ISSN No:-2456-2165

Collection, Pathotype Profiling, and Virulence Analysis of *Xanthomonas oryzae* pv. *oryzae* Causing Bacterial Leaf Blight Disease of Rice in Bangladesh

Md. Ariful Islam[1],
Mohammad Ataur Rahman[1],
Tanbin Akter[1],
Md. Monirul Islam[1],
Muhammad Abdus Salam[1],
[1]Advanced Seed Research and Biotech Centre (ASRBC), ACI Limited, Gulshan, Dhaka 1212, Bangladesh

Sharmin Sultana Misty[2],
[2]Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh 2202, Bangladesh

Mohammad Anwar Hossain[3],
Md. Ashraful Haque[3*]
[3]Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh 2202, Bangladesh

Abstract:- *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the disease-causing agent behind bacterial leaf blight (BLB) affecting the leaves at the plant growth phases of rice. It produces noticeable symptoms in infected rice, such as leaf blight, which appears on the leaves of rice plants adjacent to the leaf tips and margins. This study intends to explore bacterial *Xoo* isolates from 25 regions in Bangladesh through biochemical and molecular characterization. The bacterial isolates were grown in petri dishes on Peptone Sucrose Agar (PSA) medium, and the isolates had colonies ranging in size from 0.08 mm to 1.1 mm, and their color ranged from light yellow to yellow with a circular margin. Majority of the isolates produced raised and slimy colonies. The bacteria were then subjected to different biochemical tests resulting in positive for KOH (3%) test, gelatin liquefaction, catalase test, and while, negative for starch hydrolysis, gram reaction, and oxidase tests. Moreover, the DNA of *Xoo*-indicating bacteria was isolated, and among the 29 isolates, 15 *Xoo*-bacteria were confirmed through PCR amplification using a specific primer, *Xoo*2976. All 15 virulent *Xoo*-bacteria were subjected to a pathogenicity test on 3 rice varieties, BRRI DHAN49, IRBB66, and IRRI 154 yielded significant results in terms of disease severity. *Xoo*17 exhibits the highest virulence from the pathogenicity test. The study's outcomes may be utilized for screening BLB-resistant rice in varietal development research.

Keywords: *Bacterial Leaf Blight; Characterization; Resistance; Xoo2976; Xanthomonas oryzae pv. Oryzae.*

I. INTRODUCTION

More than half of the world's population consumes rice since it is the staple food for the majority of people [1]; [2]. Particularly in Bangladesh, rice is the most cultivated crop, and more than 165 million people consume it [3]. To meet the demand, rice farming takes up the majority of the country's arable land—11.97 million hectares, or 74.85 percent—and more than 65 percent of its irrigated land [4]; [5]; [6]. Rice cultivation is becoming more appealing to Bangladeshi farmers over other crops due to its low cultivation risk and broad adaptability. Bangladesh is ranked third, trailing only China and India, with a total production of 3.6 crore tons from 11.55 million hectares of land. However, the loss in rice production is a result of many biotic and abiotic stressors, urbanization, and climate change [7]; [8]. Furthermore, it is anticipated that it will rise along with population growth, so that by 2050, 44.6 MT of quality rice will be required for rice security [8]. One of the oldest and major biotic constraints on rice is bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae (Xoo.*), a gram-negative bacterium, can reduce rice yield by 25% by disturbing panicle emergence and grain filling [1]. In the Philippines, it was found that 22.5% yield loss in the wet season and 60,000 metric ton loss occurred in Malaysia [9]. Particularly in Asian countries, due to repeated cultivation, BLB has become endemic [10]. In Bangladesh, leaf blight has been considered one of the major diseases affecting rice, persisting from the 1907 to 2016 period, and is considered a heavy epidemic rice disease [11].

The incidence of this disease has been reported in many areas of Asia, Africa, Australia, and the U.S.A [12]; [13]; [14]. The bacterium multiplies and spreads in both directions after entering the circulatory system through

wounds on the roots and leaves. Spread occurs mostly in flood and irrigation water, but also during wind and rain [15]. The *Xoo* bacteria work by entering the plant system through any exposed wounds and pores or other natural openings and inject proteins (transcription activator-like effectors) that result in the leaf blight phase which in turn causes lesions [9]; [1]. If capable of spreading throughout the leaf, these lesions may cause grayish saprophytic growth and can also affect the grain's sterility and overall quality. Besides lesions, *Xoo* might also cause 'kresek' symptoms which include wilting, withering, and graying of the leaves [16]. Natural defense systems include the production of phenolic compounds by the plant host [17].

Although, a lot of studies have already been carried out to overcome this disease, effective regulatory practices have not yet been discovered. Some ways to overcome the pathogenicity problems are by using the biological approach, using Plant growth promoting rhizobacteria [18]; Chemical approach, by using bleach to disinfest seed and antibiotics such as penicillin [19]; developing genetic resistant rice cultivars [1]. The main objective of the study is to determine virulent *Xoo* isolates from most rice growing regions of Bangladesh through morphological and biochemical analysis followed by molecular characterization with a *Xoo* specific primer pair.

This identification and characterization of different *Xoo* genotypes and their distribution in mostly rice-growing regions of Bangladesh would highly benefit rice breeding programs aiming at BLB-resistant rice cultivar development.

In this study, BLB affected leaves were collected as isolates from all rice growing regions in Bangladesh and subjected to biochemical and molecular characterization.

II. MATERIALS AND METHODS

At the Advanced Seed Research and Biotech Centre (ASRBC), ACI Ltd. Gulshan, Dhaka, Bangladesh, investigations on isolation, morphological, biochemical, and molecular characterization were completed.

- *Collection of Diseased Leaves:*

During the T. Aman 2021 season, diseased rice leaves exhibiting the usual symptoms of BLB were collected from several rice fields across the 25 agro-ecological zones in Bangladesh (Figure 1), with the exception of AEZ-12, AEZ-15, AEZ-22, AEZ-24, and AEZ-30 (Table 1). As the disease often progresses well during these periods of plant growth, samples were taken from rice plants at the heading and nearing maturity stages using the random sampling method. Detachable disease leaves were placed inside the paper envelope. The samples were taken into the laboratory and stored in the refrigerator for further processing. These envelopes were labeled with the variety, location, sampling date, and rice environment.

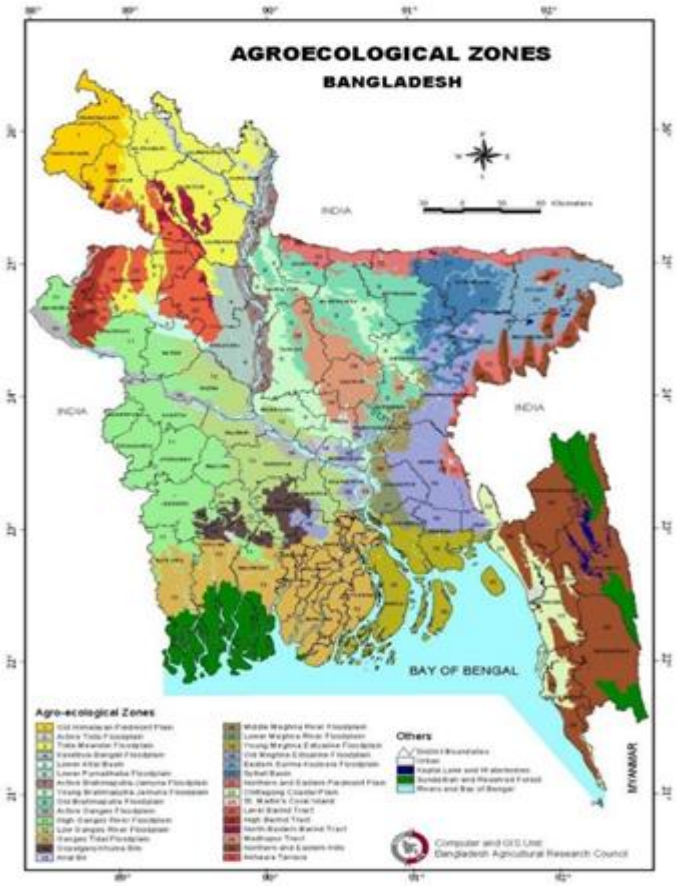

Fig 1 Disease Sample Collection Site of Agro-Ecological Zones in Bangladesh

ISSN No:-2456-2165

Table 1 List of *Xanthomonas oryzae* pv. *oryzae* Isolates Collected from Different Agro-Ecological Zones of Bangladesh

Isolate	Origin (Location)	AEZ	Agro ecological zones	Sub Regions
Xoo1	Fenchugonj, Sylhet	20	Eastern Surma-Kushiyara Floodplain	Eastern Surma-Kushiyara Floodplain
Xoo2	CRS, Mawna, Gazipur	28	Madhupur Tract	Madhupur
Xoo3	BRRI Rangpur	27	North-eastern Barind Tract	Rangpur sadar
Xoo4	Khagrachari	29	Northern and Eastern Hills	Rangamati, Bandarban
Xoo5	BAU, Mymensingh	9	Old Brahmaputra Floodplain	Mymensingh
Xoo6	Bonbelghoria, Natore	11	High Ganges River Floodplain	Rajshahi
Xoo7	BRRI, Rajshahi	10	Active Ganges Floodplain	Active Ganges Floodplain
Xoo8	Satkhira Sadar	13	Ganges Tidal Floodplain	Satkhira
Xoo9	Dinajpur	1	Old Himalayan Piedmont Plain	Dinajpur
Xoo10	Lakshmipur Sadar	17	Lower Meghna River Floodplain	Chandpur
Xoo11	Raygonj, Shirajgonj	4	Karatoya-Bangali Floodplain	Shirajgonj
Xoo12	Sonargaon, Narayangonj	16	Middle Meghna River Floodplain	Meghna river side
Xoo13	Mahadebpur, Naogaon	25	Level Barind Tract	Naogaon middle
Xoo14	Niamatpur, Naogoan	6	Lower Punarbhaba Floodplain	Naogaon border
Xoo15	Nilphamari	2	Active Tista Floodplain	Active Tista Floodplain
Xoo16	Ghatail, Tangail	8	Young Brahmaputra and Jamuna Floodplain	Tangail
Xoo17	Chittagong	23	Chittagong Coastal Plain	Feni
Xoo18	Ramu, Cox's Bazar	29	Northern and Eastern Hills	Rangamati, Bandarban
Xoo19	Jessore	11	High Ganges River Floodplain	Rajshahi
Xoo20	Khulna north	14	Gopalganj-Khulna Beels	Beel centres
Xoo21	Bhuapur, Tangail	7	Active Brahmaputra-Jamuna Floodplain	Jamuna river side
Xoo22	Nachole, Nawabgonj	26	High Barind Tract	Naogaon red soil
Xoo23	Manda, Naogaon	5	Lower Atrai Basin	Natore North
Xoo24	Laksham, Comilla	19	Old Meghna Estuarine Floodplain	Comilla
Xoo25	Mohongonj, Netrokona	21	Sylhet Basin	a) Central and Southern; b) Northern; c) Western
Xoo26	Pachagarh	1	Old Himalayan Piedmont Plain	Dinajpur
Xoo27	Bhola	18	Young Meghna Estuarine Floodplain	Bhola
Xoo28	Khuniaghas, Lalmonirhat	3	Tista Meander Floodplain	Jaypurhat, Kurigram, Gaibandha
Xoo29	Ramu, Cox's Bazar	29	Northern and Eastern Hills	Rangamati, Bandarban

➢ *Isolation of Xanthomonas Oryzae Pv. Oryzae:*

Infected leaves were washed with tap water and allowed to air dry. These leaves were divided into 5 to 7 cm-long pieces, sanitized with 1% sodium hypochlorite solution, and then rinsed in sterile distilled water. To allow the bacteria to flow out from the leaf tissue, these sections were sliced into smaller pieces 5mm x 5 mm in size and placed into a test tube filled with sterilized distilled water for 10 to 15 minutes. Apply bacterial suspension using the sterilized loop needle to modified Peptone sucrose agar in Petri plates (PSA) and the plates were incubated at 27±1°C for 2 to 3 days. PSA medium was used to transfer colonies as pure cultures. For further study, a single colony was taken as a distinctive strain and stored at 4°C.

➢ *Morphological Characterization:*

The standard approach outlined by [20] and [21] was used to examine the colony morphology of 29 *Xoo* isolates, paying particular attention to the color, size, and shape of the colonies—whether round and whole, indented, wavy, or rhizoid. Convex, flat, plate-like, or nodular elevations and appearances were noted for them. In a sterile water column, the loopful of culture was shaken after being removed from 24-hour-old culture (10 ml sterile water in a test tube). Transferring 1ml of the suspension to subsequent water columns produced 6 dilutions (10^{-1} - 10^{-6}) from this. A sample of 0.1 ml was collected from the final two series of the dilution and put onto petri plates with nutrient agar medium. The plates were then incubated for 48 hours at approximately 27±1° C before being checked for the presence of the colonies.

➢ *Biochemical Characterization:*

- *Gram Staining:*

The Gram staining process was carried out in accordance with [22] instructions. On a glass slide, bacteria were thermally fixed for 30 seconds before being rinsed with tap water after being exposed to (0.5%) crystal violet. Iodine was then applied for 1 minute, followed by another wash. Again, for decolorization, 95 percent ethanol was applied for 30 seconds. Eventually, the glass slide was counterstained with safranin after the third wash. Microscopic inspection utilized 10X and 40X magnifications. Gram +ve bacteria kept their crystal violet hue whereas Gram -ve bacteria stained red.

- *Potassium Hydroxide Test:*

The potassium hydroxide (KOH 3%) test [23] is also an excellent validation of the biochemical assay. In a drop of 3% KOH solution, the bacterial culture from the toothpick was aggressively agitated. When the toothpick was used, thread-like slime formed, which indicated the presence of Gram -ve bacteria. However, the absence of slime or thread development was a sign of the Gram +ve bacteria [24]; [21].

- *Gelatin Liquefaction:*

The sterilized petri plates were filled with fifteen milliliters of freshly made, autoclaved nutrient agar that had been mixed with 0.4% (4 g/1000 ml) gelatin (6 plates were poured with the media). Spot inoculation on the media's surface was carried out after the medium had hardened. Over the course of three days, plates were incubated at 27±1°C. Plates were then soaked once with 10 ml of mercuric chloride solution ($HgCl_2$, 12 g; Distilled water, 80 ml; Concentrated HCl, 16 ml).

- *Catalase Test:*

A loop of the test bacterium's 48-hour slant growth was spread on a slide, and a few drops of hydrogen peroxide were added. The formation of gas bubbles will ensure positive results.

- *Oxidase Activity:*

In this test, a bacterial colony that had been growing on nutrient agar for a day and had been supplemented with 1% glucose was employed. An inoculum loopful was applied to a filter paper that had been impregnated with a freshly made 1% (w/v) aqueous solution of tetramethyl-p-phenylene diamine dihydrochloride. If a purple hue appeared within 10 seconds, the isolate was evaluated as oxidase-positive, delayed positive, or negative depending on how long it took for a color to appear.

- *Identify Xoo Bacteria using PCR:*

Bacterial DNA is used directly as a PCR template in the molecular identification of microorganisms. *Xoo2976* Forward and *Xoo2976* Reverse are the particular primers used for the molecular detection of *Xoo* bacteria (Table 2). The PCR mixture used to replicate this initial detection consisted of 20 µl and contained 2 µl of 10 PCR buffer, 0.4 µl of 10 M dNTP mix, 1 µl of 5 M primary mix (Forward + Reverse), 1 µl of GC rich, and 1 unit of Taq polymerase enzyme DNA. The 1 kb of DNA ladder that was separated in 2% agarose gel electrophoresis in 1x TAE buffer was stained for the PCR findings. *Xoo* bacteria are present when colonies generate 337 bp bands [26]. The catalytic domain protein Dual Specificity Phosphatase was used to construct the primers for *Xoo2976.* For visualizing the existence of a resistant gene, the amplified product was observed by electrophoresis on 1.5% agarose gel.

➢ *Molecular Characterization:*

- *DNA extraction of Xoo Isolates:*

DNA extraction from the collected 29 *Xoo* isolates from 25 AEZ locations was carried out by following the DNA isolation method of [25] Purified *Xoo* isolates were cultivated in 3 replicated cultures in Nutrient Broth (NB) media with 10% glycerol for 72 hours at 30°C. After incubation, *Xoo* culture was transferred to 1.5 ml eppendorf tubes and centrifuged at 13,000 xg for 5 minutes. The supernatant was then discarded, and the pellets were air dried. Each tube received 200 µl of Tris 0.5M, which was used to dissolve by gently tapping the pellet. After the pellets had dissolved, 200 µl of lysis buffer was added, and the tubes were then allowed for 5–6 minutes for the lysis process. Following that, 700 µl of chloroform and isoamyl alcohol (24:1) were added to eppendorf tubes, and the centrifuge was once more run at 13,000 rpm for 10 min. To get DNA pellets, centrifuging the supernatant with 95% ethanol in each tube for an additional 10 minutes was performed. The samples were allowed to dry at room temperature after being cleaned with 70% ethanol and centrifuged once more as described above. 200 µl of distilled water was added, and the samples were kept in an incubator at 4 °C overnight. In order to allow it to polymerize, 0.8% of agarose gel was used. 10 µl of samples and 2 µl of loading dye were added to the wells after 30 minutes. Samples were stained with ethidium bromide once the gel had finished running, and DNA documentation was performed. Acetate solution (200 µl) was added to each tube after documentation followed by centrifugation at 14,800 rpm. Discard the supernatant part and 200 µl of isopropanol was added before centrifuging at 14,800 rpm. Finally, the supernatant was removed, and 200 µl of autoclaved water was added and kept at 4 °C for future investigation.

Table 2 Specific Primers for *Xanthomonas oryzae* pv. *oryzae* [26]

Primer	Sequence 5’ – 3’	Size (bp)	Description
Xoo2976 (F)	GCCGTTTTCTTCCTCAGC	337	Dual specificity phosphatase, Catalytic domain protein
Xoo2976 (R)	AGGAAAGGGTTTGTGGAAGC	337	Dual specificity phosphatase, Catalytic domain protein

➢ *Pathogenicity Test:*

- *Preparation of Bacterial Inoculums:*

From 29 *Xoo* isolates, 15 pathogenic *Xoo* isolates were found as virulent bacteria (Table 3). These isolates were grown for 72 hours at 30 degrees Celsius on a slant of peptone sucrose agar (PSA) medium. The concentration of bacterial suspension was adjusted to 3.3 x 10^8 CFU/ml by adding a total of 10 ml of sterile distilled water to the slant. The optical density (OD) was then

measured at 600 nm wavelength using a spectrophotometer (UNICO SQ-4802). The optimal concentration to produce BLB disease in rice is $OD_{600} = 1$, which corresponds to 3.3×10^8 CFU/ml of bacterial cells [27].

Table 3 Disease Reaction of 3 Check Varieties against 15 *Xoo* Isolates

SL No	Designation	Entry No	Isolates No	Block	R1	R2	R3	R4	R5	R6	AVG
1	BRRI dhan49	1	*Xoo*1	1	7	2	4	5	3	3	4.00
2	BRRI dhan49	2	*Xoo*5	1	1	6	1	5	1	2	2.67
3	BRRI dhan49	3	*Xoo*7	1	3	4	8	5	4	2	4.33
4	BRRI dhan49	4	*Xoo*9	1	1	7	1	1	1	2	2.17
5	BRRI dhan49	5	*Xoo*15	1	6	2	9	3	1	1	3.67
6	BRRI dhan49	6	*Xoo*16	1	2	2	1	1	8	6	3.33
7	BRRI dhan49	7	*Xoo*17	2	9	9	8	12	12.3	9	9.88
8	BRRI dhan49	8	*Xoo*18	2	0.9	1	1.6	0.8	2	2	1.38
9	BRRI dhan49	9	*Xoo*19	2	0.5	1	8	1	1	1	2.08
10	BRRI dhan49	10	*Xoo*20	2	1	1	1	0.9	2	1	1.15
11	BRRI dhan49	11	*Xoo*21	2	0.5	5	3	7	1	1	2.92
12	BRRI dhan49	12	*Xoo*22	2	1	1.1	1	1	1	3	1.35
13	BRRI dhan49	13	*Xoo*24	3	1	1.3	2	1	1	1	1.22
14	BRRI dhan49	14	*Xoo*25	3	3.3	3.6	4	2.2	5	7	4.18
15	BRRI dhan49	15	*Xoo*29	3	1	0.5	2	3	1	1	1.42
16	BRRI dhan49	16	Control	3	0	0	0	0	0	0	0.00
17	IRBB66	1	*Xoo*1	1	1.7	2.6	2	2	1	2	1.75
18	IRBB66	2	*Xoo*5	1	2	1	2	3	3	1	2.00
19	IRBB66	3	*Xoo*7	1	1	2.3	3	2	2	2.6	2.15
20	IRBB66	4	*Xoo*9	1	1	1	2	1	1	1	1.17
21	IRBB66	5	*Xoo*15	1	2	1	3	1	1	1	1.50
22	IRBB66	6	*Xoo*16	1	3	1	3	1	1	1	1.67
23	IRBB66	7	*Xoo*17	2	2	6	2	2	3	2	2.83
24	IRBB66	8	*Xoo*18	2	4	2	2	3	2	2	2.50
25	IRBB66	9	*Xoo*19	2	0.8	0.5	1	0.5	1	1	0.80
26	IRBB66	10	*Xoo*20	2	1	1	1	1	1	1	1.00
27	IRBB66	11	*Xoo*21	2	2	1	2.3	2	1.8	2	1.85
28	IRBB66	12	*Xoo*22	2	2	2	1	1	1	0.5	1.25
29	IRBB66	13	*Xoo*24	3	1	0.5	0.7	1	0.3	0.6	0.68
30	IRBB66	14	*Xoo*25	3	2	1	1	1.3	2.1	3	1.73
31	IRBB66	15	*Xoo*29	3	2	0.5	0.6	0.4	0.5	0.4	0.73
32	IRBB66	16	Control	3	0	0	0	0	0	0	0.00
33	IRRI 154	1	*Xoo*1	1	3.6	9	9.3	6	3	0.6	6.00
34	IRRI 154	2	*Xoo*5	1	2	4	3.5	4	2	2	2.92
35	IRRI 154	3	*Xoo*7	1	3	5	9	4	3	9	5.50
36	IRRI 154	4	*Xoo*9	1	2	1	3	5	4	2	2.83
37	IRRI 154	5	*Xoo*15	1	2	1	2	1	1	2	1.50
38	IRRI 154	6	*Xoo*16	1	3	2	3	1.8	1	1	1.97
39	IRRI 154	7	*Xoo*17	2	6	15	16	10	8	7	10.33
40	IRRI 154	8	*Xoo*18	2	3	2	2.3	4	2	2	2.55
41	IRRI 154	9	*Xoo*19	2	2	1	2.3	1	2	2	1.72
42	IRRI 154	10	*Xoo*20	2	0.5	0.7	0.3	0.3	0.5	0	0.38
43	IRRI 154	11	*Xoo*21	2	1.2	1.6	1	1	1	0.8	1.10
44	IRRI 154	12	*Xoo*22	2	0.5	0.5	0.6	0.4	0.3	0.5	0.47
45	IRRI 154	13	*Xoo*24	3	2	1	1.3	0.7	2	1	1.33
46	IRRI 154	14	*Xoo*25	3	1.3	4	3	3.5	2	1	2.47
47	IRRI 154	15	*Xoo*29	3	3	2	0.5	1	0.5	1	1.33
48	IRRI 154	16	Control	3	0	0	0	0	0	0	0.00

- *Inoculation of Xoo-Isolates:*

To prevent the spread of the seed disease, certain advanced BLB resistant lines from the Advanced Seed Research and Biotech Centre (ASRBC) were immersed in a 500 ppm streptocycline solution for 8 hours together with some susceptible and resistant lines. In 2.6 sq. feet plastic tray, clean seeds were sowed, and the seeded trays were then placed in the controlled net-house. The modified clipping method of [28] was used to inoculate 45-day-old seedlings being cultivated in double-door net

ISSN No:-2456-2165

houses with the bacterial isolate solution (Figure 2). In a nutshell, during the peak of tillering, each *Xoo* race infected twelve leaves per entry by clipping them off the tip. The scissors were disinfected by immersing them in 70% ethanol for 30 minutes before cleaning them three times with sterilized water and then dipping them into a fresh isolate solution. The development of symptoms was monitored in the infected plants. To demonstrate Koch's postulates, the bacteria was once again isolated from the artificially infected seedlings and compared to the original culture.

Fig 2 Inoculation of the Xoo Isolates

- *Disease Scoring:*

Using the leaf clip inoculation approach in a net house, artificial inoculation of the virulent *Xoo* isolates was performed to demonstrate its pathogenicity (Figure 2). An advanced line plant that was 45 days old received a clip inoculation of the bacterial suspension. After 15 days of inoculation, symptoms began to manifest as water-soaked lesions that spread down the leaf blade in a wavelike manner from the margin and grew wider and longer (Table 4). The area around the fresh leaf had water-soaked lesions that quickly spread to cover significant portions of the leaf blade, resulting in white and then turning grayish in color. Re-isolated bacteria exhibited characteristics of the *Xanthomonas oryzae* pv. *oryzae* original culture. These findings are in line with the research of [29], who used a pathogenicity test to describe a large number of isolates of *Xanthomonas oryzae* pv. *oryzae*.

Table 4 Morphological Characteristics of the *Xanthomonas oryzae* pv. *oryzae* Isolates of Bangladesh

Isolate	Colony Size Range (mm)	Avg. Colony Size (mm)	Colony Color	Shape	Appearance
*Xoo*1	0.95	0.90	Light yellow	Circular	Raised, slimy
*Xoo*2	0.38	0.34	Creamy yellow	Circular	Raised, slimy
*Xoo*3	0.79	0.72	Light yellow	Circular	Raised, slimy
*Xoo*4	0.40	0.38	Creamy yellow	Circular	Raised, slimy
*Xoo*5	0.38	0.36	Creamy yellow	Circular	Raised, slimy
*Xoo*6	0.32	0.28	Light yellow	Circular	Raised, slimy
*Xoo*7	0.36	0.34	Creamy yellow	Circular	Raised, slimy
*Xoo*8	1.20	1.10	Light yellow	Circular	Raised, bold
*Xoo*9	0.44	0.40	Creamy yellow	Circular	Raised, slimy
*Xoo*10	0.82	0.70	Light yellow	Circular	Raised, slimy
*Xoo*11	0.29	0.26	Yellow	Circular	Raised, slimy
*Xoo*12	0.40	0.36	Creamy yellow	Circular	Raised, slimy
*Xoo*13	0.48	0.45	Creamy yellow	Circular	Raised, slimy
*Xoo*14	0.52	0.48	Creamy yellow	Circular	Raised, slimy
*Xoo*15	0.28	0.26	Light yellow	Circular	Raised, slimy
*Xoo*16	0.16	0.14	Yellow	Circular	Raised, slimy
*Xoo*17	0.29	0.26	Light yellow	Circular	Raised, slimy
*Xoo*18	0.79	0.70	Light yellow	Circular	Raised, slimy
*Xoo*19	0.35	0.30	Creamy yellow	Circular	Raised, slimy
*Xoo*20	0.15	0.12	Light yellow	Circular	Raised, slimy
*Xoo*21	0.23	0.20	Creamy yellow	Circular	Raised, slimy
*Xoo*22	0.38	0.35	Creamy yellow	Circular	Raised, slimy
*Xoo*23	0.10	0.08	Light yellow	Circular	Raised, slimy
*Xoo*24	0.36	0.30	Creamy yellow	Circular	Raised, slimy
*Xoo*25	0.95	0.80	Yellow	Circular	Raised, slimy
*Xoo*26	1.00	0.97	Yellow	Circular	Raised, slimy
*Xoo*27	0.27	0.22	Creamy yellow	Circular	Raised, slimy
*Xoo*28	0.56	0.48	Light yellow	Circular	Raised, slimy

ISSN No:-2456-2165

Isolate	Colony Size Range (mm)	Avg. Colony Size (mm)	Colony Color	Shape	Appearance
*Xoo*29	0.32	0.28	Light yellow	Circular	Raised, slimy

III. RESULTS

➢ *Morphological Characterization of Xoo:*

The morphological characteristics of *Xoo* isolates demonstrate that bacterial colonies on the nutrient agar medium were smooth-surfaced, circular, and yellow to creamy yellow in color (Table 4). The colony size range from 0.08 mm (*Xoo*23) to 1.1 mm (*Xoo*8) was observed. Only one isolate, *Xoo*8, exhibited raised and bold colonies, while maximum isolates produced raised and slimy colonies.

➢ *Biochemical Characterization:*

In this study, a total of six biochemical tests were conducted to characterize the pathogen isolated from BLB infected leaf samples collected from different AEZs of Bangladesh. In this study, gram staining, KOH (3%) test, starch hydrolysis test, gelatin liquefaction, catalase test, and oxidase test were performed (Table 5). All the isolates exhibited a red color rod shape under the observation of a light microscope which indicates all of them were gram negative (Figure 3). Moreover, results obtained for KOH (3%) test (Figure 4), gelatin liquefaction, and catalase (Figure 5) tests, were all positive whereas negative results were found in gram reaction, starch hydrolysis, and oxidase tests. On the other hand, *Xoo*3 shows positive in starch hydrolysis and oxidase test whereas negative in gram reaction, KOH (3%) test, gelatin liquefaction, and catalase test.

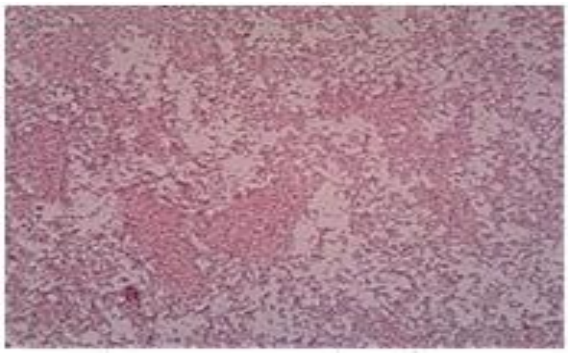

Fig 3 Gram Reaction of *Xoo*

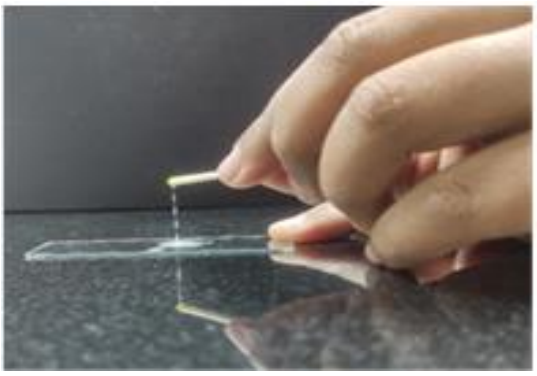

Fig 4 KOH (3%) Test

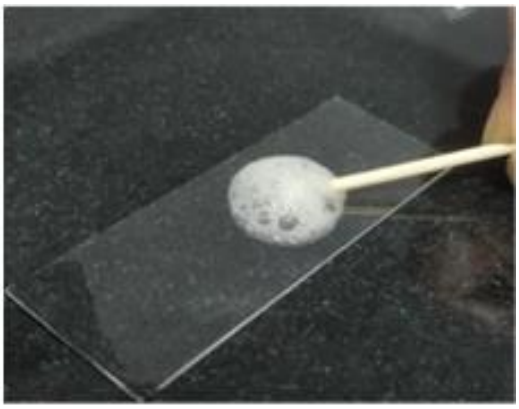

Fig 5 Catalase Test

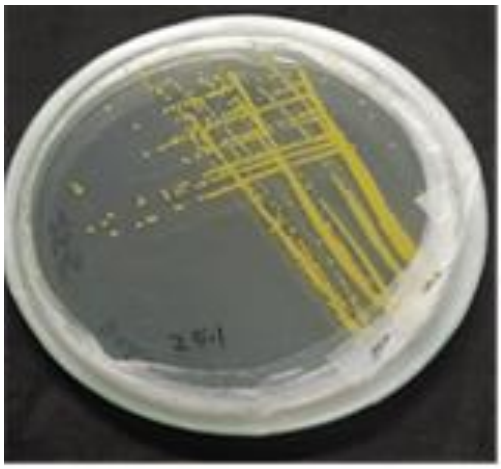

Fig 6 Colonies of *Xoo* Isolate

Table 5 Biochemical Characterization of Various *Xoo* Isolates Collected from different Rice Growing Zones of Bangladesh

Isolates	Gram Reaction	KOH Test (3%)	Starch Hydrolysis	Gelatin Liquefaction	Catalase Test	Oxidase Test
*Xoo*1	-	+	-	+	+	-
*Xoo*2	-	+	-	+	+	-
*Xoo*3	-	-	+	-	-	+
*Xoo*4	-	+	-	+	+	-
*Xoo*5	-	+	-	+	+	-
*Xoo*6	-	+	-	+	+	-
*Xoo*7	-	+	-	+	+	-

Isolates	Gram Reaction	KOH Test (3%)	Starch Hydrolysis	Gelatin Liquefaction	Catalase Test	Oxidase Test
*Xoo*8	-	+	-	+	+	-
*Xoo*9	-	+	-	+	+	-
*Xoo*10	-	+	-	+	+	-
*Xoo*11	-	+	-	+	+	-
*Xoo*12	-	+	-	+	+	-
*Xoo*13	-	+	-	+	+	-
*Xoo*14	-	+	-	+	+	-
*Xoo*15	-	+	-	+	+	-
*Xoo*16	-	+	-	+	+	-
*Xoo*17	-	+	-	+	+	-
*Xoo*18	-	+	-	+	+	-
*Xoo*19	-	+	-	+	+	-
*Xoo*20	-	+	-	+	+	-
*Xoo*21	-	+	-	+	+	-
*Xoo*22	-	+	-	+	+	-
*Xoo*23	-	+	-	+	+	-
*Xoo*24	-	+	-	+	+	-
*Xoo*25	-	+	-	+	+	-
*Xoo*26	-	+	-	+	+	-
*Xoo*27	-	+	-	+	+	-
*Xoo*28	-	+	-	+	+	-
*Xoo*29	-	+	-	+	+	-

➢ *Molecular Characterization:*

PSA medium which contains 0.001% Cu $(NO_3)_2$ was used to grow the isolated *Xoo* samples. This PSA medium is specific for growing *Xanthomonas oryzae* pv. *oryzae*, stated [30]. *Xoo* bacteria have oxidizing properties [21], gram negative [31], with single flagellum polar and pathogenic [21]. After the PCR procedure, not all yellow colonies were suspected as *Xanthomonas oryzae* pv. *oryzae* when employing a particular primer pair (Table 2). Figure 6 shows that all isolates with DNA bands plotting at 337 base pairs (bp) are *Xanthomonas oryzae* pv. *oryzae*. The 29 bacterial isolates examined by PCR resulted in 15 *Xoo* bacteria and 14 non-bacterial *Xoo* isolates (Table 6). The colony that generates a 337 bp band with the *Xoo*2976 primer is *Xoo* bacterium, according to [26].

Fig 7 *Xoo* Amplification Results using *Xoo*2976 Primers

M=1kb DNA ladder, 1= *Xoo*1, 2= *Xoo*2, 3= *Xoo*3, 4= *Xoo*4, 5= *Xoo*5, 6= *Xoo*6, 7= *Xoo*7, 8= *Xoo*8, 9= *Xoo*9, 10= *Xoo*10, 11= *Xoo*11, 12= *Xoo*12, 13= *Xoo*13, 14= *Xoo*14, 15= *Xoo*15, 16= *Xoo*16, 17= *Xoo*17, 18= *Xoo*18, 19= *Xoo*19, 20= *Xoo*20, 21= *Xoo*21, 22= *Xoo*22, 23= *Xoo*23, 24= *Xoo*24, 25= *Xoo*25, 26= *Xoo*26, 27= *Xoo*27, 28= *Xoo*28, 29= *Xoo*29

Table 6 *Xoo* Identification Results using Primer *Xoo*2976

S/L	Isolate	Amplification result	S/L	Isolate	Amplification result	S/L	Isolate	Amplification result
1	Xoo1	Yes	11	Xoo11	No	21	Xoo21	Yes
2	Xoo2	No	12	Xoo12	No	22	Xoo22	Yes
3	Xoo3	No	13	Xoo13	No	23	Xoo23	No
4	Xoo4	No	14	Xoo14	No	24	Xoo24	Yes
5	Xoo5	Yes	15	Xoo15	Yes	25	Xoo25	Yes
6	Xoo6	No	16	Xoo16	Yes	26	Xoo26	No
7	Xoo7	Yes	17	Xoo17	Yes	27	Xoo27	No
8	Xoo8	No	18	Xoo18	Yes	28	Xoo28	No

9	Xoo9	Yes	19	Xoo19	Yes	29	Xoo29	Yes
10	Xoo10	No	20	Xoo20	Yes	-	-	-

➢ *Pathogenicity Test:*

According to lesion length, the maximum lesion length in BRRI dhan49, *Xoo*17 was 12.3 cm, compared to an average of 9.88 cm. Following that, *Xoo*7 and *Xoo*25 each displayed an average lesion length of 4.33 and 4.18 cm, respectively. A maximum lesion length of 16 was seen on IRRI 154, *Xoo*17, compared to an average of 10.33 cm. Furthermore, *Xoo*1 displayed an average length of 6 cm whereas *Xoo*7 displayed 5.5 cm. Moreover, all isolates from the resistant variety IRBB66 displayed at least some damage. Here, *Xoo*17 exhibits a brief lesion with an average length of 2.83 cm.

IV. DISCUSSION

The causal organism was isolated from the infected leaf, presenting the common symptoms of BLB disease. Isolated samples were cultured using Peptone Sucrose Agar (PSA) media. After 48 hours of incubation at 27 ±1 °C, isolation from infected leaves produced typical yellow and mucoid colonies of the bacterium on media (Figure 7). The colonies were purified by streaking the isolated colony on nutrient agar media. Pure colonies were streaked onto nutrient agar medium and incubated for 48 hours at 27 ±1°C. Cultures were stored in the refrigerator at 4°C, which served as a stock culture for future investigation.

On the nutrient agar medium, however, the bacterium colonies appeared circular, yellow to creamy yellow in color, and with a smooth surface (Table 4). In terms of colony sizes, the maximum size of the colony was observed at 1.1 mm in the *Xoo*8 isolate, whereas the minimum colony size of 0.08 mm was observed in isolate *Xoo*23. On the other hand, most isolates were circular in shape with raised and slimy colonies, except isolate *Xoo*8 produced raised and bold colonies. The present results are supported by [32], where *Xoo* colonies were slightly convex, and smooth, with regular to irregular margins and colonies of the bacterium on a nutrient agar medium appeared yellow to creamy yellow in color with a smooth surface and were opaque against transmitted light after 48 hours.

A total of 6 biochemical tests were conducted to characterize the pathogen isolated from BLB-infected samples collected from different AEZ in Bangladesh. The tests included gram staining, KOH (3%) test, starch hydrolysis test, gelatin liquefaction test, catalase test, and oxidase test (Table 5). All isolates exhibited gram negative reactions with a red color and rod shape when examined through a light microscope (Figure 3). Positive results were obtained for the KOH (3%) test (Figure 4), gelatin liquefaction, and catalase tests (Figure 5), but negative for starch hydrolysis and oxidase tests except for *Xoo*3, similar result has been reported by [33]. All isolates tested negative for oxidase and gram reaction [34], but [29]found only gram negative reaction in their isolates. [35] reported similar results of biochemical studies indicating a positive reaction for gelatin liquefaction, while the few isolates showed a negative reaction for starch hydrolysis. [36] reported the bacterium was gram-negative, rod-shaped, and produced a red color after counterstaining with safranin. In the present study, based on such biochemical tests, it was detected as *Xanthomonas oryzae* pv. *oryzae* isolates.

However, for virulence study, PCR technology is rapidly becoming an important tool in molecular biology because of its capability to rapidly screen a huge number of samples with a lower amount of DNA. Nowadays, primers were developed after a thorough sequencing analysis of numerous bacterial isolates from *Xoo* [37]; [38]. Primers are constructed using the *Xoo* bacteria conservation sequence's conservative site [39]; [40]. The stability of the amplified results from the *Xoo*-specific primers suggests that a specific primer may be utilized to identify *Xoo* bacteria in a variety of environments. Previous research employing a variety of primers has shown that each *Xoo*-specific primer consistently identifies *Xoo* bacteria [41]. It is also suggested that only one specific primer would be enough for the identification of *Xoo* bacteria [42]. In this study, after morphological and biochemical analysis of the 29 *Xoo* isolates, 15 virulent *Xoo* isolates were identified by using *Xoo* specific primer (Table 6), *Xoo*2976 according to [26]; [42].

Moreover, artificial inoculation of the identified 15 virulent *Xoo* isolates was carried out to prove the pathogenicity using the leaf clip inoculation technique under net house conditions (Figure 2). Inoculation was carried out on 45 days old plants of three rice varieties: BRRI Dhan49, IRRI 154, and IRBB66. BLB symptoms started to appear as water-soaked lesions that spread in a wavy pattern from the leaf blade's margin and increased in both width and length after 15 days of inoculation. The region adjoining the healthy part showed water-soaked blighted lesions extended rapidly to cover large areas of the leaf blade, which turned white to greyish. These findings are in line with research by [29] in which they used a pathogenicity test to describe several isolates of *Xanthomonas oryzae* pv. *oryzae.*

In this investigation, 15 virulent isolates were used for the pathogenicity test after the validation of the PCR assay. We chose three types of rice for the pathogenicity test: The IRRI 154 variety was a very common or standard variety all throughout the world, while BRRI dhan49 was a popular variety in Bangladesh during the wet season but highly BLB vulnerable. Last but not least, IRRI introduced IRBB66, a type that is BLB resistant. According to lesion length, the maximum lesion length in BRRI dhan49, *Xoo*17 was 12.3 cm, compared to an average of 9.88 cm. Following that, *Xoo*7 and *Xoo*25 each displayed an average lesion length of 4.33 and 4.18 cm, respectively. A maximum lesion length of 16 was seen on IRRI 154 by *Xoo*17, compared to an average of 10.33 cm. Furthermore, *Xoo*1 displayed an average length of 6 cm whereas *Xoo*7 displayed 5.5 cm. Moreover, all isolates from the resistant

variety IRBB66 displayed at least some damage. Here, *Xoo*17 exhibits a brief lesion with an average length of 2.83 cm. Based on the differential reactivity of the isolates, it was determined in the current research indicated the most virulent *Xanthomonas oryzae* pv. *oryzae* isolates were *Xoo*7, *Xoo*17, and *Xoo*25.

V. CONCLUSION

Identification of *Xanthomonas oryzae* pv. *oryzae* using the *Xoo*2976 molecular marker as a specific primer is very operational. This primer produces 337 bp band sizes, which indicates 15 isolates were *Xoo* bacteria and 14 were non-*Xoo* bacteria among the 29 isolates. The finding from molecular characterization also indicates that the other 14 non-*Xoo* bacteria isolates would be another *Xanthomonas* spp. The *Xoo*2976 molecular marker may be used to identify *Xoo* bacteria specifically and biochemical and morphological studies would be also essential for screening BLB resistant rice cultivars. Eventually, this study also shows that different *Xoo* bacteria exist within Bangladesh, and this is evidence of the presence of *Xanthomonas oryzae* pv. *oryzae* within the country along with other *Xanthomonas* spp.

REFERENCES

[1]. J.A. Khan, S. Afroz, H.M.I. Arshad, N. Sarwar, H.S. Anwar, K. Saleem, M.M. Babar, F.F.J.A.l.s. Jamil, Biochemical basis of resistance in rice against Bacterial leaf blight disease caused by Xanthomonas oryzae pv. oryzae, 1(3) (2014) 181-190.

[2]. B. Sharma, M.J.B.J.o.A.R. Pandey, Identification of rice germplasm with resistance to bacterial leaf blight (Xanthomonas oryzae pv. oryzae), 37(2) (2012) 349-353.

[3]. M.A.B. Siddique, M.A.R. Sarkar, M.C. Rahman, A. Chowdhury, M.S. Rahman, L.J.A.J.o.A. Deb, R. Development, Rice farmers' technical efficiency under abiotic stresses in Bangladesh, 7(11) (2017) 219-232.

[4]. S. Saha, A. Sharmin, R. Biswas, M.J.I.J.o.E. Ashaduzzaman, Agriculture, Biotechnology, Farmers' perception and adoption of agroforestry practices in Faridpur district of Bangladesh, 3(6) (2018) 268280.

[5]. M.M.J.P.A. Rahman, Policy, Achieving Sustainable Development Goals of Agenda 2030 in Bangladesh: the crossroad of the governance and performance, 24(2) (2021) 195-211.

[6]. M.S. Rahaman, S. Haque, M.A.R. Sarkar, M.C. Rahman, M.S. Reza, M.A. Islam, M.A.B.J.F. Siddique, A. Agriculture, A cost efficiency analysis of boro rice production in Dinajpur district of Bangladesh, 6(1) (2021) 67-77.

[7]. I.J. Shelley, M. Takahashi-Nosaka, M. Kano-Nakata, M.S. Haque, Y.J.J.o.I.C.f.A.D. Inukai, Rice cultivation in Bangladesh: present scenario, problems, and prospects, 14 (2016) 20-29.

[8]. M. Kabir, M. Salam, A. Chowdhury, N. Rahman, K. Iftekharuddaula, M. Rahman, M. Rashid, S. Dipti, A. Islam, M. Latif, Rice vision for Bangladesh: 2050 and beyond, Bangladesh Rice Journal 19(2) (2015) 1-18.

[9]. N. Jonit, Y. Low, G. Tan, Xanthomonas oryzae pv. oryzae, biochemical tests, rice (Oryza sativa), Bacterial Leaf Blight (BLB) disease, Sekinchan, Appl. Environ. Microbiol 4 (2016) 63-69.

[10]. C. Mondal, M.R. Islam, K.G. Dastogeer, M. Atiqur, R. Khokon, M. Wazuddin, M.A.I. Khan, Screening of parental lines of three-line rice hybrid against Xanthomonas oryzae pv. Oryzae, Journal of Agricultural Technology 10(2) (2014) 407-421.

[11]. M. Khatun, B. Nessa, M. Salam, M. Kabir, Strategy for rice disease management in Bangladesh, Bangladesh Rice Journal 25(1) (2021) 23-36.

[12]. T.B. Adhikari, C. Cruz, Q. Zhang, R. Nelson, D. Skinner, T. Mew, J. Leach, Genetic diversity of Xanthomonas oryzae pv. oryzae in Asia, Applied and Environmental Microbiology 61(3) (1995) 966-971.

[13]. Y. Sere, A. Onasanya, V. Verdier, K. Akator, L. Ouedraogo, Z. Segda, M. Mbare, A. Sido, A. Basso, Rice bacterial leaf blight in West Africa: preliminary studies on disease in farmers' fields and screening released varieties for resistance to the bacteria, Asian Journal of Plant Sciences 4(6) (2005) 577-579.

[14]. G.-H. Jiang, Z.-H. Xia, Y.-L. Zhou, J. Wan, D.-Y. Li, R.-S. Chen, W.-X. Zhai, L.-H. Zhu, Testifying the rice bacterial blight resistance gene xa5 by genetic complementation and further analyzing xa5 (Xa5) in comparison with its homolog TFIIAγ1, Molecular genetics and Genomics 275 (2006) 354-366.

[15]. A. Dath, S. Dath, Role of inoculum in irrigation water and soil in the incidence of bacterial blight of rice, Indian Phytopathology (India) (1983).

[16]. G. Laha, R. Singh, D. Ladhalakshmi, S. Sunder, M.S. Prasad, C. Dagar, V.R. Babu, Importance and management of rice diseases: a global perspective, Rice production worldwide (2017) 303-360.

[17]. M.A. Khan, M. Naeem, M. Iqbal, Breeding approaches for bacterial leaf blight resistance in rice (Oryza sativa L.), current status and future directions, European Journal of Plant Pathology 139 (2014) 27-37.

[18]. J.F. Murphy, G.W. Zehnder, D.J. Schuster, E.J. Sikora, J.E. Polston, J.W. Kloepper, Plant growth-promoting rhizobacterial mediated protection in tomato against Tomato mottle virus, Plant Disease 84(7) (2000) 779-784.

[19]. A. Ontiveros-Cisneros, O. Moss, A. Van Moerkercke, O. Van Aken, Evaluation of antibiotic-based selection methods for Camelina sativa stable transformants, Cells 11(7) (2022) 1068.

[20]. J. Bradbury, Isolation and preliminary study of bacteria from plants, PANS Pest Articles & News Summaries 16(4) (1970) 632-637.

[21]. N.W. Schaad, J.B. Jones, W. Chun, Laboratory guide for the identification of plant pathogenic bacteria, American Phytopathological society (APS press)2001.
[22]. P. Gerhardt, R. Murray, R. Costilow, E.W. Nester, W.A. Wood, N.R. Krieg, G.B. Phillips, Manual of methods for general bacteriology, (1981).
[23]. T. Suslow, M. Schroth, M. Isaka, Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining, Phytopathology (USA) (1982).
[24]. E. Ryu, A simple method of differentiation between Gram-positive and Gram-negative organisms without staining, Kitasato Archives of Experimental Medicine 17 (1940) 58-63.
[25]. A.F. Sandhu, J.A. Khan, S. Ali, H.I. Arshad, K. Saleem, Molecular characterization of Xanthomonas oryzae pv. oryzae isolates and its resistance sources in rice germplasm, PSM Microbiology 3(2) (2018) 55-61.
[26]. J.M. Lang, J.P. Hamilton, M.G.Q. Diaz, M.A. Van Sluys, M.R.G. Burgos, C.M. Vera Cruz, C.R. Buell, N.A. Tisserat, J.E. Leach, Genomics-based diagnostic marker development for Xanthomonas oryzae pv. oryzae and X. oryzae pv. oryzicola, Plant Disease 94(3) (2010) 311-319.
[27]. M.M. Rashid, S.A.I. Nihad, M.A.I. Khan, A. Haque, A. Ara, T. Ferdous, M.A.I. Hasan, M.A. Latif, Pathotype profiling, distribution and virulence analysis of Xanthomonas oryzae pv. oryzae causing bacterial blight disease of rice in Bangladesh, Journal of Phytopathology 169(7-8) (2021) 438-446.
[28]. H. Kauffman, An improved technique for evaluating resistance to rice varieties of Xanthomonas oryzae pv. oryzae, Plant Dis. Rep. 57 (1973) 537-541.
[29]. E. Ghasemie, M. Kazempour, F. Padasht, Isolation and identification of Xathomonas oryzae pv. oryzae the causal agent of bacterial blight of rice in Iran, Journal of Plant Protection Research (2008).
[30]. D.O. NIÑO-LIU, P.C. Ronald, A.J. Bogdanove, Xanthomonas oryzae pathovars: model pathogens of a model crop, Molecular plant pathology 7(5) (2006) 303-324.
[31]. P.C. Fahy, G.J. Persley, Plant bacterial diseases: a diagnostic guide, (1983).
[32]. J. Han, L. Sun, X. Dong, Z. Cai, X. Sun, H. Yang, Y. Wang, W. Song, 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, Systematic and applied microbiology 28(1) (2005) 66-76.
[33]. A. Sreeramulu, M. Nayudu, Biochemical characters and pathogenic variation of different isolates of paddy bacterial leaf blight pathogen, Oryza 24 (1987) 363-367.
[34]. N. Muneer, A. Rafi, M.A. Akhtar, Isolation and characterization of Xanthomonas oryzae pv. oryzae isolates from North West Frontier Province (NWFP), Pakistan, Sarhad Journal of Agriculture 23(3) (2007) 743.
[35]. P. Thimmegowda, A.R. Sataraddi, M. Patil, L. Geeta, V. Prabhu, Biochemical and nutritional studies of Xanthomonas oryzae pv. oryzae, Journal of Plant Disease Sciences 3(1) (2008) 9-12.
[36]. R. Jabeen, T. Iftikhar, H. Batool, Isolation, characterization, preservation and pathogenicity test of Xanthomonas oryzae pv. oryzae causing BLB disease in rice, Pak. J. Bot 44(1) (2012) 261-265.
[37]. B.-M. Lee, Y.-J. Park, D.-S. Park, H.-W. Kang, J.-G. Kim, E.-S. Song, I.-C. Park, U.-H. Yoon, J.-H. Hahn, B.-S. Koo, The genome sequence of Xanthomonas oryzae pathovar oryzae KACC10331, the bacterial blight pathogen of rice, Nucleic acids research 33(2) (2005) 577-586.
[38]. H. Ochiai, Y. Inoue, M. Takeya, A. Sasaki, H. Kaku, Genome sequence of Xanthomonas oryzae pv. oryzae suggests contribution of large numbers of effector genes and insertion sequences to its race diversity, Japan Agricultural Research Quarterly: JARQ 39(4) (2005) 275-287.
[39]. A. Onasanya, A. Basso, E. Somado, E. Gasore, F. Nwilene, I. Ingelbrecht, J. Lamo, K. Wydra, M. Ekperigin, M. Langa, Development of a combined molecular diagnostic and DNA fingerprinting technique for rice bacteria pathogens in Africa, Biotechnology 9(2) (2010) 89-105.
[40]. N. Furuya, S. Taura, T. Goto, B.T. Thuy, P.H. Ton, K. Tsuchiya, A. Yoshimura, Diversity in virulence of Xanthomonas oryzae pv. oryzae from Northern Vietnam, Japan Agricultural Research Quarterly: JARQ 46(4) (2012) 329-338.
[41]. T. Tasliah, M. Mahrup, J. Prasetiyono, Identifikasi molekuler hawar daun bakteri (Xanthomonas oryzae pv. oryzae) dan uji patogenisitasnya pada galur-galur padi isogenik, (2013).
[42]. Z. Noer, D. Suryanto, Molecular identification xanthomonas oryzae pv. Oryzae causes of bacterial blight for rice with a specific primers, (2018).