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Isolation and identification of Phylloplane Bacteria from rice ecosystem of Andhra Pradesh against *Magnaporthe oryzae*

K Mounika, P Madhusudhan, M Ramabhadra Raju and I Paramasiva
Department of Plant Pathology, Acharya N G Ranga Agricultural University,
Agricultural College, Bapatla - 522101, Andhra Pradesh, India.

ABSTRACT

A foliar pathogen causing blast symptoms on rice was collected from Bapatla district. The disease is caused by pathogen *Manaporthe oryzae* was identified based on the morphological characters. Healthy leaf samples were collected from four districts of Andhra Pradesh. Phylloplane bacteria was isolated from the leaf samples by leaf imprinting method. Based on Gram's staining method and cultural characters of the bacterial isolates, *Bacillus sps* were identified and maintained the cultures for further experiments.

Key words:*Identification, Isolation, Magnoparphac oryzac, Phylloplane loacteria and Rice ecosystem*

Rice (*Oryza sativa* L.) being the major staple food and one of the main sources of income and employment, is an important crop all over the world. Almost 90% of the global production and consumption of rice is reported from Asia, where a considerably large part of the world's population resides (www.fao.org; accessed on 20 January 2022).

Rice crop is challenged by a number of biotic and abiotic stresses in the various rice-growing regions of the world. It is reported to be attacked by about 36 fungal, 21 viral and 6 bacterial diseases (Ou, 1985). Among fungal diseases, blast disease caused by *Magnaporthe oryzae* B.C. Couch is one of the important fungal diseases affecting considerable loss in rice production. Phyllosphere is one of the hostile environments and there has been a lot of interest in the life forms that inhabit it. Mwajita *et al.* (2013) evaluated the rhizosphere, rhizoplane and phyllosphere bacteria from rice fields in Kenya for PGPB and reported that over 50% of bacterial isolates from phyllosphere were able to solubilize phosphates. Production of indole-3-acetic acid (IAA) is common among bacterial epiphytes (Brandl *et al.,* 2001).

The disease can be controlled by the use of fungicides, botanicals and biological control methods. The use of native biocontrol agents that coexist along with the pathogen (epiphytes) in disease management is considered as the eco-friendly and sustainable approach. As the use of fungicides may harm the environment, the present study is taken up focusing on the biological control of the disease.

MATERIAL AND METHODS

The present study was carried out at the department of Plant Pathology, Agricultural College, Bapatla.

Sample collection

The samples were collected from four districts, *viz*. from Nellore, Tirupati, Bapatla and West godavari. The samples for isolating phyllospheric organisms were characterized by the deep green and disease free leaves from upper part of rice plants.

Isolation of phyllospheric biocontrol agents

For isolation of phylloplane bacteria, Hicrome Bacillus agar was used. Based on the chromogenic nature of the media, bacterial colonies with different colours such as blue, green, pink etc., For isolation, the samples that were collected from the four districts were imprinted in Petri plate onto their respective selective media. Thus inoculated Petri plates were incubated at 25^0C- 28^0C for 24-48h. For further identification colonies with different colours streaked on nutrient agar and cultural characters were identified. (Plate 1).

Gram's staining

The morphological characteristics of the isolated bacteria were tested by Gram's staining. It was done using the standard protocol. A thin bacterial smear was fixed on a clean glass slide. The smear was stained

Isolation through leaf imprint method

Pure culture of bacillus on nutrient agar

Plate 1. Isolation and cultural characters of phylloplane bacteria

Table 1. List of locations surveyed for collection of leaf samples for isolation of phylloplane bacteria

Location number	Name of the District	Name of the Mandal	Name of the Village	Latitude (degrees)	Longitude (degrees)	Name of the Variety	Stage of the crop
1	Nellore	Nellore Rural	Chintareddypalem	14.42833	79.99692	KNM 1638	Tillering
2			Kakupalli	14.39075	80.0348	KNM 733	Tillering
3		T.P.Gudur	Chinna Cherukuru	14.43837	80.05397	KNM 1638	Tillering
4			Pottepalem	14.46472	79.92405	KNM 1638	Tillering
5			Anjaneyapuram	14.45339	80.07468	BPT 5204	Tillering
6	Tirupati	Naidupeta	L.A.Sagaram	13.90933	79.9004	NLR 1436	Panicle initiation
7			Anamedu	13.95398	79.92997	BPT 5204	Panicle initiation
8		Pellakuru	Chavali	13.87601	79.86457	NLR 1436	Panicle initiation
9			Talvaipadu	13.89054	79.88591	RNR 15048	Tillering
10			Sirsnambedu	13.8692	79.8625	RNR 15048	Tillering
11		Ozili	Josulavarikandriga	13.91601	79.88121	RNR 15048	Panicle initiation
12	Bapatla	Bapatla	Basivireddypalem	15.87178	80.46138	MTU 1262	Grain filling
13			Madiboinivaripalem	15.88772	80.46511	BPT 5204	Panicle initiation
14			Apikatla	15.96759	80.51069	BPT 5204	Tillering
15			Egavaripalem	15.832	80.43026	MTU 1262	Vegetative stage
16			Matysavaripalem	15.89136	80.49346	MTU 1271	Near harvesting
17			Mulapalem	15.92291	80.48245	BPT 5204	Panicle initiation
18		Karlapalem	Karlapalem	15.91481	80.49321	MTU 1262	Grain filling
19		Tsunduru	Edlapalli	16.21358	80.61108	BPT 5204	Harvesting stage
20		Nagaram	Repalle	15.99198	80.70813	BPT 2782	Grain filling
21		Chirala	Devinuthala	15.85717	80.46236	MTU 1262	Panicle intiation
22	West Godavari	Penugonda	Penugonda	16.64803	81.74253	MTU 1318	Harvesting stage
23		Tanuku	Tetali	16.74953	81.69986	MTU 1318	Harvesting stage
24			Duvva	16.7806	81.62839	MTU 1262	Harvesting stage
25		Poduru	Vedangi	16.56678	81.72709	MTU 1014	Harvesting stage
26		Penumantra	Maruteru	16.63114	81.7457	MTU 1262	Grain filling stage
27		Palakollu	Palakollu	16.5258	81.73541	MTU 1318	Harvesting stage
28		Gopalapuram	Gopalapuram	16.70889	81.8255	MTU 1318	Harvesting stage
29		Pentapadu	Prathipadu	16.81014	81.573	MTU 7029	Harvesting stage

Table 2. List of Phylloplane bacterial isolates from rice ecosystem of Andhra Prade

S. No	Isolate code	District	Mandal	Village
1	PNL-01	Nellore	Nellore Rural	Chintareddypalem, Kakupalli
2	PNL-02			
3	PNL-03			
4	PNL-04			
5	PNL-05		T.P Gudur	Pottepalem, Anjaneyapuram
6	PNL-06			
7	PNL-07			
8	PTP-08	Tirupati	Naidupeta	Anamedu
9	PTP-09			
10	PTP-10		Pellakuru	Chavali, Talvaipadu,
11	PTP-11			Sirsnambedu
12	PTP-12			
13	PTP-13			
14	PTP-14			
15	PBT-15	Bapatla	Bapatla	Madiboinivaripalem,
				Apikatla,
16	PBT-16			Egavaripalem,
17	PBT-17			Matysavaripalem,
18	PBT-18			
19	PBT-19		Karlapalem	Karlapalem
20	PBT-20			
21	PBT-21			
22	PBT-22		Chirala	Devinuthala
23	PBT-23			
24	PBT-24			
25	PWG-25		Penugonda	Penugonda
26	PWG-26		Tanuku	Tetali
27	PWG-27			
28	PWG-28		Poduru	Vedangi
29	PWG-29		Penumantra	Maruteru
30	PWG-30		Palakollu	Palakollu
31	PWG-31			
32	PWG-32			
33	PWG-33		Gopalapuram	Gopalapuram
34	PWG-34		Pentapadu	Prathipadu

with crystal violet for 60 s, then wash with water. After this, the smear was treated with Lugol's iodine for 30 s, then washed with water, further with decolorization agent 70% of alcohol for 15 s, and washed with water. Finally, the smear was stained with safranin (counterstain), then washed with water and air-dried. The Gram's-stained smear was observed under a microscope. The bacterial cells appeared purple, called Gram's positive, and the bacterial cells appeared pink, referred to as Gram's negative.

Identification of the biocontrol agents

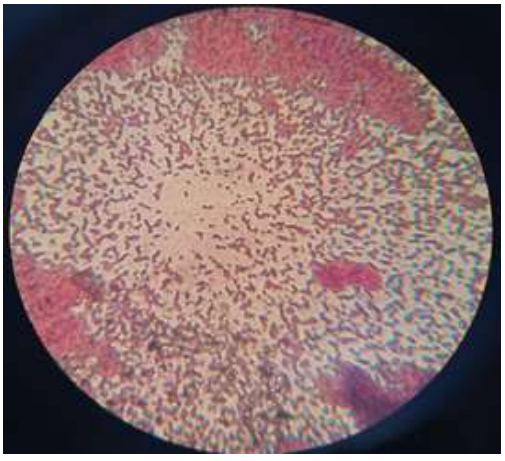
After incubation period, *Bacillus sps* were identified based on colony colour like white, creamy white and greyish white.

Maintenance of cultures

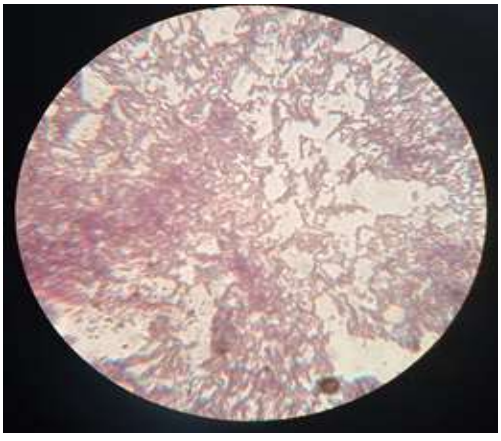
First identified bacterial cultures were sub-cultured on Nutrient Agar. Then loopful of each isolate was transferred on to NA slants aseptically and incubated for two days at 26^0C. The cultures thus obtained were stored in refrigerator at 4^0C and revived monthly for further studies.

Table 3. Gram staining reaction of phylloplane bacterial isolates

S. No	Isolate code	Gram staining reaction
1	PNL-01	-ve
2	PNL-02	-ve
3	PNL-03	-ve
4	PNL-04	-ve
5	PNL-05	-ve
6	PNL-06	-ve
7	PNL-07	+ve
8	PTP-08	+ve
9	PTP-09	+ve
10	PTP-10	-ve
11	PTP-11	-ve
12	PTP-12	+ve
13	PTP-13	+ve
14	PTP-14	-ve
15	PBT-15	-ve
16	PBT-16	+ve
17	PBT-17	-ve
18	PBT-18	-ve
19	PBT-19	+ve
20	PBT-20	-ve
21	PBT-21	-ve
22	PBT-22	+ve
23	PBT-23	-ve
24	PBT-24	-ve
25	PWG-25	+ve
26	PWG-26	+ve
27	PWG-27	+ve
28	PWG-28	-ve
29	PWG-29	+ve
30	PWG-30	+ve
31	PWG-31	-ve
32	PWG-32	-ve
33	PWG-33	-ve
34	PWG-34	+ve



Gram negative bacteria at 10x



Gram positive bacteria at 10x

Plate 2. Gram staining reaction of isolated phylloplane bacteria

RESULTS AND DISCUSSION

Isolation

Twenty-nine leaf samples were collected from rice ecosystem of Nellore, Tirupati, Bapatla, West Godavari districts of different mandals at different stages of crop. During the sample collection, data such as geographical location, crop variety and stage of the crop was recorded (Table 1). In this study, 34 bacterial isolates were isolated from the phylloplane region of rice ecosystem of Nellore, Tirupati, Bapatla and West Godavari districts. In those districts, seven isolates from Nellore, 7 isolates from Tirupati, 10 isolates from Bapatla and 10 isolates from West Godavari districts were isolated. (Table 2).

Cultural characterization

Among the 34 isolates, 14 isolates were tested Gram positive and appeared as rod shaped. These Bacillus isolates were mentioned as PNL, PTP, PBT, PWG based on district names as Phylloplane Bacillus Nellore, Phylloplane Bacillus Tirupati, Phylloplane Bacillus Bapatla, Phylloplane Bacillus West Godavari respectively. These isolates were used for further experiments. (Table 3) of the 14 isolates assessed for their cultural characters, 5 isolates PTP-09, PTP-12, PTP-13, PWG-25, PWG-29 had round colonies while 9 isolates PNL-07, PTP-08, PBT-16, PBT-19, PBT-22, PWG-26, PWG-27, PWG-30, PWG-34 had irregular shaped colonies. Colony colour of four isolates to be creamy, 4 were white, four isolates were in yellow and three are slightly yellow in colour. Margins of 14 isolates were entire margin observed in 2 isolates while 9 isolates was undulate, 2 isolates have rhizoid margin and remaining as filamentous margin. Elevation of the colony was raised in 7 isolates while 7 isolates were flat mentioned in (Table 3) (plate 2).

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