

Isolation and cultural characterization phylloplane bacteria from rice ecosystem of Andhra Pradesh

K Mounika, P Madhusudhan, M Ramabhadra Raju, I Paramasiva and N Kiran Kumar

Department of Plant Pathology, Acharya N G Ranga Agricultural University,
Agricultural College, Bapatla-522101, Andhra Pradesh, India

ABSTRACT

A total of 34 phylloplane bacterial isolates were isolated from the rice phylloplane on Hicrome Bacillus specific media by leaf imprinting method. Among these 14 gram positive rod shaped bacteria were selected and cultural characters like colony colour, shape, elevation and margins were characterized. All the 14 isolates are creamy white to white in colour, colony shape was irregular to round, flat or with raised elevation. Based on these cultural characters they were identified as *Bacillus* spp.

Keywords: *Bacillus*, *Bacteria*, *Phylloplane* and *Rice*,

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 in India, yield losses due to blast could be as high as 50% when the disease attains an epidemic proportion (Lavanya and Gnanamanickam, 2000).

Different approaches have been developed to manage the rice blast disease and to increase the productivity. Chemical fungicides can be used to control diseases, however repeated use of these products has unintended consequences and puts the environment at risk of fungicidal resistance. Consumer demand for environmentally friendly and safe food items is currently quite high. The biocontrol agents will pave the alternative way and will reduce the usage of fungicides in turn can reduce the fungicide contamination and simultaneously promotes the growth of plants. Biological agents are promising tool

for managing disease that can be used alone and in conjunction with other strategies for crop production that are sustainable (Dubey *et al.*, 2015). As blast disease causes infection on the aerial parts of rice crop then, it would be wise to find suitable phylloplane colonizing antagonistic microbes that could survive in the dynamic environmental conditions of phylloplane region and also suppress the pathogen. Phylloplane bacteria are the bacteria that can affect plant growth positively by several mechanisms like nutrient solubilization, siderophore production, biological nitrogen fixation, quorum sensing (QS) signal interference and inhibition of biofilm formation, phytohormone production, exhibiting antagonism towards plant pathogens by secreting cell wall degrading enzymes, antibiotics and secondary metabolites, induction of systemic resistance (ISR), promoting beneficial plant-microbe symbiosis, and many others mechanisms. Hence the present investigation is planned to isolate and characterize the phylloplane bacteria for the management of rice blast

MATERIAL AND METHODS

Sample Collection: Fresh and healthy leaf samples were collected from fields of four different districts viz., West Godavari, Bapatla, Nellore and Tirupati. During sample collection, name of the variety, stage of the crop, geographical location such as village name, district, latitude and longitude details were

West Godavari districts of Andhra Pradesh. The Bacterial isolates were isolated on Hicrome Bacillus agar media by leaf imprinting method. Because of the chromogenic nature of the media, bacterial colonies observed in distinct colours as white, pink, blue, green and orange as shown in (Plate 1(a & b)). The distinct colonies of bacterial isolates on specific media picked up and cultured on nutrient agar media for Gram's staining and their cultural characters.

Gram's staining of phylloplane Bacterial isolates

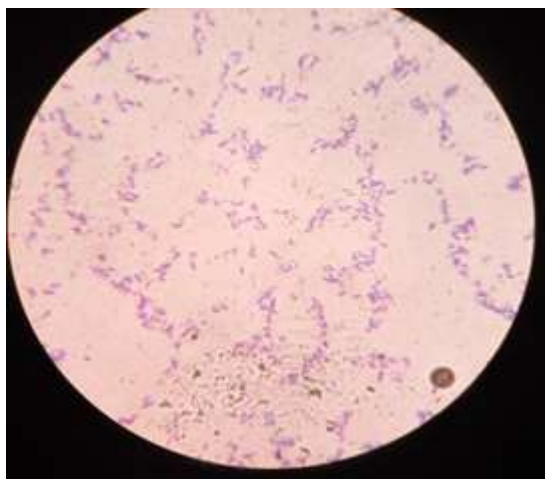
Gram's staining was conducted for all the 34 phylloplane bacterial isolates. Out of 34 isolates, 14 isolates have shown positive reaction to Gram's staining (Plate 2) and remaining 20 isolates showed negative reaction. All the 14 Gram positive bacteria *i.e.*, PNL-07, PTP-08, PTP-09, PTP-12, PTP-13, PBT-16, PBT-19, PBT-22, PWG-25, PWG-26, PWG-27, PWG-29, PWG-30, PWG-34 are found rod shaped under microscopic observations. The 14 Gram positive bacterial isolates were selected for further studies.

Cultural Characterization of Phylloplane Bacterial isolates

The 14 test bacterial isolates were cultured on nutrient agar medium and incubated for 24 to 48 hours in order to perform the cultural characterization of each isolate. Colony characteristics like colour, shape, elevation and margin were investigated. When grown on NA media, each isolate had a different growth pattern. Of the 14 isolates assessed for their cultural characters, 5 isolates; PTP-09, PTP-12,

PTP-13, PWG-25, and 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 isolates varied such as white and creamy white; 9 isolates (PNL07, PTP-08, PTP-12, PTP-16, PBT-22, PWG-26, PWG-29, PWG-30, PWG-34) were white in color and 5 isolates (PTP-09, PTP-13, PBT-19, PWG-25 and PWG-27) were found to be creamy white. Entire margin observed in 2 isolates (PTP13 and PBT22) while in 9 isolates (PNL-07, PTP-09, PTP-12, PBT-16, PBT-19, PWG-25, PWG-26, PWG-27 and PWG-29) it was undulate, 2 isolates (PWG-30 and PWG-34) have rhizoid margin and PTP-08 was with filamentous margin. Elevation of the colony was raised in 7 isolates (PTP-09, PTP-12, PBT-22, PWG-27, PWG-29, PWG-30, PWG-34) while in 7 isolates (PNL-07, PTP-08, PTP-13, PBT-16, PBT-19, PWG-25, PWG-26) it was flat (Table 1; Plate 3). As all the 14 phylloplane bacterial isolates are Gram positive, rod shaped, colony colour is creamy white to white hence they were identified provisionally as *Bacillus* spp. In further studies they were characterized based on biochemical tests, 16S rRNA gene sequence analysis and their antagonistic efficacy against *Magnaporthe oryzae* B.C.Couch was evaluated.

The results are in accordance with Joo *et al.* (2007) who studied 7 strains from Korean soyabean –fermented food, Chungkookjang. All the



Gram positive rod shaped bacterial cells at 40x



Gram positive rod shaped bacterial cells at 100x

Plate 2. Photomicrographs of Gram staining reactions

- Pharmacology and Life Sciences*. 6 (1):194-200.
- Bathlomew J W 1962.** Variables influencing results and precise definition steps in Gram staining as a means of standardizing the results obtained. *Stain Technology*. 37: 139-55.
- Corpe W A 1985.** A method for detecting methylotrophic bacteria on solid surfaces. *Journal of Microbiological Methods*. 3: 215-221.
- Dubey S C, Singh V, Priyanka K, Upadhyay B K and Singh B 2015.** Combined application of fungal and bacterial bio-agents, together with fungicide and Mesorhizobium for integrated management of Fusarium wilt of chickpea. *BioControl* 60: 413-424.
- FAO STAT DATABASE (2022)** <http://www.fao.org/faostat/en/#data/QC>
- Joo M H, Hur S H, Han Y S and Kim J Y 2007.** Isolation, identification, and characterization of *Bacillus* strains from the traditional Korean soybean-fermented food, Chungkookjang. *Journal of Applied Biological Chemistry*. 50(4): 202-210.
- Lavanya B, Gnanamanickam SS 2000.** Molecular tools for characterization of rice blast pathogen (*Magnaporthe grisea*) population and molecular marker-assisted breeding for disease resistance. *Current Science*. 78 (3): 248-257
- Ou S H 1985.** *Rice Disease*. (2nd Edition), Commonwealth Mycological Institute, Kew, Surrey, England. 201.
- Renuka R and Sudhakar R 2017.** Biological control of sheath blight (*Rhizoctonia solani* Kuhn) and stem rot (*Sclerotium oryzae* Catt.) diseases of rice. *Msc Ag (Thesis)*, professor jayashankar Agricultural university of Telanagana Hyderabad.
- Shambhavi Ravindra K, Tomar A, Purushottam Jitender S and Shishu Pal S 2020.** Isolation and Identification of *Bacillus* Species from Soil for Phosphate, Potassium Solubilisation and Amylase Production. *International Journal of Current Microbiology and Applied Sciences*. 9(05): 415- 426.

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