Oryza Vol. 57 Issue No. 1, 2020 (64-69)
DOI https://doi.org/10.35709/ory.2020.57.1.8

Studies on suppression of bacterial leaf blight by rice endophytic bacteria under field condition

K Kavitha[1], P Nagamani[1*], P Madhu Sudhan[2], K Viswanath[3] and NP Eswara Reddy[3]

[1]S.V. Agricultural College (ANGRAU), Tirupati, Andhra Pradesh, India

[2]Agricultural Research station (ANGRAU), Nellore, Andhra Pradesh, India

[3]Regional Agricultural Research Station (ANGRAU), Tirupati, Andhra Pradesh, India

**Corresponding author e-mail: manipath28@gmail.com*

Received : 6 November 2019 Accepted: 27 March 2020 Published : 31 March 2020

ABSTRACT

A total of 45 endophytic bacterial isolates were isolated from the healthy rice cultivars viz; MTU 1010, BPT 5204 and NLR 34449 cultivated in Nellore and Chittoor districts of Andhra Pradesh. All the isolated endophytic bacterial isolates were evaluated for their antagonistic effect against bacterial leaf blight (BLB) pathogen Xanthomonas oryzae pv. oryzae by agar well diffusion method. The diameter of inhibition zone formation ranged from 0 mm to 16.8 mm. Among 45 isolates, EMP-5 and EBK-3 isolates showed highest antagonistic effect with inhibition zone of 16.8 mm and 16.6 mm, respectively. Isolate EMP 5 was used for the management of BLB under field condition. Among all the treatments, treatment T5 (i.e., seed treatment with EMP-5 @ 10^9 cells ml^{-1} + foliar application of EMP-5 @10^9 cells ml^{-1}) exhibited highest reduction in disease index (4.92 %), increased plant growth promoting ability with plant height of 80.8 cm, increase in the no. of effective tillers (97.4 %), grain yield (5340 kg ha^{-1}) and straw yield (6013 kg ha^{-1}) over the untreated control. The results strongly emphasize that endophytic bacteria (EMP-5) could be efficiently used for management of bacterial leaf blight.

Key words: *Bacterial leaf blight, bacterial endophyte, antagonism, plant growth parameter, rice*

INTRODUCTION

Many bacterial diseases are known to infect rice *viz*., Bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), Bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*), Bacterial panicle blight (*Burkholderia glumae*), Bacterial brown stripe (*Acidovorax avenae* subsp. *avenae*) etc. Out of these bacterial leaf blight of paddy (BLB) is of economic importance. Even though the occurrence of BLB in rice is reported worldwide, it is of economic importance mainly in Asia and in some parts of Western Africa where rice is cultivated under irrigated and low land ecosystems. Srinivasan et al. (1959) have first reported the disease in India from Maharashtra with the widespread cultivation of semi dwarf, high-yielding and nitrogen-responsive varieties like Taichung Native 1. The severity and significance of damage due to BLB necessitated the development of strategies to manage the disease in rice. In the control of BLB, combination spray of streptomycin and copper oxychloride resulted in inhibition of bacterial growth during the initial stages of infection (Hori, 1973) while streptomycin was used effectively for the disinfection of rice seeds (Srivastava, 1972). But no chemicals are available for complete control (Ou 1985, Reddy and Nayak, 1985), as the chemicals are effective only at the initial stage of the disease; chances of development of resistant strains, in addition to residual toxicity and environmental pollution. Use of bacterial antagonists against *Xanthomonas oryzae* pv. *oryzae* might be an effective strategy, of bacterial to disease suppression by biological

control.

MATERIAL AND METHODS

Isolation of endophytic bacteria

Healthy leaves washed with water to remove the dust and there after 2 g of leaves chopped into pieces of 4-6 mm used for isolation. The disinfection and isolation was done as the procedure of Araujo et al. (2001).

Isolation of bacterial leaf blight pathogen from disease sample

Xanthomonas oryzae pv. *oryzae*, the causal agent of bacterial leaf blight was isolated from the diseased plants. Infected leaves of rice were excised with sterile scalpel and made into small bits. The leaf bits were solution surface sterilized with one per cent sodium hypochlorite for three minutes and then washed with sterile distilled water. Later the infected leaf bits after proper drying on sterile blotting paper were transferred to Nutrient Agar (NA) medium and incubated at room temperature (25-27 ^{0}C) for 72 h (Jabeen et al., 2012). The single yellow round, smooth margin, mucous colonies were selected and transferred onto NA plates for pure culture. The single colony selected as a pure culture and maintained at 4 ^{0}C for further studies.

Evaluating the antagonistic effect of endophytic bacteria against *Xanthomonas oryzae* pv. *oryzae*

The antagonistic effect of endophytic bacterial isolates against *Xanthomonas oryzae* pv. *oryzae* was evaluated by using agar well diffusion method. One ml of *Xanthomanas oryzae* pv. *oryzae* culture suspension (10^7 cells ml^{-1}) obtained from 24 h old culture grown on Peptone Sucrose Broth medium was added to 20 ml of 0.3 per cent peptone sucrose agar medium and inoculated on a petriplate spreading evenly with a L-rod. After solidification, 4 wells were formed with the help of 6 mm diameter cork borer and 10 µl of each endophytic bacterial isolate was poured into the wells created on the medium. Inoculated petriplates were incubated at room temperatures (28 ± 2^0 C) for 24 h after which the diameter of the inhibition zone was measured. The level of *Xanthomanas oryzae* pv. *oryzae* growth inhibition was determined by measuring the difference between the inhibition zone formed and diameter of the pathogen growth (Yasmin et al., 2016).

Management of BLB with potential endophyte under field condition

A field experiment with cv. NLR-34449 was conducted during *rabi* season (November-April 2019) with different treatments in randomized block design with three replications at Agricultural Research Station, Nellore. The potential endophytic bacterial isolate (EMP-5) was evaluated by applying through various methods like seed treatment, seedling root dip and foliar spray. Application of streptomycin sulphate @ 600 ppm+ Copper oxychloride @ 0.25% was kept as as standard check. The details of treatments is given in Table 1.

Mass multiplication of BLB pathogen and potential endophytic bacterium

Potential endophytic bacterium was inoculated in nutrient broth medium and incubated (25 ± 2 ^{0}C) for 48 h with constant shaking at 180 rpm in a rotary shaker yielding 10^9 cells ml^{-1}. Then used for seed treatment, seedling root dip, foliar spray treatments in field conditions. Similarly bacterial leaf blight pathogen was multiplied in nutrient broth. Loopful of pathogenic bacterial culture was added to the medium and incubated in a rotary shaker at room temperature (25±2 ^{0}C) for 72 h. Cell density was determined using a spectrophotometer. The pathogenic culture was arrived at a concentration of 10^9 cells ml^{-1} and the suspension thus prepared was used for clip inoculation to healthy plants for the occurrence of the disease and evaluation of the efficacy treatments under field conditions.

Percent disease index (PDI)

The effectiveness of the treatments on the intensity of bacterial leaf blight was observed 15 days after

Table 1. Details of treatments.

Treatment	Detail treatment
T_1	Seed bacterization with EMP-5 @10^9 cells ml^{-1}
T_2	Seedling root dip with EMP-5@10^9 cells ml^{-1}
T_3	Foliar spray with liquid suspension of EMP-5 @10^9 cells ml^{-1} at 21 DAT
T_4	T_1+T_2
T_5	T_1+T_3
T_6	Streptomycin sulphate @ 600ppm + COC 0.25% (Standard check)
T_7	Un treated control (pathogen alone)

pathogen inoculation by scoring 10 leaves from each replication with a 0-9 scale of the Standard Evluation System of IRRI. Pathogenic bacterial suspension was clip inoculated to 45 days old plants of rice cultivar NLR 34449 by excising 2-3 cms of tips of rice leaves with a sterilized scissor which was immersed in the bacterial pathogen suspension (10^8 cells ml^{-1}) prepared in sterile distilled water. The control plants were inoculated with sterile phosphate buffer Per cent disease index was calculated for bacterial leaf blight infected plants formula given by McKinney (1923).

Per cent disease index (% PDI) =

$$\frac{\text{Sum of all numerical ratings}}{\text{Total number of leaf graded}} \times \frac{100}{\text{Maximum grade}}$$

Plant growth parameters *viz*., plant height and number of effective tillers 10 $hills^{-1}$ and grain yield and straw yield was recorded per plot (5x3 m^2).

RESULTS AND DISCUSSION

Isolation of the pathogen

Bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) isolated from the diseased plants resulted in mucoid, round and smooth bacterial colonies on NA medium incubated at 28 ± 2 ^{0}C for 48-72 hours. The pathogen was purified by streak plate method and preserved at -20 ^{0}C in glycerol stocks.

Isolation of endophytic bacteria

A total of 45 endophytic bacterial isolates were isolated from the healthy paddy cultivars *viz*., MTU 1010, BPT 5204 and NLR 34449 which are popularly cultivated in Nellore and Chittoor districts of Andhra Pradesh. Nineteen endophytic bacterial isolates were isolated from the samples collected from nine villages spread across six mandals of Nellore district and 26 endophytic bacteria from the leaf samples collected from four villages spread across three mandals of the Chittoor district.

In vitro evaluation of endophytic bacteria against bacterial leaf blight (BLB) pathogen

A total of 45 endophytic bacterial endophyte isolates were evaluated for their antagonistic effect against bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Table 2, Fig. 1). The diameter of inhibition zone formation ranged from 0 mm to 16.8 mm. The highest antagonistic effect was exhibited by EMP-5 and EBK-3 isolates with inhibition zone of 16.8 mm and 16.6 mm, respectively. This was followed by EBA-5 (15.9 mm) isolate, whereas, the isolates EUD-2, EUD-8, EUD-10, EBL-6 and EBP-1 had no antagonistic effect against BLB pathogen with inhibition zone of 0 mm.

Management of bacterial leaf blight of rice with potential endophytic bacteria (EMP-5) under field condition

The isolate EMP-5 showed highest antagonistic activity was selected and evaluated under different treatments for its efficacy in the management of Bacterial Leaf Blight of rice under field condition. The experiment was conducted with cultivar NLR-34449 in a Randomized Block design with three replications. The experiment was sown during *rabi* 2018-19 at Agricultural Research Station, Nellore.

Effect on percent disease index (PDI)

Per cent disease index was significantly reduced in all the treatments (T_1 to T_6) as compared to untreated control (T_7). Among the treatments, T_5 (seed treatment with EMP- 5 @ 10^9 cells /ml + foliar spray with suspension of EMP-5 @10^9 cells /ml) recorded lowest percent disease index (4.9 %), which was at par with treatment T_6 (Streptomycin sulphate @ 600 ppm + COC 0.25%). Highest PDI (20.7 %) was recorded in the untreated control (T_7).

Effect on plant growth parameters in field experiment

The effect of various treatments on plant height, number of effective tillers, grain and straw yields was also studied and results are presented in Table 3.

The maximum plant height was recorded in T_5 (80.8 cm) (seed bacterization with EMP-5 @ 10^9 cells ml^{-1} + foliar spray with suspension of EMP-5 @10^9 cells ml^{-1}) which was on par with treatment T_4 (seed treatment with EMP-5 @10^9 cells ml^{-1} + seedling root dip with EMP-5 @10^9 cells ml^{-1}) and T_6 (Streptomycin sulphate @ 600 ppm + COC 0.25%). Lowest plant height (74.1 cm) was recorded in untreated control (T_7).

The number of tillers significantly increased in

Table 2. Antagonistic effect of bacterial endophytes against bacterial leaf blight.

S.no.	Name of the bacterial isolates	Inhibition zone of antagonistic activity (mm)*
1	MB-1	14.3cd **
2	MB2	3.0^{r}
3	MB-3	5.9^{pq}
4	MB-4	13.9^{cd}
5	EMP-1	11.1^{g}
6	EMP-2	13.3^{ef}
7	EMP-3	7.8^{nml}
8	EMP-4	8.0^{mlkj}
9	EMP-5	16.8^{a}
10	EMP-6	8.7^{ijkl}
11	EMP-7	7.5^{nm}
12	EMP-8	8.4^{mlkj}
13	EMP-10	12.1^{f}
14	EUD-1	6.8^{on}
15	EUD-2	0.0^{s}
16	EUD-3	15.5^{b}
17	EUD-4	7.6^{nml}
18	EUD-6	3.1^{r}
19	EUD-7	7.5^{nm}
20	EUD-8	0.0^{s}
21	EUD-9	8.1^{mlkj}
22	EUD-10	0.0^{s}
23	EP1	6.8^{nm}
24	EP2	5.5^{pq}
25	EP3	6.0^{on}
26	EP4	5.3^{p}
27	EBA-1	9.7^{ih}
28	EBA-2	10.0^{gh}
29	EBA-5	15.9^{ab}
30	EBL-1	7.9^{klm}
31	EBL-2	9.0^{kjih}
32	EBL-3	8.7^{ijkl}
33	EBL-6	0.0^{s}
34	EBP-1	0.0^{s}
35	EBP-3	9.7^{ij}
36	EBV-1	9.0^{g}
37	EBV-2	4.8^{q}
38	EBV-4	6.1^{pq}
39	EBV-8	12.8^{fg}
40	EBN-1	10.0^{gh}
41	EBN-2	9.1^{jih}
42	EBR-1	7.6^{nml}
43	EBR-2	14.3^{c}
44	EBK-1	3.3^{r}
45	EBK-3	16.6^{a}
46	Control	0.0^{s}
	C.D at 5 %	1.010
	SEm(±)	0.361
	C.V. (%)	8.96

Mean of four replications. **Means in a column followed by same super script letters are not significantly different according to DMRT.

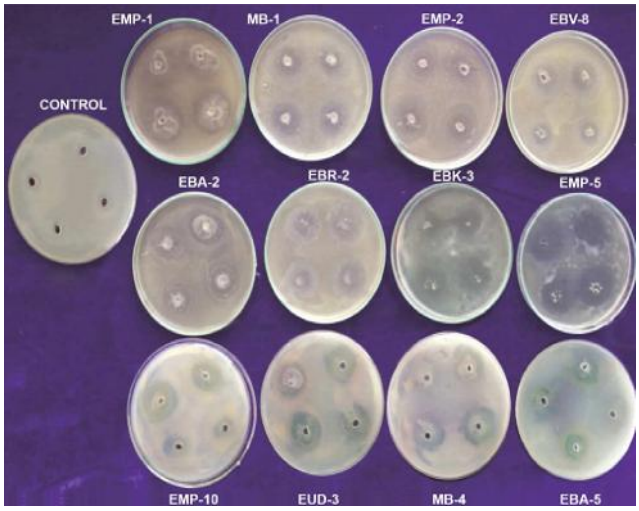

Fig. 1. Efficacy of endophytic bacteria against bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) by agar well diffusion method.

all the treatments (T_1 to T_6) as compared to untreated control (T_7). However, the highest per cent of effective tillers (97.4%) was recorded in (seed treatment with EMP-5 @ 10^9 cells ml^{-1} + foliar spray with suspension of EMP-5 @10^9 cells ml^{-1}) which was at par with treatment T_6 (95.6 %). Lowest per cent effective tillers recorded in untreated control (88.3 %). Treatments T_1, T_2, T_3 and T_4 recorded 90.6, 92.6, 93.6 and 94.0 per cent, respectively.

Similarly, all the treatments (T_1 to T_6) significantly increased grain yield. Highest grain yield (5340 kg ha^{-1}) was recorded in T_5 (seed treatment with EMP-5 @ 109 cells ml^{-1} + foliar spray with suspension of EMP-5 @10^9 cells ml^{-1}) as compared to untreated control (T_7) which was on par with treatment T_4 (5270 kg ha^{-1}). Lowest grain yield recorded (3299 kg ha^{-1}) in T_7. Treatments T_1, T_2, T_3, T_6 recorded 4592, 4655, 4500 and 4740 grain yield kg ha^{-1}, respectively.

Similar trend was observed for straw yield. Highest straw yield (6013 kg ha^{-1}) was recorded in T_5 which was significantly different from all other treatments.

Bacterial endophytes colonize plant tissue same as that of plant pathogens, which act as biocontrol agent as well as plant growth promoting agents (Berg et al., 2005). The strong union amidst host plant and endophytes is mediated through the action of secondary metabolites produced by the microorganisms and the

Table 3. Management of bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*) with potential endophytic bacterial isolate (EMP-5) under field condition.

Treatment	Per cent disease index (PDI)	*Plant height (cm)	Per cent of effective tillers	*Grain yield (Kg ha^{-1})	*Straw yield (Kg ha^{-1})
T_1	11.3^{e} (19.6)	75.8^{ab}	90.6 ^{ab} (71.8)	4592^{bc}	5095^{b}
T_2	9.7^{d} (18.15)	76.3^{ab}	92.6 ^{bc} (73.8)	4655^{bc}	5258^{b}
T_3	8.4^{c} (16.79)	77.1^{ab}	93.6 ^{c} (75.8)	4500^{b}	5023^{b}
T_4	5.9^{b} (14.01)	79.2^{bc}	94.0 ^{c} (75.9)	5270^{cd}	5456^{b}
T_5	4.9^{a} (12.81)	80.8^{c}	97.4 ^{d} (81.1)	5340^{d}	6013^{c}
T_6	5.4^{ab} (13.41)	78.3^{bc}	95.6 ^{cd} (77.9)	4740^{bc}	5413^{b}
T_7	20.7^{f} (27.07)	74.1^{a}	88.3 ^{a} (70.0)	3299^{a}	3972^{a}
SEm(±)	0.86	0.76	1.19	216.89	158.61
C.D at 5 %	2.68	2.37	3.70	675.79	494.16
C.V %	3.58	0.70	2.74	8.11	5.30

* Values in the parenthesis are angular transformed values. *Mean of three replications. **Means in a column followed by same super script letters are not significantly different according to DMART.

host cells (Reinhold and Hurek, 2011).

Endophytes have shown significant disease control of plant diseases. Nagendran et al. (2013) reported that endophytic bacterium, *Bacillus subtilis* var *amyloliquefaciens* (FZB24) applied through seed treatment @ 4g kg^{-1} + seedling dip @ 4g l^{-1} + soil application @ 500 g ha^{-1} + foliar application @ 500g ha^{-1} recorded lowest severity of bacterial leaf blight of rice (31.36) with a per cent reduction of 40 over control under glass house condition.

Lowest PDI in treatment T_5 (seed treatment with EMP-5 @ 10^9 cells ml^{-1} + foliar spray with liquid suspension of EMP-5 @10^9 cells ml^{-1}) is attributed due to the seed treatment of rice plants that might have resulted in induction of resistance through the secretion of certain bioactive compounds, which are inhibitory to the pathogens for certain period which again strengthened by foliar application. Alam et al. (2017) reported that seedling dip (BRRI dhan-29) before transplanting and subsequent foliar application with BRRh-5 (*Pseudomonas aeruginosa*) at the tillering and flowering stages along with varying doses of NPK fertilizers significantly promoted growth and grain yield. Yasmin et al. (2017) reported that BRp3 (*Pseudomonas aeruginosa*) inoculated to rice plants decreased the per cent diseased leaf area (3.7%) against *Xoo* and also enhanced grain and straw yields by 51 and 55 per cent under *in vivo* conditions. Khoa et al. (2016) demonstrated that CT-78 induced plant resistance to bacterial leaf blight disease by application of bacterial suspensions to rice seeds before sowing and also observed that inoculated plants showed greater protection against the pathogen up to 45 DAS is due to efficient induction of resistance by colonization of endophytic bacteria, which might secrete bioactive compounds to inhibit the pathogen or systemically induce resistance in the host. These substances could also be transported in to rice plant through stomata pores on leaf surface (by foliar spraying) or via roots (by soil drenching). Lower infected tillers, leaves and reduction in lesion lengths on leaves could help rice plants to preserve enough leaf surfaces for photosynthesis resulting in healthy plants and fewer yield losses.

The isolate (EMP-5) used in field experiment resulted in significant disease reduction and increase in various plant growth parameters. This strain may have the potential to be used as bioinoculant for sustainable rice production.

REFERENCES

Alam KM, Haque E, Paul NC, Khaleque MA, Al-garniSMS, Rahman M and Islam MT (2017). Enhancement of growth and grain yield of rice in nutrient deficient soils by rice probiotic bacteria. Rice Science 24(5): 264-273

Araujo WL, Saridakis HO, Barroso PAV, Aguilar-Vildoso CI and Azevedo JL (2001). Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Canadian Journal of Microbiology. 47: 229-236

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J

(2005). Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiology Ecology 51: 215-229

Hori M (1973). Nippon Shin - noyaku Mongatri, Japan Plant Protection Association, Tokyo, Japan pp. 622

Jabeen R, Iftikhar T and Batool H (2012). Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. Pakistan Journal of Botany 44(1): 261-265

Khoa ND, Giàu NDN and Tuan T Q (2016). Effects of Serratia nematodiphila CT-78 on rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. Biological Control 103: 1-10

McKinney HH (1923). Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum. Journal of Agriculture Research 26: 195-217

Nagendran K, Karthikeyan G, Peeran MR, Prabakar K and Raguchander T (2013). Management of bacterial leaf blight disease in rice with endophytic bacteria. World Applied Sciences Journal 28(12): 2229-2241

Ou SH (1985). Rice diseases, 2nd edition. Common wealth mycological institute, Kew, Surrey, England pp. 61-69

Reddy PR and Nayak P (1985). Spread pattern of bacterial blight disease in rice crop. Indian Phytopathology 38: 39-44

Reinhold-Hurek B and Hurek T (2011). Living inside plants: bacterial endophytes. Current Opinion in Plant Biology 14: 435-443

Srinivasan MC, Thirumalachar MJ and Patel MK (1959). Bacterial leaf blight of rice. Current Science 28: 469-470

Srivastava DN (1972). Bacterial blight of rice. Indian Phytopathology 25: 1-16

Yasmin S, Hafeez FY, Mirza MS, Rasul M, Arshad HMI, Zubair M and Iqbal M (2017). Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by rhizospheric Pseudomonas aeruginosa BRp3. Frontiers in Microbiology 8: 1895 DOI: https://doi.org/10.3389/fmicb.2017.01895

Yasmin S, Zaka A, Imran A, Zahid MA,Yousaf S, Rasul G, Arif M and Mirza MS (2016). Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. PLoS One 11(8): 1-19 DOI:https://doi.org/10.1371/journal.pone.0160688