Journal of Biological Control, 37(2): 73-79, 2023, DOI: 10.18311/jbc/2023/34946

Research Article

In vitro assessment of bacterial endophytes for antagonistic activity against *Magnaporthe oryzae* and *Cochliobolus miyabeanus* in rice

ADITYA KUKRETI[1], B. S. CHETHANA[1], M. K. PRASANNAKUMAR[1*], C. MANJUNATHA[2], NAMBURI KARUNAKAR REDDY[1], M. E. PUNEETH[1] and PARTH GULATI[3]

[1]*Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru – 560065, Karnataka, India*
[2]*Division of Genomic Resources, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru – 560024, Karnataka, India*
[3]*Biomaterials Laboratory, Department of Biosciences and Biomedical Engineering, IIT Bhilai, Kutelabhata – 492013, Chhattisgarh, India*
**Corresponding author E-mail:* babu_prasanna@rediffmail.com

ABSTRACT: Rice is the global staple food, contributing over half of the world's agricultural production. Excessive fungicide use in rice farming poses environmental, fungicide resistance, and beneficial microbial disruption concerns, making biocontrol, particularly bacteria, a promising biocontrol agent due to their rapid growth, ease of handling, and robust colonization attributes. Endophytes are endosymbionts residing within host plants, playing a pivotal role in plant health without inciting any pathogenic effects. In this study, we investigated the antagonistic potential of four characterized bacterial endophytic strains, *viz*., *Bacillus velezensis* strains A6 and P42, *B. pseudomycoides* HP3d and *Paenibacillus polymyxa* PGSS-1 against major foliar rice pathogens *viz*., *Magnaporthe oryzae* and *Cochliobolus miyabeanus.* Against *M. oryzae*, all the endophytic strains exhibited significant per cent inhibition (50.00-66.67%) and the highest inhibition of 66.67% was achieved by *B. pseudomycoides* strain HP3d, while the lowest inhibition of 50 and 51.11% was by *B. velezensis* strain A6, P42, and *P. polymyxa* PGSS1, respectively. These endophytes outperformed the positive control, *P. fluorescens* which showed 44.44% inhibition. Similarly, against *C. miyabeanus,* 31.25-43.75% inhibition was recorded, of which *B. velezensis* strain A6 exhibited the highest inhibition (43.75%), while *B. velezensis* strain P42 showed the lowest (31.25%). Furthermore, a double Petri dish assay was conducted to evaluate the volatile compounds produced by these endophytes against the two rice pathogens. It was observed that the volatile compounds produced by *B. pseudomycoides* strain HP3d and *P. polymyxa* strain PGSS1 significantly and effectively inhibited the growth of *M. oryzae* by 88.89% when compared to *P. fluorescens* (50.00%), whereas *B. velezensis* strain A6 showed the lowest inhibition (33.33%). Against the brown spot pathogen, *C. miyabeanus*, the endophytes demonstrated inhibition ranging from 56.25-87.50%, with *B. pseudomycoides* strain HP3d and *P. fluorescens* achieving the highest inhibition (87.50%) and *B. velezensis* strain P42 showing the lowest (56.25%). These results highlight the significant and varied inhibitory effects of volatile compounds released by these endophytes against fungal pathogens of rice. Overall, our findings highlight the promising biocontrol potential of these endophytic strains under *in vitro* conditions with *B. pseudomycoides* strain HP3d and *P. polymyxa* PGSS1 showing exceptional efficacy and they can be deployed in the field for the management of foliar fungal pathogens in rice.

KEYWORDS: Biocontrol, dual culture, double petri dish assay, phytopathogens, volatile organic compounds

(Article chronicle: Received: 04-05-2023; Revised: 29-06-2023; Accepted: 30-06-2023)

INTRODUCTION

Rice stands as the primary staple food for a significant portion of the global population, with India being one of the leading rice producers globally and contributing over half of its total yield (Childs and LeBeau, 2022). Rice diseases consistently pose a substantial threat to global rice production, with an estimated 1-5% annual loss worth billions of dollars. As a result, it is crucial to mitigate outbreaks of diseases and minimize yearly losses to maintain sustainable rice productivity (Mew *et al*., 2004). Among the array of rice diseases, rice blast (*Magnaporthe oryzae*) and brown spot in rice (*Cochliobolus miyabeanus*) are economically important on a global scale (Samal and Parida, 2021). These diseases collectively incur an estimated annual loss of 10–15% in paddy cultivation across the world, with blast disease alone leading to yield losses ranging from one to hundred per cent (Kato, 2001).

Unregulated application of fungicides against these pathogens aimed at augmenting rice yields gives rise to a host of concerns, encompassing environmental hazards, the emergence of pathogen resistance, losses through leaching, and the disruption of beneficial microflora (Slaton *et al*., 2003). Nature hosts a diverse array of biocontrol agents (BCAs), and within this spectrum, bacteria stand out as particularly advantageous. Their rapid growth, ease of handling, and robust colonization attributes position them as an ideal choice for biocontrol efforts.

Plant Growth Promoting Rhizobacteria (PGPRs) are free-living and root-colonizing bacteria which offer valuable benefits to plants. They mitigate disease impact and boost yields across numerous crops. The global emphasis on food quality and environmental health has led to the widespread application of PGPR in various crops, enhancing growth, seedling emergence, and crop yield. Some PGPR products have even reached commercial markets. Additionally, rhizobial inoculants have demonstrated positive effects, including improved nutrient uptake, growth, seedling vigour, and yield in rice cultivation (Chithrashree *et al*., 2011). While the prospect of utilizing PGPR for the biological control of rice diseases has been extensively explored, its implementation has unveiled several limitations, chiefly linked to the vulnerability of PGPRs to various biotic and abiotic stresses (Malfanova *et al*., 2011). Endophytes are beneficial endosymbionts that inhabit host plants, actively contributing to plant well-being without causing any harmful pathogenic effects. Beyond fundamental biocontrol strategies such as the production of siderophores, Hydrogen Cyanide (HCN), volatile and non-volatile organic compounds, as well as lytic enzymes, there exists an intriguing proposition that endophytic bacteria might even trigger plant defence mechanisms *via* a phenomenon termed Induced Systemic Resistance (ISR) (Amruta *et al.* 2018; Prasanna *et al.*, 2021). This approach capitalizes on their unique capability to induce antagonistic effects precisely at the site of infection, facilitated by their remarkable colonization abilities (Hardoim *et al*., 2008). Hence, the present research is directed towards harnessing the potential of endophytic bacteria as potent biocontrol agents**.**

Emerging endophytes like *Bacillus velezensis*, *Paenibacillus* sp., and *B. pseudomycoides* are gaining popularity as biocontrol agents. A Gram-positive bacterium, *B. velezensis*, identified in 2005, promotes plant root development through nutrient uptake and the production of secondary metabolites like indole-3-acetic acid. It also inhibits fungal growth by synthesizing various compounds such as polyketides, glucanase, lipopeptide antibiotics, and iron carriers (Ruiz-Garcia *et al*., 2005; Kim *et al.*, 2017). *Paenibacillus* sp., another endophyte, is known for its release of hydrolyzing enzymes that aid in plant tissue colonization. It forms protective biofilms around plant roots, acting as a barrier against pathogens. Additionally, it emits volatile compounds that inhibit pathogen growth and induce systemic resistance in plants (El-Deeb *et al*., 2013; Timmusk *et al*., 2005). *Bacillus pseudomycoides* NBRC 101232 is a facultative anaerobic Gram-positive endophyte bacterium isolated from tomato roots and was found to be highly effective against *R. solanacearum* (Yanti *et al*., 2018). In the present study, we investigated the potential of endophytic bacteria, *B. velezensis* strains A6 and P42, *P. polymyxa* PGSS-1, and *B. pseudomycoides* HP3d as novel and effective biocontrol agents against rice pathogens *M. oryzae* and *C. miyabeanus*.

MATERIALS AND METHODS

Source of endophytic bacteria and pathogens

Four characterized endophytic bacterial strains *viz*. *B. velezensis* strain A6 (accession number: MSXZ01000332.1), *B. velezensis* strain P42 (accession number: KC692168.3), *P. polymyxa* strain PGSS-1, and *B. pseudomycoides* strain HP3d (accession number: MH465502) were procured from the Bacteriology Laboratory, Department of Plant Pathology, UAS, GKVK, Bengaluru. The samples were initially preserved at a temperature of 4°C and subsequently stored at -20°C in a solution containing 20% glycerol for subsequent investigation. Simultaneously, we procured molecularly characterized phytopathogens *M. oryzae* and *C. miyabeanus* from VC Farm of Mandya, Karnataka, for the study.

In vitro assay

Evaluation of bacterial endophytes against rice pathogens *M. oryzae* and *C. miyabeanus* was carried out by dual culture technique. The six-day-old pathogens grown on Potato Dextrose Agar (PDA) and 48-hour-old bacterial cultures grown on nutrient agar were used for the test. The actively growing pathogen mycelial discs (5 mm) were placed on one side, and bacterial endophytes were streaked on the other side of the petri plate containing PDA media. Three biological replications were maintained, and inoculated plates were incubated at 27±1 °C. The pathogens alone were placed on one end of the petri plate and served as control. The results on the performance of endophytic bacteria against test fungi were recorded after the complete growth of pathogens on control plates. The colony diameter was measured and compared with control plates. Per cent inhibition of the pathogens over control was calculated by adopting the formula given by Vincent, 1947:

$$\text{PIRG} \times \frac{\text{R1 - R2}}{\text{R1}} 100$$

where, PIRG - Per cent inhibition of radial growth, R1- Radial growth of test fungi in control plate, R2 - Radial growth of test fungi in dual culture with endophyte.

Double petri dish assay

The assay was carried out to assess the antifungal activity of Volatile Organic Compounds (VOCs) produced by the bacterial endophytes against rice pathogens *M. oryzae* and *C. miyabeanus*. A 5 mm agar plug from the edge of an actively growing colony of each pathogen was inoculated on the base plate of the Petri plate containing 15 ml PDA. Subsequently, the base plate of another petri plate containing 15 ml NA was streaked with potential bacterial endophytes, and the two base plates were sealed immediately, using a double layer of parafilm to make a closed chamber plate. All the plates were incubated at 27 ±1 °C until maximum growth was observed in control plates (Rouissi *et al*., 2013). The per cent inhibition was calculated by using the formula of Vincent, 1947.

Statistical analysis

The experiments were laid out as a completely randomised design; all data were subjected to ANOVA, and Duncan's Multiple Range Test (DMRT) was performed to separate the group means when ANOVAs were significant at $P<0.05$. The alphabets, given as superscripts in the tables, denote the ranking of treatments based on the DMRT test. All analyses were carried out using the Agricolae package in R version 4.3.1.

RESULTS AND DISCUSSION

Dual culture assay

The performance of different endophytic bacteria against test fungi was recorded after the complete growth of the pathogens in control. All results attained were compared to positive (*Pseudomonas fluorescens*) and negative control (only pathogen). The rice blast pathogen, *Magnaparthe oryzae*, recorded per cent inhibition ranging from 50.00-66.67 (Table 1, Figure 1A) over negative control. Among the tested endophytes maximum per cent of inhibition of 66.67 was recorded with *Bacillus pseudomycoides* HP3d while minimum inhibition of 50.00 and 51.11 was recorded with *B. velezensis* A6, P42 and *Paenibacillus polymyxa* PGSS1 respectively. All bacterial endophytes were significantly effective over *P. fluorescens* as it recorded the least per cent inhibition of 44.44.

Evaluation of endophytes against brown spot pathogen of rice, *Cochliobolus miyabeanus* (Table 1, Figure 1B), showed varied per cent of inhibition ranging from 31.25- 43.75% over negative control. Among endophytes, *B. velezensis* A6 has recorded a maximum inhibition of 43.75%, whereas it was minimum of 31.25% in *B. velezensis* P42. *Pseudomonas fluorescens* recorded 50% and was significantly effective over

Table 1. Effect of endophytic bacterial strains against *Magnaporthe oryzae* and *Cochilobolus miyabeanus* of rice by dual culture and double petri dish assay

	Dual culture				Double petri dish			
Bacterial endophytes	***Magnaporthe oryzae***		***Cochliobolus miyabeanus***		***Magnaporthe oryzae***		***Cochliobolus miyabeanus***	
	RMG (mm)*	% Inhibition **	RMG (mm)*	% Inhibition **	RMG (mm)*	% Inhibition **	RMG (mm)*	% Inhibition **
Bacillus velezensis P42	45^{c}	50.00 $(44.98)^{b}$	55^{b}	31.25 $(38.56)^{e}$	25^{d}	72.22 $(58.17)^{b}$	15^{d}	81.25 $(64.37)^{b}$
Bacillus pseudomycoides HP3d	30^{d}	66.67 $(54.72)^{a}$	52^{c}	35.00 $(40.51)^{d}$	10^{e}	88.89 $(70.52)^{a}$	10^{e}	87.50 $(69.28)^{a}$
Paenibacillus polymyxa PGSS1	45^{c}	50.00 $(44.98)^{b}$	48^{d}	40.00 $(43.07)^{c}$	10^{e}	88.89 $(70.52)^{a}$	35^{b}	56.25 $(48.57)^{d}$
Bacillus velezensis A6	44^{c}	51.11 $(45.62)^{b}$	45^{e}	43.75 $(44.98)^{b}$	60^{b}	33.33 $(35.25)^{d}$	30^{c}	62.50 $(52.22)^{c}$
Pseudomonas florescens (Positive control)	50^{b}	44.44 $(41.79)^{c}$	40^{f}	50.00 $(48.17)^{a}$	45^{c}	50.00 $(44.98)^{c}$	10^{e}	87.50 $(69.69)^{a}$
Control (Negative control)	90^{a}	-	80^{a}	-	90^{a}	-	80^{a}	-

* RMG-Average radial mycelial growth, **% Inhibition-% inhibition of mycelial growth over control ***Means followed by same letters are not significantly different according to DMRT at 5% level. Values in parentheses are arcsine-transformed values

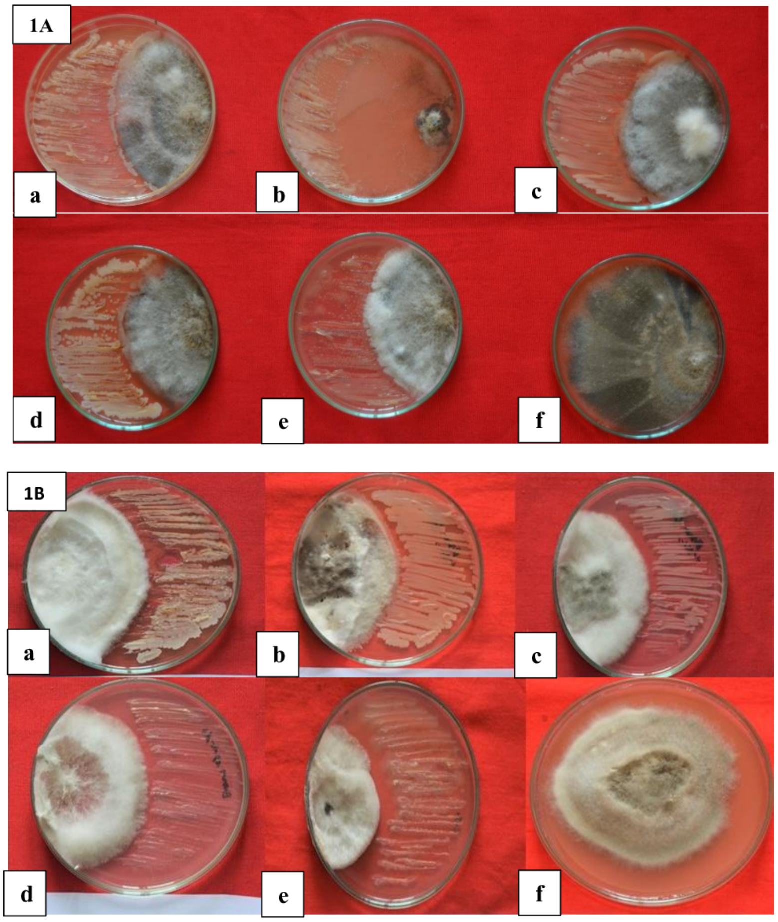

Fig. 1: *In vitro* evaluation of antagonistic potential of bacterial endophytic strains a) P42, b) HP3d, c) PGSS1, d) A6, e) *Pseudomonas fluorescens*, f) Control against A) *Magnaporthe oryzae* and B) *Cochliobolus miyabeanus*, by dual culture assay

all the tested endophytes. The results indicated that all the tested endophytes were effective in inhibiting the mycelial growth of phytopathogens.

Bacteria as endophytes hold great potential as agents for biological control of foliar plant pathogens and for enhancing overall plant growth. To effectively identify and isolate these beneficial indigenous strains, it is imperative to establish a suitable *in vitro* assessment system (Wang *et al.*, 2019). *Bacillus* sp. are well known for their ability to produce antibiotics possessing toxicity against pathogens. Additionally, *Bacillus* sp. can generate endospores capable of surviving in extreme environmental conditions, rendering them valuable for potential applications in biological control (Prasanna *et al.*, 2021). In the present study in dual culture assay, among endophytes, *B. pseudomycoides* HP3d was most effective against *M. oryzae* and showed significant inhibition not only over control but also over *P. fluorescens,* whereas *B. velezensis* A6 was most effective against *C. miyabeanus* but showed less inhibition compared to *P. fluorescens.* The results indicate that for the control of *M. oryzae*, the endophytes were more effective than *P. fluorescens,* a common and one of the most efficient biocontrol commercially available. However, in the case of *C. miyabeanus*, while the endophytes showed varied levels of effectiveness in inhibiting the pathogen, their performance was not as significant as that of *P. fluorescens.* The ability of endophytic strains in the present study to inhibit fungal growth may be attributed to their capacity to produce antibacterial metabolites and hydrolases. These substances come into direct contact with the fungal cell wall, disrupting it and consequently impeding the radial expansion of the fungi (Ruqiya *et al*., 2022). These actions ultimately result in the inhibition of microbial growth or even cell death. The inhibitory capacity of antagonistic bacteria arises from their advantageous characteristics in competitive environments, particularly in terms of spatial competition, nutrient utilization, and oxygen availability. These attributes enable antagonistic bacteria to thrive and effectively suppress the growth of the pathogen (Karim *et al.,* 2018).

Double petri dish assay

In this experiment, a double petri dish assay was used to determine the effectiveness of VOCs produced by the bacterial endophytes in suppressing the growth of

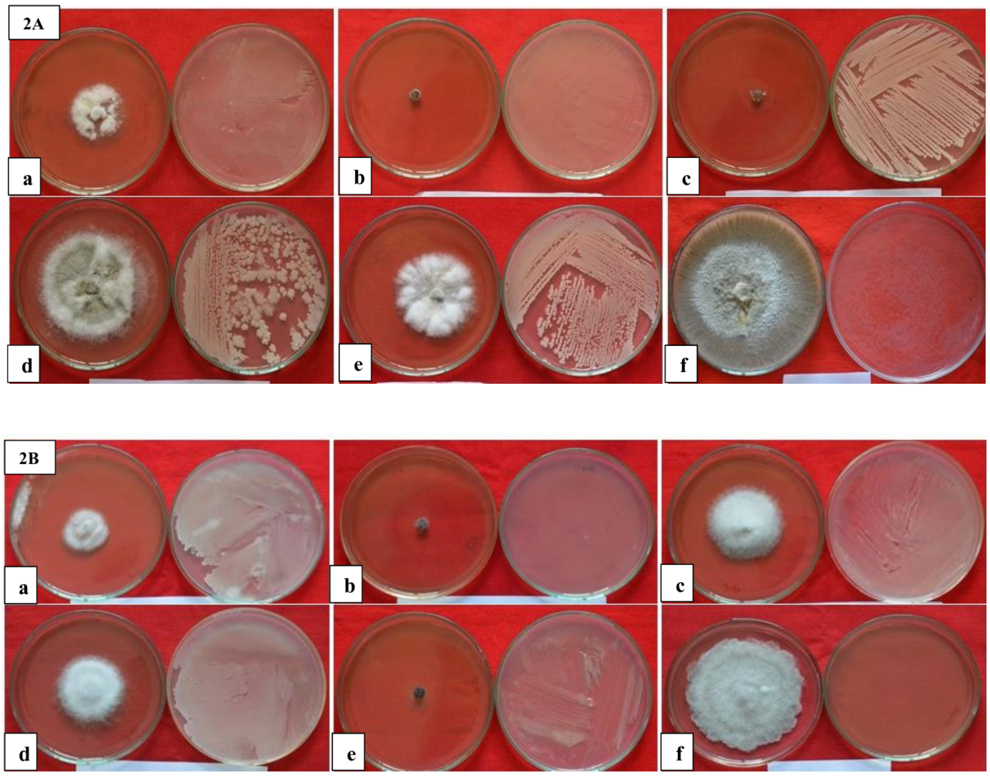

Fig. 2: *In vitro* evaluation of antagonistic potential of bacterial endophytic strains a) P42, b) HP3d, c) PGSS1, d) A6, e) *Pseudomonas fluorescens*, f) Control against A) *Magnaporthe oryzae* and B) *Cochliobolus miyabeanus*, by double petri dish assay

phytopathogens in rice. The bacterial endophytes significantly suppressed the growth of the rice blast pathogen, *M. oryzae*, with per cent inhibition ranging from 33.33 – 88.89 (Table 1, Figure 2A). Among the four bacterial endophytes, *B. pseudomycoides* HP3d and *P. polymyxa* PGSS1 recorded a maximum per cent inhibition of 88.89 and was significant over *P. fluorescens*, which inhibited only 50.00% of fungal growth. *Bacillus velezensis* A6 recorded a minimum of 33.33% inhibition. Evaluation of endophytes against brown spot pathogen of rice, *C. miyabeanus*, showed varied per cent of inhibition ranging from 56.25-87.50 (Table 1, Figure 2B). Among endophytes, *B. pseudomycoides* strain HP3d and *P. fluorescens* recorded a maximum per cent inhibition of 87.50, whereas *B. velezensis* P42 recorded a minimum of 56.25% inhibition. The results revealed that volatiles released by the four bacterial endophytes suppressed the growth of fungal pathogens significantly.

The VOCs produced by endophytic bacteria are of great interest as they play a crucial role in inhibiting the growth and spore germination of plant pathogens. VOCs have a distinct advantage over diffusible antibiotics as they can spread over long distances, creating fungistatic microenvironments in the vicinity of antagonist communities. Consequently, microbial antagonist strains that can produce potent volatile compounds are more likely to prevent pathogenic fungi from infecting plants, eliminate surviving spores in the soil, and curtail both the production and establishment of diseases (Wang *et al.*, 2019). In the present study in double petri dish assay, *B. pseudomycoides* HP3d was most effective against both *M. oryzae* and *C. miyabeanus* and showed significant inhibition over control. The results obtained aligned with those obtained from the dual plate assay and confirmed that for *M. oryzae*, the endophytes proved to be more efficient compared to *P. fluorescens*. However, in the case of *C. miyabeanus*, although the endophytes displayed effectiveness in inhibiting the pathogen, performance was either on par with that of *P. fluorescens* as in the case of *B. pseudomycoides* HP3d or less significant than in the case of other endophytes.

CONCLUSION

The present study underscores the significant biocontrol potential of the characterized bacterial endophytic strains (*Bacillus velezensis* A6 and P42, *Paenibacillus polymyxa* PGSS-1, and *B. pseudomycoides* HP3d) against *Magnoparthe oryzae* and *Cochliobolus miyabeanus*. Through dual culture and volatile assays, these strains exhibited remarkable inhibitory effects on both pathogens, comparable to the performance of the commonly used biocontrol agent

P. fluorescens under *in vitro* conditions, particularly *B. pseudomycoides* HP3d demonstrated exceptional efficacy. These findings hold immense promise for the development of sustainable and effective strategies for rice blast and brown spot disease management, and our research will be further directed towards the identification of volatile compounds responsible for antagonistic activity and exploring the practical applicability of these endophytic strains in the field conditions.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Plant Pathology, UAS, GKVK, Bengaluru for the support received for the smooth conduct of the research.

REFERENCES

Amruta, N., Kumar, M. P., Puneeth, M. E., Sarika, G., Kandikattu, H. K., Vishwanath, K., and Narayanaswamy, S. 2018. Exploring the potentiality of novel rhizospheric bacterial strains against the rice blast fungus *Magnaporthe oryzae*. *Plant Pathol J,* **34**(2): 126. https://doi.org/10.5423/PPJ.OA.11.2017.0242

Childs, N., and LeBeau, B. 2022. *Rice Outlook: August 2022*. Amber Waves: The Economics of Food, Farming, Natural Resources, and Rural America.

Chithrashree, Udayashankar, A. C., Nayaka, S. C., Reddy, M. S., and Srinivas, C. 2011. Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *Biol Control*, **59**(2): 114-122. https://doi.org/10.1016/j.biocontrol.2011.06.010

El-Deeb, B., Fayez, K., and Gherbawy, Y. 2013. Isolation and characterization of endophytic bacteria from *Plectranthus tenuiflorus* medicinal plant in Saudi Arabia desert and their antimicrobial activities. *J Plant Interact*, **8**(1): 56-64. https://doi.org/10.1080/17429145.2012.680077

Hardoim, P. R., van Overbeek, L. S., and van Elsas, J. D. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol*, **16**(10): 463-471. https://doi.org/10.1016/j.tim.2008.07.008

Karim, H., Hamka, L., Kurnia, N., and Junda, M. 2018. Effectivity of antagonistic bacteria in controlling of Fusarium wilt diseases of banana (*Musa paradisiaca*) by *in vitro*. *J Phys*, **1028**: Article 012014. https://doi.org/10.1088/1742-6596/1028/1/012014

Kato, H. 2001. Rice blast disease. *Pestic Outlook*, **12**(1): 23-25. https://doi.org/10.1039/b100803j

Kim, S. Y., Song, H., Sang, M. K., Weon, H. Y., and Song, J. 2017. The complete genome sequence of *Bacillus velezensis* strain GH1-13 reveals agriculturally beneficial properties and a unique plasmid. *J Biotechnol*, **259**: 221-227. https://doi.org/10.1016/j.jbiotec.2017.06.1206

Malfanova, N., Kamilova, F., Validov, S., Shcherbakov, A., Chebotar, V., Tikhonovich, I., and Lugtenberg, B. 2011. Characterization of *Bacillus subtilis* HC8, a novel plant-beneficial endophytic strain from giant hogweed. *Microb Biotechnol*, **4**(4): 523-532. https://doi.org/10.1111/j.1751-7915.2011.00253.x

Mew, T. W., Leung, H., Savary, S., Vera Cruz, C. M., and Leach, J. E. 2004. Looking ahead in rice disease research and management. *Crit Rev Plant Sci*, **23**(2): 103-127. https://doi.org/10.1080/07352680490433231

Prasanna, S., Prasannakumar, M. K., Mahesh, H. B., Babu, G. V., Kirnaymayee, P., Puneeth, M. E., Narayan, K. S., and Pramesh, D. 2021. Diversity and biopotential of *Bacillus velezensis* strains A6 and P42 against rice blast and bacterial blight of pomegranate. *Arch Microbiol*, **203**(7): 4189-4199. https://doi.org/10.1007/s00203-021-02400-9

Ruiz-Garcia, C., Bejar, V., Martinez-Checa, F., Llamas, I., and Quesada, E. 2005. *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river Vélez in Málaga, southern Spain. *Int J Syst Evol*, **55**(1): 191-195. https://doi.org/10.1099/ijs.0.63310-0

Ruqiya, S., Girisha, H., Manjunatha, C., Rangeshwaran, R., Kandan, A., Sivakumar, G., Kumar, M. K. P., Pramesh, D., Shivakumara, K., Venu, H., and Nanditha, S. 2022. Biocontrol potential and molecular characterization of lipopeptides producing *Bacillus subtilis* against *Sclerotinia sclerotiorum*. *Biol Control*, **36**(4): 215-221.

Rouissi, W., Ugolin, L., Martini, C., Lazzeri, L., and Mari, M. 2013. Control of postharvest fungal pathogens by antifungal compounds from *Penicillium expansum*. *J Food Prot*, **76**(11): 1879-1886. https://doi.org/10.4315/0362-028X.JFP-13-072

Samal, S., and Parida, S. 2021. Major fungal diseases of rice: A case study. *Asian J Biol Sci*, **10**(2):469. https://doi.org/10.5530/ajbls.2021.10.62

Slaton, N. A., Cartwright, R. D., Meng, J., Gbur, E. E., and Norman, R. J. 2003. Sheath blight severity and rice yield as affected by nitrogen fertilizer rate, application method, and fungicide. *J Agron*, **95**(6): 1489-1496. https://doi.org/10.2134/agronj2003.1489

Timmusk, S., Grantcharova, N., and Wagner, E. G. H. 2005. *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl Environ *Microbiol,* **71**(11): 7292-7300. https://doi.org/10.1128/AEM.71.11.7292-7300.2005

Vincent, J. M. 1947. The esters of 4-hydroxybenzoic acid and related compounds. Part I. Methods for the study of their fungistatic properties. *J Chem Soc*, **66**(5): 149-155. https://doi.org/10.1002/jctb.5000660504

Wang, X., Li, Q., Sui, J., Zhang, J., Liu, Z., Du, J., Xu, R., Zhou, Y., and Liu, X. 2019. Isolation and characterization of antagonistic bacteria *Paenibacillus jamilae* HS-26 and their effects on plant growth. *Biomed Res Int.* https://doi.org/10.1155/2019/3638926

Yanti, Y., Warnita, W., Reflin, R., and Nasution, C. R., 2018. Characterizations of endophytic *Bacillus* strains from tomato roots as growth promoter and biocontrol of *Ralstonia solanacearum*. *Biodiversitas*, **19**(3): 906-911. https://doi.org/10.13057/biodiv/d190320