Journal of Animal & Plant Sciences, 34(4): 2024, Page: 927-935
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2024.4.0777

In planta ANALYSIS OF SALICYLIC ACID MEDIATED MANAGEMENT OF BACTERIAL LEAF BLIGHT OF RICE

A. Sudha[1,*], M. F. Firdouse[1], A. S. Amala[1], S. Arunprakash[1], I. Johnson[1], R. Anandham[2] and N. Saranya[3]

[1]Department of Plant Pathology, TNAU, Coimbatore, Tamil Nadu, India
[2]Department of Agricultural Microbiology, TNAU, Coimbatore, Tamil Nadu, India
[3]Department of Plant Molecular Biology and Bioinformatics, TNAU, Coimbatore, Tamil Nadu, India
*Corresponding author's email - sudha.a@tnau.ac.in

ABSTRACT

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a catastrophic bacterial disease of rice, and researchers are facing difficulties in developing the management practices, whereas the continued use of bactericides *viz*, streptomycin, copper oxychloride leads to the development of resistant *Xoo* strains. As a consequence, the development of an environmentally safe bactericide for the management of this devastating disease is needed. The present study focused on identification of effective synthetic activators against bacterial leaf blight in rice during their interaction. Survey was conducted to collect different isolates of *Xoo* and their morphological characters and biochemical properties were determined. All the 10 isolates exhibited circular to flattened slimy yellow colour colonies of the pathogen and showed positive reaction for citrate utilization, lysine utilization and ornithine utilization test. All the ten isolates showed negative reaction for sorbitol utilization. The collected isolates were subjected to PCR for the amplification of 16S-23S intergenic region with the species specific primer and obtained amplicon at ~470 bp for the confirmation at molecular level. The virulent isolate was identified by measuring biofilm formation and xanthomonadin pigment which revealed that the isolate ADT 53 strain T3 with the accession number OR121377 recorded maximum OD value of 0.356 for biofilm formation at 570 nm and 0.686 for xanthomonadin pigment at 445 nm. *In planta* studies revealed that salicylic acid at 100 ppm has the maximum inhibitory effect against bacterial leaf blight disease with the minimal lesion size of 0.7 cm compared to negative control (water).

Keywords: Activators, BLB, Biofilm, Characterization, *in planta,* Xanthomonadin.

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Published first online June 04, 2024 Published final August 25, 2024

INTRODUCTION

Rice (*Oryza sativa* L.) is considered as one of the staple-food crops for about half of the world's population. The global production of rice was 512.8 million metric tonnes on a milled basis during 2022-23(Fao.org 2021). Numerous biotic and abiotic components are the key constraints for reduction of the production and productivity of rice. Among the biotic factors, diseases (26%), insects (20%), and weeds (23% each) are the major limitations. The utmost crucial bacterial disease in rice production system is bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The BLB is widely present in Southeast Asia and Japan, causing average crop loss of 20–50% and up to 100% in severe stages (Mizukami and Wakimoto, 2003). Farmers in Japan's Fukuoka region first reported this disease in 1884-1885. The disease was epidemic in Bihar (Shahabad district) in 1963, and it became a devastative disease of rice in Punjab (Srivastava, 1967). In India, during 1951, a very important rice disease was found on a widespread basis in different areas of Maharashtra state, which was first reported by Mizukami and Wakimoto (2003). An important epidemic of this disease was happened during 1979-1980 in the significant rice growing states of India, *viz.,* Andhra Pradesh, Punjab, Haryana, and western Uttar Pradesh. According to Mew (1987), the increased production loss due to the disease depends on locality, season, weather, cultivar and the application of high amounts of nitrogen fertiliser that tend to cause 60% of yield loss.

Strategies for managing BLB include cultivating disease-resistant varieties, applying chemicals and use of bio-control agents. However, these components may have some influence on human being, environment and leads to development of resistant strains of the pathogen. Therefore, it is most important to carry out research on effective eco-friendly and healthy agrochemicals to control the BLB. Plant defense activators are chemicals that provokes the defense related genes by providing signals in plants through signal transduction pathway which is mediated through salicylic acid (Sreeja, 2014).

These agents modifies plant–pathogen interaction by various mechanism related to defense, prior to or after challenge and the plant must be protected by the agent against a broad range of pathogens. Plant defense activators render plants resistant to a wide variety of pathogens by activating systemic acquired resistance (SAR) (Kim *et al*., 2016). Although several biological, chemical, and genetic control methods have improvements in rice protection from bacterial leaf blight, there was still a requirement for an effective approach that would facilitate large-scale protection. Therefore, this study was conducted to screen out the known chemicals (synthetic activators) for their effects on rice leaf blight.

MATERIALS AND METHODS

Collection of *Xoo* infected samples: Roving survey got done for collection of paddy leaves exhibiting characteristic symptoms of blight disease at the tillering-stage as described by Raveloson *et al*. (2023) from Bhavanisagar, Aduthurai, Ambasamuthiram, Thirupathisaram, Nagapattinam, Vaigai dam, Wetland and Paddy Breeding Station (PBS) of Tamil Nadu Agricultural University (TNAU), Coimbatore during January to March of 2023. The labelled samples were packed and refrigerated at 4°C for further analysis. Then, following experiments were carried out in Bacteriological laboratory of Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India

Isolation of the pathogen and selection of suitable medium: The leaves showing the bacterial blight symptom were cleansed with sterile-distilled water; air-dried and chopped into small leaf-bits then soaked in sterile distilled water in eppendorf tube (1.5 ml); kept undisturbed to exudate the bacterial cells into water. After 30 minutes, the ooze observed is taken in a loop: streaked in a plate with nutrient agar (NA) medium and incubated at 28±1°C for 36-48 hrs. For the selection of suitable media for the growth of the pathogen, the isolated bacterial-culture was inoculated in three distinct broths namely nutrient broth, peptone sucrose broth (Ghasemie *et al*., 2008), and luria-bertani broth (Yasmin *et al*., 2017) that have been shown to support the growth of Xanthomonads, incubated for 24 hours in a shaker at 28°C. At regular time interval, the spectrophotometer reading (OD value) was taken to detect the rate of bacterial growth at OD 595 nm. One ml of *Xoo* inoculated broth were added to 60% glycerol for the maintenance of glycerol stock at -80°C (Van Hulten *et al*., 2019) and single colonies were transferred to agar slants and stored at -4°C for further use.

Assessment of pathogenicity: The rice variety TN 1 (Taichung Native 1) was obtained from PBS, TNAU, Coimbatore. The pathogenicity of the isolates was assessed using the clip inoculation technique in three fully expanded leaves at 35-40 days after sowing and disease incidence was observed after 14-16 days after inoculation (Ghasemie *et al*., 2008).

Characterization of the pathogen

Morphological characterization: The different isolates of the incitant was characterized morphologically through visual interpretation based on colour, average diameter of the colonies, shape and appearance of the colonies.

Biochemical characterization: The Biochemical test kit (KB002) purchased from Hi Media, India was utilized to do biochemical characterization of the pathogenic-isolates as described by Chowdappa *et al*. (2018). It was carried out as per the protocol described by the manufacturer. Each kit is a standardised colorimetric identification system that comprises of citrate-utilization, lysine-utilization, ornithine-utilization, urease, phenylalanine deamination, nitrate-reduction, H_2S production, glucose, adonitol, lactose, arabinose and sorbitol tests. Each test was obtained based on pH change due to utilization of the substrate by the pathogen after inoculation. After incubation, the colour change can be visually interpreted.

Molecular characterizationof the pathogen: The isolates were inoculated in the LB broth and incubated in shaker for 48hrs at 25°C. The DNA extraction was carried out in accordance with the guidelines set forth by Neumann *et al*. (1992). The primers *Xoo*F 5' CATCGTTAGGACTGCCAGAAG 3', *Xoo*R 5' GTGAGAACCACCGCCATCT 3' were used for amplification of 16S-23S intergenic region by polymerase chain reaction as elucidated by Lang *et al*. (2010) with some refinements. With the help of thermo cycler (Veritipro 96 well thermo cycler, Thermo fisher scientific), PCR amplification was carried out. According to the following cycles the PCR program was edited as: initial denaturation of 95°C for 75 minutes, denaturation of 94°C for 1 minute, annealing time of 55°C for 1 minute, extension time of 72°C for 10 minutes of 35 cycles, final extension time of 72°C for 15 minutes and finally incubated at 4 °C.

To check the amplified products, agarose gel electrophoresis was performed with 0.8% agarose at 75 V for 45 min as per the protocol given by Lee *et al*. (2012). Then the amplified products were sent for Sanger sequencing to Bio kart India Ltd. To assess the gene-sequence homology NCBI-BLAST server was used (Joshi *et al* 2023). The gene sequence was submitted to GenBank, and accession number was obtained.

Investigation of the pathogen's pathogenicity

Biofilm Assay: The formation of Biofilm by the pathogen isolates was quantified by following the protocol of Sahu *et al*., 2018. The bacterial isolates were inoculated in LB broth and incubated at 35-37℃ for 24 hrs in shaker to attain the mid-exponential growth phase. It is then diluted in the ratio of 1:100 using a fresh broth. About 4 ml of suspension after dilution was poured in another glass tube and incubated at 28℃ for 72 hrs without any shaking. After incubation, the adhering bacterial cells at the bottom of the tube were gently washed thrice after the broth was emptied out. The cells were then stained with 0.1% crystal violet (4 ml) for 15 mins and unbound crystal violet was removed, followed by three water washes. The cells were finally solubilised in 4 ml of DMSO (Dimethyl Sulfoxide). The amount of biofilm that would be formed was determined by measuring the absorbance (OD value) at 570 nm using spectrophotometer. The entire experiment was done in triplicates.

Quantification of Xanthomonadin: The Xanthomonadin pigment produced by the pathogen was measured in accordance with the procedure outlined by Sahu *et al*., 2018. Eight ml broth of different *Xoo* isolates was centrifuged at 8000 g for 10 mins to collect *Xoo* cells. Cells were then mixed with 2 ml of 100% methanol and kept in darkness for 10 mins on rotary shaker. After incubation, centrifugation was done at 12000 g for 8 minutes to collect the supernatant. The absorbance at 445 nm was measured in spectrophotometer to quantify the amount of xanthomonadin pigment.

In planta* study of the synthetic activators against *Xoo

Activators and solvents: The activators are prepared at different concentration of 50, 100 and 250 ppm listed in **Table 1**. Chloramphenicol at 50,100 and 250 ppm and distilled water was utilized as a positive and negative control.

***In planta* Assay:** *In vitro* protection and curative activity of synthetic activators against rice BLB, was assessed by as mentioned by Wang *et al*., 2019. The paddy seeds of TN 1 variety was soaked in water for 24 hrs prior to sowing and the seedlings were raised in pots under glasshouse condition. Growing temperature ranged from 27-30℃ with the photoperiod of 12 hrs and relative humidity of 85% was maintained. After 21 days, the seedlings were transferred to pots with 15 cm diameter containing 1 kg clay soil from wetland of Tamil Nadu Agricultural University, Coimbatore. After 14 days of transplanting, the test compounds were uniformly sprayed on the leaves at the titre of 50, 100 and 250 ppm. Three replications were maintained for each activator. After a day of spray, the suspension of pathogen at 1×10^{-8} CFU/ml was inoculated in the clipped leaf tip (4-5 cm) using scissor dipped in pathogenic bacterial suspension. Again test compounds were sprayed after 24 hrs of pathogen inoculation.The disease index of the inoculated rice leaves were assessed after 14 days of inoculation.

Statistical analysis: The experiments were carried out in a completely randomized design with three replications for each activator studied (Satheesan *et al*., 2012). A statistical analysis was conducted to determine the activators on pathogen growth (Joshi *et al*., 2023). One-way ANOVA was performed to discover significant differences between each treatments using SAS 9.4 Software (Statistical Analysis System). DMRT test was used to compare the mean values of triplicates at $P\leq.0.05$.

RESULTS

Isolation and characterization of *Xoo:* From the rice BLB infected leaf samples collected during survey; pathogen was isolated and named as given in Table 2. Among the different broth tested, luria-bertani (LB) broth was found to support the growth of the incitant with highest OD value of 2.492 after 30 hrs of inoculation in contrast with peptone sucrose broth (PSB) and nutrient broth (NB) which had the OD value of 0.458 and 1.231 (Fig. 1a). The outcome of the study indicates that the cultures of *Xoo* were quite stable over a period of time in LB medium.

The isolates collected from various regions slightly differ in their morphological character such as average diameter, color, shape and appearance of the colony that is interpreted visually are listed in Table 3. Most of the isolates are in bright yellow with circular, raised and have slimy appearance (Fig. 1b).

Variations in biochemical behavior were determined using the biochemical test kit (KB002) purchased from Himedia. In this study, all the isolates showed the positive reaction for the entire biochemical test. All the ten isolates showed negative reaction for sorbitol utilization. The isolate T3 collected from the variety ADT 54 showed positive reaction for all the tests except H_2S production and sorbitol utilization. No colour change was observed in control (un-inoculated broth) (Table 4).

These isolates were further confirmed by molecular characterization by amplifying 16S-23S rDNA intergenic region through PCR analysis. All the isolates were successfully amplified at 470 bp (Fig. 1c). Then, accession number for the genomic sequence of some pathogen isolates was obtained v*iz*., OR050503, OR121377, OR121386, OR231284, OR121387, OR231927, OR231928.

Selection of virulent isolate based on Biofilm formation and Xanthomonadin pigment production: To select the virulent isolate of the pathogen biofilm and xanthomonadin was measured. The results of this

analysis revealed that (Fig. 2a) the isolate T3 collected from Tanjore district from ADT 54 showed the maximum OD value of 0.356 for biofilm formation and 0.686 for Xanthomadin pigment followed by the isolate CO55 strain B3 collected from Bhavanisagar with the OD value of 0.353 for biofilm and 0.513 for xanthomonadin. Hence the isolate ADT 54 strain T3 with the accession number OR121377 was chosen as a virulent isolate to carry out *in planta* studies.

***In planta* screening of activators:** *In planta* assay of activators against BLB disease exhibited that the plants sprayed with salicylic acid (100 ppm) has the minimal lesion growth of 0.7cm whereas 5.2 cm in the inoculated control and the per cent disease inhibition was 87% for salicylic acid when compared to positive control (93%) chloramphenicol (Fig. 2b). On comparing the results of *in planta* assay, salicylic acid at the titre of 100 ppm was discovered to be optimal for suppressing the BLB disease in rice.. Among the different activators salicylic acid showed the maximum inhibition under *in planta* screening. The efficacy of SA is depends on the deliberation and it determines the efficacy of the treatment in controlling the disease. The studies representing that 100 ppm are an ideal for their inhibition activity. Low concentrations of salicylic acid i.e., not more than 100 ppm stand observed to be unproductive in contradiction of rice BLB.

Table 1: List of compounds, solvents and concentrations used in the experiment are listed below.

Compounds	Solvents	Concentration (ppm)	Reference
Salicylic acid	DMSO	50, 100, 250	Liang *et al*., 2022
Cinnamic acid	DMSO	50, 100, 250	Wang *et al*., 2019
Barbituric acid	Water	50, 100, 250	Sreeja, 2014
Thiamine HCl	Water	50, 100, 250	Ahn *et al*., 2005
Nicotinic acid	Water	50, 100, 250	Sreeja, 2014
Chloramphenicol	Water	50, 100, 250	

Table 2: Collection of *Xanthomonas oryzae* pv. *oryzae* at different paddy varieties and their location.

S. No.	Variety	Isolate	Location
1.	CO 52	PC1	Paddy breeding station (PBS), TNAU, Coimbatore
2.	TN 1	PC2	Paddy breeding station (PBS), TNAU, Coimbatore
3.	ADT 38	T1	Aduthurai, Thanjavur
4.	CO 38	T2	Aduthurai, Thanjavur
5.	ADT 54	T3	Aduthurai, Thanjavur
6.	CO 55	B1	Bhavanisagar, Erode
7.	ADT 45	B2	Bhavanisagar, Erode
8.	CO 51	B3	Bhavanisagar, Erode
9.	ASD 16	CW1	Wetland, TNAU, Coimbatore
10.	IMF 9	CW2	Wetland, TNAU, Coimbatore

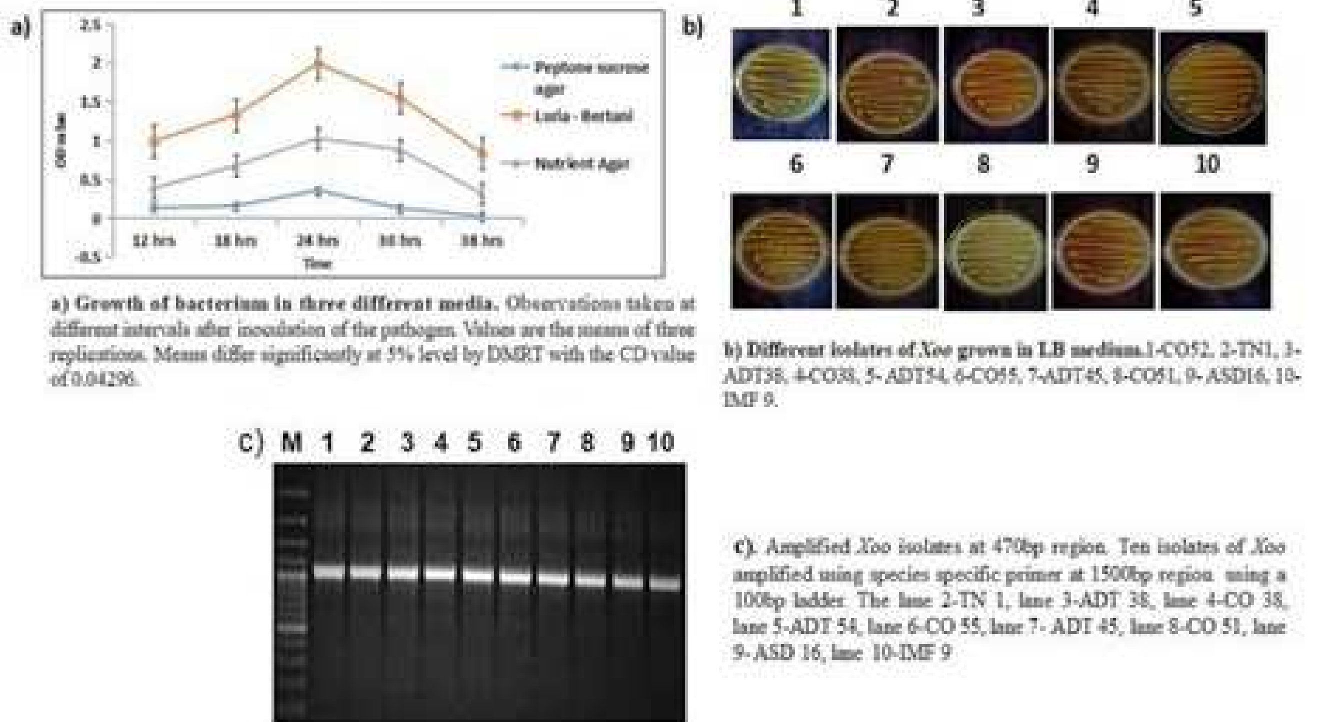
Table 3: Morphological characters of different isolate.

S. No.	Isolates	Diameter of colony (mm)	Colony tint	Shape	Appearance
1.	PC1	1.5	Yellow	Circular to unequal	Elevated
2.	PC2	2.1	Golden yellow	Spherical	Raised
3.	T1	1.2	Yellow	Spherical	Raised
4.	T2	1.4	Creamy yellow	Spherical	Flattened
5.	T3	3.0	Yellow	Spherical	Raised
6.	B1	2.2	Yellow	Irregular	Raised
7.	B2	1.5	Yellow	Spherical	Flattened
8.	B3	2	Light yellow	Spherical to irregular	Raised
9.	CW1	2.5	Yellow	Spherical	Raised
10.	CW2	1.2	Yellow	Spherical	Raised
C.D (P≤0.05)		0.073			

Table 4: Biochemical characterization of different isolates.

Test	PC1	PC2	T1	T2	T3	B1	B2	B3	CW1	CW2
Citrate utilization	☑	☑	☒	☑	☑	☒	☑	☒	☑	☑
Lysine utilization	☑	☒	☒	☑	☑	☑	☑	☑	☑	☑
Ornithine utilization	☑	☑	☑	☑	☑	☑	☒	☑	☑	☑
Urease	☑	☒	☑	☑	☑	☒	☑	☑	☑	☑
Phenylalanine Deamination	☑	☑	☒	☒	☑	☑	☒	☑	☒	☑
Nitrate reduction	☒	☑	☑	☑	☑	☒	☑	☑	☑	☒
H_2S production	☑	☒	☑	☑	☒	☑	☒	☑	☒	☑
Glucose	☒	☑	☑	☒	☑	☑	☑	☒	☑	☑
Adonitol	☒	☑	☒	☑	☑	☑	☒	☒	☑	☒
Lactose	☒	☒	☑	☑	☑	☒	☑	☑	☒	☑
Arabinose	☑	☑	☒	☒	☑	☑	☒	☒	☑	☒
Sorbitol	☒	☑	☑	☑	☒	☑	☒	☑	☒	☑

Fig.1 Growth of the Xanthomonas oryzae pv.oryzae in different media and their characterization

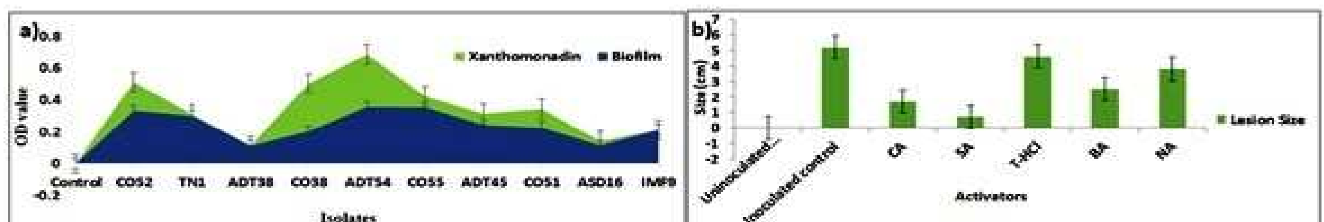


a) Growth of bacterium in three different media. Observations taken at different intervals after inoculation of the pathogen. Values are the means of three replications. Means differ significantly at 5% level by DMRT with the CD value of 0.04296.

b) Different isolates of *Xoo* grown in LB medium. 1-CO52, 2-TN1, 3-ADT38, 4-CO38, 5- ADT54, 6-CO55, 7-ADT45, 8-CO51, 9- ASD16, 10-IMF 9.

c). Amplified *Xoo* isolates at 470bp region. Ten isolates of *Xoo* amplified using species specific primer at 1500bp region using a 100bp ladder. The lane 2-TN 1, lane 3-ADT 38, lane 4-CO 38, lane 5-ADT 54, lane 6-CO 55, lane 7- ADT 45, lane 8-CO 51, lane 9-ASD 16, lane 10-IMF 9

Fig.2 Study of virulence and selection of activator based on the size of lesion in *in planta* assay



a) Quantification of biofilm and xanthomonadin in different isolates of *Xoo*. Observations taken after 24hrs after inoculation of the pathogen. Values are the means of three replications. Means differ significantly at 5% level by DMRT wit the CD value 0.0052 for biofilm and 0.0062 for xanthomonadin.

b) Lesion size on pathogen inoculated leaves treated with different synthetic activators. Observations taken after 14 days after inoculation of the pathogen. Values are the means of three replicationsMeans differ significantly at 5% level by DMRT with the CD value of 0.0409. CA- Cinnamic acid, SA- Salicylic acid, T- HCl -Thiamine hydrochloride, BA- Barbituric acid, NA- Nicotinic acid

DISCUSSION

The leaf blight disease in rice is unique diseases and is a key risk to production of rice in equally temperate and tropical areas since its high epidemic potential. Foliar application of salicylic acid treated rice plants exhibited a important decrease in incidence of > 80 % observed in green house. The current work was directed to curtain the synthetic activators against *Xoo*. In order to carry out the study the identification of virulent isolate is essential. The choice of the media also decides the virulent factor of the strains under laboratory condition. Hence, the pathogen viability is known to be affected by the medium in which they are cultivated and by conditions of storage (Raju *et al*., 2006). From the results of selection of appropriate media for the growth of the pathogen, LB medium was found to best. Similarly, yellow mucoid colonies were reported by Jabeen *et al*. (2012). The results correlate with the findings mentioned by Sezonov *et al*. (2007) in which LB media documents rapid growth and better yield for many species. Moreover, the LB medium also used in *X. campestris* production to increase the productivity of xanthan gum (Djenar *et al*., 2018). Sezonov *et al*. (2007) described that, *E. coli* bacterial, cells grown in LB, having better growth because it contains amino acid-rich and also poor in saccharides.

Morphological characterization: On isolating the pathogen *Xoo* in LB medium majority of the isolates revealed bright yellow with circular, raised and slimy appearance and this was coordinated by the outcomes of Kim *et al*. (2021). In a comparable manner, Shankara *et al*. (2016) reported that in Xoo isolated from Karnataka rice fields, the pathogenic bacteria had a colony size of 0.4 mm and a structure that was round, convex, yellow to creamy yellow in colour with an even surface area on the nutrient-agar medium. The same results also obtained for Patil *et al*. (2023) with the maximum colony size of 5.0mm. Also, different isolates of *Xoo* exhibits different physiological properties due to various enzymatic reactions. Similarly, Yang *et al*., 2020 reported the physiological variations for *Xoo.* Those isolates exhibited different physiological properties due to various enzymatic reactions.

Biochemical characterization: This result was observed to be correlated with the biochemical tests of Bhutto *et al*. (2018) catalase; amylase (starch hydrolysis) showed variations among the isolates. Patil *et al*. (2023) reported that all the isolates showed Gram- negative reaction through red tint once measured under bright light optical microscope and yielded optimistic positive for catalase and it decomposes hydrogen peroxide to water and oxygen. Also, Patidar and Ranjan (2022) did an experiment for biochemical characterization and studies shows that *Xoo* is a small bar rod shaped, gram-negative, gave positive result for KOH string test, catalase test and negative result for indole test and nitrate reduction test which is found to be similar to our present investigation. During 2018 Pradhan *et al*. characterised *Xoo* isolates from Chattisgarh and reported that the enzyme sorbitol and mannitol was not used by the isolates. Hence, utmost the *Xoo* isolates gave progressive/positive reaction for KOH and catalase test.

Molecular characterization: All the DNA samples from pathogenic bacterial isolates were noticed to be successfully amplified at 470 bp. Similarly, Sandhu *et al*. (2018) characterized the *Xoo* isolates through PCR analysis using the same primers and amplified at the same base pairs. The outcomes associate with the findings of Khan *et al*. (2022). Once amplification was done with *Xoo*-specific primers the base pairs of 470 band was amplified in North western Pakistan (Rafi *et al*., 2013). Divya *et al*. (2021) reported that the *Xoo* isolate isolated from rice was amplified with the base pairs of 470 in Telangana state. The amplified region was found to be specific for *Xoo* and capable to detect different strains that produces BLB symptoms *in vivo* (Lang *et al*., 2010). Additionally, the current findings clearly demonstrated the primer pair's sensitivity for the detection of *Xoo* in artificially infected plants.

Quantification of biofilm formation and xanthomonadin pigment production: Xanthomonadin is membrane-bound creamy pigments that are characteristically formed by bacteria *Xanthomonas* spp., plays an essential part in host-pathogen interactions and epiphytic survival. (He *et al.,* 2020; Poplawsky *et al*., 2000). Xanthomonadin is also essential for growth and development of biofilm (Yu *et al*., 2019). Biofilm formation which is important to survive epiphytically in plants for the pathogen prior to the induction of bacterial symptoms (Li *et al*., 2011). The active isolate which exhibited the highest OD value for biofilm formation and xanthomonadin pigment was noted to be correlated by the outcomes of Sahu *et al*. (2018) and Shi *et al*. (2021). Since, the above results were confirmed that to screen the virulent isolate the biofilm and xanthomonadin parameters were essential.

Screening of synthetic activators: Salicylic acid (100 ppm) was observed to be effective against rice BLB disease (lesion size – 0.7cm). This could be because the appropriate concentration of elicitor is needed for eliciting the efficient plant defence mechanism. Similarly, Thepbandit *et al*. (2021) showed similar a test to ascertain the ideal concentration of SA-Rice mate to elicit the defense mechanism in plants and found that 100-300 ppm optimal for their defense role by producing extrapolysaccharides and inhibition of biofilm production. According to Wani *et al*. (2017), applying SA to plants also enhanced the production of defensive

chemicals complicated in universal and native acquired resistance as well as the start of pathogenesis-related gene expression. The reason behind the results confirmed that the utilization of SA induce the expression of defense-related genes such as PR1a, PAL, PR5 and HSP90 on paddy leaves (Yang *et al*., 2019) and involved with cinnamate and SA pathway with phenylpropanoid compounds. During rice LB resistance, the SA path is a key signaling medium that enables cross-talk between signals, perhaps delivering well-organized energy and the accumulation of likely enzymes. (Adam *et al*., 2018; Norouzi *et al*., 2015). Because the SA signalling pathway is connected to systemic acquired resistance (SAR), which can happen when endogenous SA accumulates and is activated during pathogenesis, exogenous SA can initiate the pathway and boost disease resistance. (Lopez-Gresa *et al*., 2016).

Conclusion: The present study revealed that salicylic acid (100 ppm) can be employed for the management of rice BLB with minimal impact on environment. As the salicylic acid was found to enhance the defense mechanism by activating the pathogenesis-related gene efficiently, futuristic investigation on changes at transcriptome level may reveal the most in-depth understanding

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgements: We sincerely thank Department of Plant Pathology, TNAU, Coimbatore, Tamil Nadu, India, for providing facilities.

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