant pathogenic bacteria. Dept. Plant Pathology Univ. of Georgia. p. 28 (1980).
17. Schaad, N. W., Laboratory guide for identification of plant pathogenic bacteria. 2nd edition. *International Book Distributing Co*., Lucknow, India. pp. 78-83 (1992).
18. Sreeramulu, A. and Nayudu, M. V., Biochemical characters and pathogenic variation of different isolates of paddy bacterial leaf blight pathogen. *Oryza* **24(4):** 363-367 (1987).
19. Suresh, R., Epidemiology and management of bacterial leaf blight of paddy caused by *Xanthomonas oryzae* pv. *oryzae*. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci. Raichur. pp. 45-47 (2012).
20. Suslow, T. V., Schroth, M. N. and Isaka, M., Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology* **72:** 917-918 (1982).
21. Thimmegowda, P. R., Arun, R. S., Patil, M. B., Geeta, L. B. and Virupaksha, P., Biochemical and nutritional studies of *Xanthomonas oryzae* pv. *oryzae*. *Journal of Plant Disease Sciences* **3(1):** 9-12 (2008).