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Research Article

CHARACTERIZATION AND EVALUATION OF ANTIFUNGAL POTENTIAL OF **RHIZOSPHERE BACTERIAL COMMUNITIES OF RICE AGAINST** FUSARIUM MONILIFORME AND RHIZOCTONIA SOLANI

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Abstract

Rice (Oryza sativa L.) is one of the staple food crop of Pakistan as well as in the whole world. A number of phytopathogens including bacteria, fungi, nematodes and viruses along with environment associated stresses such as nutrient deficiency, drought, salinity and etc. have a detrimental effect on rice yield. In order to overcome the attack of pathogenic microbes, use of rhizosphere associated bacteria as bio-control agent is an attractive way to minimize the use of toxic agrochemicals. In this study, we screened seven strains of bacterial endophytes from the rhizosphere of rice plants. A colony PCR was performed using universal primers to characterize the isolated bacterial cultures followed by sequence analysis. On the basis of molecular characterization, bacterial isolates were identified as Pantoea sp., Pseudomonas sp., Bacillus megaterium, Pseudomonas flourescens, Lysinibacillus fusiformis, Delftia sp., and Acinetobacter baumnii. Additionally, bacterial isolates were assayed for In vitro effects against Fusarium moniliforme and Rhizoctonia solani causing bakanae and sheath blight disease of rice, respectively. Pantoea sp., Burkholderia sp., Bacillus megaterium and Delftia sp. moderately suppressed Fusarium moniliforme but Pseudomonas flourescens, Lysinibacillus fusiformis both showcased a strong inhibiting activity against Fusarium moniliforme. On the other hand, Pantoea sp., Pseudomonas sp., Bacillus megaterium, Pseudomonas flourescens and Lysinibacillus fusiformis had a strong inhibitory effect against Rhizoctonia solani. The isolated endophytic bacteria were also found to be good producers of phyto-hormones such as hydrogen cyanide (HCN) and catalase. On the basis of our results, we conclude that the endophytic bacteria from rice rhizosphere possess antifungal activity against economic important pathogenic fungi.

Keywords: Bacterial communities, Bio-control, Sustainable agriculture, Rhizosphere, Cash crop

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INTRODUCTION 1

Rice (Oryza sativa L.) is among the world's most important cereal crops, feeding 60% of the world's population (Nawaz et al., 2022).

Fusarium moniliforme causing bakanae disease of rice was first reported in 1990 in Pakistan by Khokhar (Khokhar, 1990). Due to the bakanae disease, about 70% loses were

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estimated in last few years, thus evolving as a major risk factor to food security (Bashyal et al., 2022). While, sheath blight (*Rhizoctonia solani*) of rice, was first recorded from Japan in 1910 by Miyaki (Singh et al., 2015). Several million kilograms per year of fungicides being used by Korea on Rice crop to control sheath blight-disease (Mew and Rosales, 1992; Bhalli et al., 2001).

Microbes have long been recognized to coexist with plants. In certain circumstances, this partnership is beneficial to both parties. Among them, endophytes or rhizosphere inhabiting microbes live in mutualistic interactions inside their hosts without harming them, sharing and trading the advantages of metabolic and physiological processes' products (Reinhold-Hurek and Hurek, 2011; Kraiser et al., 2015). About 30,000 plant species growing on unknown places globally are inhabitant of microbes (Strobel et al., 2004). According to characterization, the organisms which live in a close association on the plants or colonize the internal tissue of plant in some part of their entire life cycle no matter whether it is beneficial, harmful or neutral, are named as Endophytes. The term was coined by De bary (1866) and first reported in plant tissues in 1926, is a large group of ubiquitous microorganisms that have a close association with their host in their life cycle (Kusari and Spiteller, 2012; Hallmann et al., 2012). Microbes make integral part of plant-micro ecology and are not specific to single plant species (Li and Hu, 2005; Rosenblueth and Martínez-Romero, 2007).

Plant associated microbes provide protection to plant and improves plant health and growth (Malhadas et al., 2017). Direct plant growth activation by plant associated bacteria is mostly due to production of hormones and increasing nutrients availability (Mercado-Blanco and Lugtenberg, 2014). Rhizoremediation prevents the seed germination by reducing the pollutant effects as 1aminocyclopropsne-1-carboxylate (ACC) deaminase a bacterial enzyme reported to make plants tolerant to biotic and abiotic stress (Glick et al., 2016; Santoyo et al., 2016).

The thin layer of soil beneath the power of plant roots is known as rhizosphere which is known as hotspot of microbial activities (Hiltner, 1904). Plants roots show more effect on the on soil microbes communities by altering root morphology and root exudation (Berg and Smalla, 2009). These effect microbial diversity, can their population and biological activity around the roots, the so-called rhizosphere effect (Hartmann et al., 2008; Mendes et al., 2013). In root, the amount of microbes is higher than any other part of the plant organ. Average density is 10⁵ cfu per g of fresh weight. The average amount of 10^4 and 10^3 are reported for the stem and leaf portions, respectively (Hallmann and Berg, 2006; Compant et al., 2021). As, various previous studies has reported the inhibitory effect of endophytes against bacterial and fungal phyto-pathogens (Ramesh et al., 2012; Mingma et al., 2014). Therefore, the present work was planned to

isolate. characterize the bacterial communities associated with rice rhizosphere. Determination of In vitro antifungal potential of isolated bacteria against Rhizoctonia solani and Fusarium moniliforme causing sheath blight and bakanae diseases of rice, respectively. The extracted bacterial isolates from the rhizosphere of rice has the potential to serve as a biocontrol agents against economic important patho-syestems of rice, while reducing the reliance of farmers on synthetic toxic pesticides.

2 MATERIALS AND METHODS 2.1 Sampling and Experimental Site

The rhizospheric soil [5.61% clay (< 2 mm), 41.73% silt (> 2 mm), 50.88% sand (> 63 mm)] samples from rice (vegetative growth collected from stage) were Agriculture Farm (31°29'43.0"N 74°17'49.2"E) of University of the Punjab, Lahore, Pakistan (Rasool et al., 2021). Samples were collected in define patterns W, M, X and Z patterns. These samples were collected in plastic sampling bags and stores at 4°C for further use. Plot number was also mentioned on the plastic sampling bags and sealed with rubber band (Forster et al., 1995).

2.2 Culture Media

In order to isolate bacteria, Bertani (1951) Luria Bertani Agar (LBA) bacterial media preparation technique was followed. While, two types of general fungal culture media potato dextrose agar (PDA) were prepared to Fusarium moniliforme culture and Rhizoctonia solani by methods previously reported (Aryal, 2018). For fungal pathogens propagation, PDA was autoclaved at 121°C for 15-20 min at 15 psi on liquid cycle (Mazhar et al., 2021). Pouring of both the media were done in sterilized laminar flow. About 20 mL of media was poured into presterilized disposable plastic plates. All the plates were labeled and stored upside down in refrigerator.

2.3 Isolation of Bacteria

Serial dilution method was used to isolate the rhizospheric microbes associated with the rice roots. The rhizospheric soil samples were diluted upto 10 folds as described by Ben-David *et al.* (2014). The diluted samples were inoculated with the help of dropper on the LB agar plates and spread uniformly with the help of drigalski spatula. Afterwards, the parafilm was used to seal the plates and incubated at 25°C for 24 hrs (Wafula et al., 2015). Streaking method was used to purify the bacterial colonies. Pure bacterial isolates were obtained (Jagessar et al., 2008). Seven pure cultures with different morphological characters were selected for further analysis.

2.4 Fungal Pathogens

Rhizoctonia solani and Fusarium moniliforme culture was taken from FCBP

(First Fungal Culture Bank of Pakistan) University of the Punjab Lahore, Pakistan.

2.5 Bio-chemical characterization of bacterial isolates

2.5.1 Gram staining

Gram staining was performed to differentiate between two major groups of bacteria namely Gram positive and/or gram negative. All the seven fresh cultured strains of bacteria were tested. Results were assessed on the base of colony color after staining. Bacterial colony which shows purple color after staining belongs to gram positive group and colony gives pink color after staining belongs to gram negative group of bacteria (Coico, 2006).

2.5.2 Catalase test

To check the availability of catalase enzyme in the interested strains of bacteria according to Coico (2006) catalase test was performed. Formation of O_2 indicates that bacterial isolates produces catalase enzyme and lack of O_2 indicates no catalase production.

2.5.3 Hydrogen cyanide test

Hydrogen cyanide test was performed to check the potential of bacterial isolates to synthesize the hydrogen cyanide. Bakker and Schipper (1987) method was adopted to perform the hydrogen cyanide test.

2.6 Morphological Characterization of Bacterial isolates

Macro-character such as colony color, colony appearance, colony texture were observed with naked eye as well as with the help of compound microscope micro-characters such as bacterial cell size and shape were observed (Holt et al., 1994).

2.7 Molecular characterization

Colony PCR method was used to amplify the bacterial colonies. Fresh colony of bacteria was added into PCR reaction mixture tube. For the amplification of bacterial gene coding universal primers 63F (5⁻-CAGGGCCTAACACATGCAAGTC-3⁻) and 1387R (5⁻-CGGCGGWGTGTACAAGGC-3⁻) for 16s

RNA were used (Marchesi et al., 1998) for the sequencing of amplified bacterial DNA in PCR tubes was sent to Advance bioinformatics lab located in Lahore. The sequenced DNA was then used to identify the Unknown bacterial isolates. The DNA was analyzed by blasting the sequence on NCBI (<u>https://blast.ncbi.nlm.nih.gov/Blast.cgi?PR</u> <u>OGRAM=blastn&PAGE_TYPE=BlastSearc</u> <u>h&LINK_LOC=blasthome</u>). With most similar strains in data bank were analyzed, selected and studied with their accession numbers.

2.8 In-vitro anti-fungal assays

An *in-vitro* antagonistic effect was observed between all the seven bacterial isolates and pathogenic fungus *Rhizoctonia solani* and *Fusarium moniliforme* causing sheath blight and bakanae diseases, respectively. To study the antagonistic activity isolates were subjected to dual culture technique (Francisco et al., 2011). Three replicates of each reaction were prepared (Kumar et al., 2002). A negative control was also placed for both of the pathogenic fungi, which includes only fungal plug in the center of PDA plate with no bacterial treatment (Shobha and Kumudini, 2012). All the plates were made air tight by wrapping with parafilm. Experimental plates were incubated at 30°C for 5 days. After 5 days reading and pictures were taken and percentage inhibition was calculated.

% inhibition = $\underline{\text{Control}} - \underline{\text{growth of fungi with bacteria}} \times 100$ Control

2.9 Statistical Analysis

Data were analyzed at $P \le 0.05$ by Statistix 8.1 software. Percentage data were transformed and then analyzed.

3 RESULTS

Morphological Characterization of Bacterial Isolates

Isolated bacterial isolates were morphologically characterized on the basis of their colony appearance or texture, shape, growth pattern and color as shown in table 1.

Isolates	Colony Color	Colony Shape	Colony Appearance	Bacteria Shape
Isolate 1	Whitish yellow	Muciod	Irregular	Spherical
Isolate 2	White	Circular	Opaque	Small rod shape
Isolate 3	Off white	Irregular	Crystalline	Rod shaped
Isolate 4	Pale yellow	Spherical	Opaque	Rod shaped
Isolate 5	Off white	Round	Wrinkled	Slender rod shaped
Isolate 6	Creamy	Circular	Smooth	Rod shaped
Isolate 7	Off white	Round	Transparent	Rod shaped

 Table 1: Morphological characters of isolated bacteria.

3.1 Molecular Characterization of Bacterial isolates

3.1.1 Colony PCR amplification of isolates

Amplification of size (1-1.3 kb) was obtained from isolated colonies. The PCR results were confirmed in 1% agarose gel using standard 1 kb DNA marker. The PCR product analyses are shown in Figure 1.

3.1.2 Sequence Analysis

According to NCBI blast results (<u>https://blast.ncbi.nlm.nih.gov/Blast.cgi</u>), an isolate 1 has a maximum homology (100%) with the *Pantoea sp.* (Genbank accession id: MF52168.1). Isolate 2 has a maximum homology (100%) with the *Pseudomonas sp.*

(Genbank accession id: ON624237.1), Isolate 3 has a maximum homology (100%)



Figure 1: PCR amplification of isolated endophytic bacterial isolates using universal primers. Lane M: marker ladder. Lane 5, 8, 9, 10, 11, 12, and 15 no product. Lane 1 and 18: negative control. Lane 3, 4, 6, 7, 13, 14 and 17 amplified PCR product of desired size (1.3kb).

Table 2: NCBI Blast results of bacterial isolates with homology to the strain, and the record of GenBank accession numbers of the sequences deposited at NCBI databank.

Isolates	Isolate Source	Homology	Strain	Genbank		
				accession		
				Nos.		
1	Rice rhizosphere	100%	Pantoea sp. (MF52618.1)	OP686478		
2	Rice field Soil	100%	Pseudomonas sp. (ON624237.1)	OP686479		
3	Rice rhizosphere	100%	Bacillus megaterium (CP035098.1)	OP686484		
4	Rice Field Soil	97%	Acinetobacter baumani	OP686483		
			(HQ645939.1)			
5	Rice rhizosphere	100%	Pseudomonas flourescens	OP686482		
			(MN099290.1)			
6	Rice rhizosphere	99%	Lysinibacillus fusiformis (KP13773)	OP686481		
7	Rice	100%	Delftia sp. (LC474083)	OP686480		
	rhizospheric Soil					

with the *Bacillus megaterium* (Genbank accession id: CP035098.1). Isolate 4 has a maximum homology (97%) with the *Acinetobacter baumanni* (Genbank accession id: MF52168.1). Isolate 5 has a maximum homology (100%) with the *Pseudomonas flourescens* (Genbank accession id: MN099290.1). Isolate 6 has a maximum homology (99%) with the *Lysinibacillus* (Genbank accession id: KP813773). Isolate 7 has a maximum homology (100%) with the

Delftia sp. (Genbank accession id: LC474083). NCBI results and GenBank accession numbers are summarized in Table 2.

3.2 Biochemical assays of Bacterial Isolates

3.2.1 Gram staining

Gram staining of isolated bacterial isolates revealed that all of the bacterial isolates (*Pantoea* sp, *Pesudomonas* sp, *Bacillus* megnaterium, Acinetobacter baumani, Pesudomonas flourescens, Delftia sp.) belong to the gram negative group of bacteria except Lysinibacillus fusiformis (a gram positive bacteria). Occurrence of pink colony color under the microscope identified the grame negative bacteria and purple colony color indicated the gram positive bacteria (Table 3).

3.2.2 Hydrogen cyanide test

Hydrogen cyanide (HCN) production test of bacterial isolates proves that all seven bacterial isolates produce a significant amount of HCN (Table 3). Hydrogen cyanide was determined by observing the yellow to reddish color of filter paper placed on the lid of petri plate. All the bacterial isolates including Pantoea sp, Pesudomonas sp, **Bacillus** *megnaterium*, Acinetobacter baumani. Pesudomonas flourescens, Lysinibacillus fusiformis and Delftia sp. were capable of producing phyto-hormone HCN which is major consistuent in plant protection and growth.

3.2.3 Catalase test

Catalase enzyme was produced by all rhizospheric bacterial strains. When a bacterial colony was inoculated into the hydrogen per-oxide solution, bacteria produced oxygen (bubble formation) in the precense of substrate. The formation of bubbles indicated that the isolated bacterial strains, in the absence oxygen, converted hydrogen per-oxide into oxygen gas. All of the bacterial isolates had shown a positive catalase test result (Table 3).

3.2.4 *In-vitro* anti-fungal assay

Results of bio control ability of bacterial isolates were evaluated on the basis of a scale

ranging from 1 to 3, where 1 = highly suppressed, 2= moderately suppressed and 3= little or no effect as shown in Table 4, as well as by using percentage inhibition formula: percentage inhibition = obtained growth of fungi/ total growth of fungi (control) \times 100 as shown in Table 5. All the data was calculated after 5 days of inoculation. Pantoea sp. has shown moderate effect against Fusarium moniliforme and Rhizoctonia solani with 51% and 57 % suppression, respectively. Pseudomonas sp. has also produced moderate results against Fusarium moniliforme and Rhizoctonia solani with 57% and 62 % of suppression. Bacillus megaterium has shown 43 % inhibition of Fusarium moniliforme but strong suppressing ability of 74% against Rhizoctonia solani. Acinetobacter baumnii little effect on has very Fusarium moniliforme, while moderate effect against Rhizoctonia solani with 26% and 57% inhibition. respectively. Pseudomonas flourescens was very suppressive against both Fusarium moniliforme and Rhizoctonia solani having 62% and 65% inhibition respectively. Lysinibacillus ability. fusiformis also has shown 63% and 64% of suppressing effect against Fusarium moniliforme and Rhizoctonia solani. Delftia sp. shows a moderate 54% and 58%, respectively antifungal effect against Fusarium moniliforme and Rhizoctonia solani.

	Bio-Chemical Assays						
Bacterial Endophytes	Gram staining		Catalase test		HCN		
	Positive	Negative	Positive	Negative	Positive	Negative	
Pantoea sp.		~	~		~		
Pseudomonas sp.		~	~		~		
Bacillus megnaterium		~	✓		~		
Acinetobacter baumani		~	✓		~		
Pesudomonas		~	~		~		
flourescens							
Lysinibacillus	~		~		~		
fusiformis							
Delftia sp.		~	✓		~		

Table 3: The biochemical assays of isolated bacterial endophytes.

Table 4: Interaction effect of bacterial isolates against *Rhizoctonia solani* and *Fusarium moniliforme*.Results were evaluated according to the scale standardized as, 1=Suppressed, 2=moderately suppressed and 3=little or no effect.

Treatments	Interaction Effect						
	Fusarium moniliforme			Rhizoctonia solani			
	1	2	3	1	2	3	
Pantoea sp.		√			✓		
Pesudomonas sp.		√			✓		
Bacillus megnaterium		√		×			
Acinetobacter baumanii			~		✓		
Pesudomonas flourescens	✓	\checkmark		×			
Lysinibacillus fusiformis	✓			✓			
Delftia sp.		\checkmark			✓		

T	Percentage Inhibition					
Treatments	Fusarium moniliforme	Rhizoctonia solani				
Pantoea sp.	51%	57%				
Bukholderia sp.	57%	62%				
Bacillus megnaterium	43%	74%				
Acinetobacter baumani	26%	57%				
Pesudomonas flourescens	62%	65%				
Lysinibacillus fusiformis	63%	64%				
Delftia sp.	54%	58%				

 Table 5: Percentage inhibition of bacterial isolates against Rhizoctonia solani and Fusarium solani.

4 DISCUSSION

Rice associated bacterial strains were isolated, from University of the Punjab, Lahore, Pakistan and characterized for their antifungal potential against the plant pathogenic fungi causing bakanae and Sheath Blight of rice to encourage the bio-pesticide development and use. Pant or rhizosphere associate bacteria are known to not only increase the resistance against pathogenic microbes but also provides better growth and maximize the yield. Beneficial microflora has the potential in managing the abiotic and biotic stress inducing factors through growth promotion and instigating defense response by producing specific metabolites (Majeed et al., 2018; Berendsen et al., 2018; Atiq, 2022). Soil associated microbiomes are involved in number of important ecosystem functions including nutrient cycling, soil organic matter decomposition, soil formation and thereby indirectly affecting plant growth. Among the soil micro biota, bacteria are abundant in number and diverse as one gram of soil contains up to 109 bacteria consisting of 10,000 to 50,000 bacterial taxa (Bulgarelli et al., 2013). The bacterial communities composition in the soil was known to greatly affected by biotic and abiotic factors mainly soil pH (Lauber et al., 2009), salinity (Rajaniemi and Allison 2009), type (Griffiths et al., 2011), structure (Sessitsch et al., 2001), moisture (Cruz-Martínez et al., 2009) and soil organic matter (Blaud et al., 2015).

Bacterial isolates (Pantoea sp.) inhibit pathogenic influence of Rhizoctonia solani, Phythium mytrotyl and Fusarium moniliforme (Hallmann et al., 1997). Four types of phyto-hormones (IAA, abscisic acid, gibberelic acid, and cytokinin) along with phyto-stimulants, and fix atmospheric N₂ in rice (Mano and Morisaki, 2008). According to Verma et al. (2001) Pantoea sp. had shown highly aggressive endophytic antagonistic influence through colonization in deep water rice. While, Bacillus megaterium depicted a inhibitions against Fusarium strong moniliforme and Rhizoctonia solani. As reported to produce extracellular metabolites suppresses that fungal sporulation, elongation and mycelial growth. Bacillus effectively megaterium has reduced Ralstonia solanacearum induced bacterial wilt disease (Nguyen et al., 2011). Rghunath et al. (2012) identified the antibacterial as well as antifungal potential of Bacillus megaterium (Rao et al., 2011). The bacterial population found especially in the roots of Putterlickia verrucosa and Putterlickia retrospinosa plants is responsible for the manufacture of the significant anticancer and cytotoxic chemical maytansine (Kusari et al., 2014).

According to our findings Psudomonas sp. have shown strong bio-control ability against the *Fusarium moniliforme* and *Rhizoctonia solani* causing bakanae and Sheath blight disease on rice plants by producing phytohormons and biostimulants.

Burkholderia sp. take over the pathogen by secreting specific enzymes and hormones to control disease. For example, *Burkholderia gulmae* a pathogen causing root rot and grain rot in rice is inhibited by the Burkholderia sp. Most species of Burkholderia shows antagonistic activity and producing siderophores against the pathogens to reduce disease in rice crop (Loaces et al., 2011).

Pseudomonas flouorescens has been widely used for controlling sheath blight of rice (Vidhyasekaran and Muthamilan, 1999). The bacterium has the ability to produce certain antifungal compounds such as cell lytic and enzymes (3-glucanase degrading and chitanses) (Lim et al., 1991; Velazhahan et al., 1999). Through siderophore production it the chlamydospores growth suppresses (Bakker et al., 1986) and Wheat foliar pathogens (Septoria tritici, Puccinia recondita) tobacco black root by HCN production (Voisard et al., 1989; Flaishman, 1996). Pseudomonas flouorescens has been involved in the ISR induction of host plants (Maurhofer et al., 1998). Pseudomonas flouorescens proves to be best biological control agent which successfully decreases the disease incidence and increases the plant weight (Srivastava et al., 1999).

Acinetobacter baumanii produce metabolites that inhibit fungal growth and can serve as efficient bio-control agent (cook, 1993; Raaijmakers et al., 2002; Ranjbariyan et al., 2011). Delftia sp were first reported as PGPR of rhizosphere of rice (Han et al., 2005). These endophytic bacteria contributed towards the biological control of fungal pathogens (Mukhopadhyay et al., 1996) and plant parasitc nematodes (Hallmann et al., 1995). In our study Delftia sp. had shown strong antifungal potential against the *R*. *solani* and *F. moniliforme*. Delftia sp. was also reported as antagonistic to *Phytopathora nicotianae* (kummar et al., 2002).

Lysinibacillus fusiformis has nitrogenase activity and shown radial growth inhibition against *F. oxysporum*, *F. moniliforme*, *F.* solani and *M. phaselonia* (Singh et al., 2015). Lysinibacillus fusiformis produces numerous hormones (Kumar et al., 2002); that promote plant growth, chitinase which make it efficient biocontrol of fungal pathogens (Hoster et al., 2005). In our study, we also found a significant antifungal activity of *L. fusiformis* against *R. solani* and *F. moniliforme*.

The plant host and bacterial endophytes interaction plausibly resulted into the raise of plant growth and health. Endophytic bacteria can play an important role in the regions wish to practice low input sustainable agriculture (Robert et al., 2008). The most useful way for the application of bacterial endophytes in agriculture is still unknown. The method of inoculating bacteria was mostly unsuccessful on large fields due to their association with other biological agents (O`Callaghan, 2016). Application of beneficial microbes to minimize the threats to agriculture is not possible as most of the conventional agriculture relying on use of pesticides, fungicides and inorganic fertilizers because these factor reduces the plant dependency on bacterial and fungal endophytes (Hardoim et al., 2015). Till now there is no commercial product of available in market but in the coming years the bacterial isolates can be used as plant growth promoters and bio fertilizers to increase the yield of various crops.

5 Conclusion and Future Perspectives

Plant associated bacterial communities are poorly studied group of microorganisms that represent an abundant and significance phyto-hormones. source of bioactive compounds, metabolites and their resistance against the pathogenic microbes with a potential to serve in wide range of agriculture, medical and industrial areas. Bacterial isolates have the potential to control the present and emerging pathogens, also the biotic stresses like climate change. In our study, seven selected strains of isolated bacterial had shown a strong antifungal potential against two main fungal pathogen causing bakanae disease and sheath blight of rice.

Isolated bacteria can serve as potential biocontrol agents against fungal pathogens of economic important cash crops. They will certainly help in protecting the environment from toxic chemicals and providing a healthy living environment. Future work is required to understand the mechanism of action and how they interact with surrounding microbial diversity in order to be more efficient and productive in their use. Thus, bacterial isolates obtained have shown the potential to be developed as commercial bio-control products with the cooperation of indigenous agri-based industry.

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7 Conflict of interest

The authors have declared no conflict of interest.

8 REFERENCES

- Atiq, M. (2022). A way forward towards the management of chilli anthracnose - a review. Agricultural Sciences Journal, 4(1), 1-10.
- Aryal, S. 2018. Potato Dextrose Agar (PDA)-Principle. *Uses*, Composition,

Procedure and Colony Characteristics.

- Aurangzeb, M., J. Ahmed, and M. B. Ilyas. 1998. Chemical control of Bakanae disease of rice caused by Fusarium moniliforme. Pakistan.J. Phytopathol. 10:14-17.
- Bakker, A. W. and B. O. B. Schippers. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas sppmediated plant growthstimulation. Soil. Biol. Biochem. 19:451-457.
- Bashyal, B. M., K. Rawat, P. Parmar, A. K. Gupta, S. Gupta, S. G. Krishnanan, R. Choudhary, S. Ercisli, A. Kovacevic, and R. Aggarwal. 2022. Transcriptomic analysis of bakanae disease resistant and susceptible rice genotypes in response to infection by Fusarium fujikuroi. Molecular Biology Reports, 1-14.
- Ben-David, A. and C. E. Davidson. 2014. Estimation method for serial dilution experiments. J. Microbiol. Methods. 107:214-221.
- Berendsen, R. L., G. Vismans, K. Yu, Y. Song, R. de Jonge, W. P. Burgman, M. Burmølle, J. Herschend, P. A. Bakker, and C. M. Pieterse. 2018. Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME. J. 12:1496-1507.
- Berg, G. and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68:1-13.
- Bertani, G. 1951. Studies on lysogenesis I: the mode of phage liberation by lysogenic Escherichia coli. J. Biol. Chem. 62:293-300.
- Blaud, A., F. Diouf, A. M. Herrmann, and T. Z. Lerch. 2015. Analysing the effect

of soil organic matter on bacterial communities using T-RFLP fingerprinting: different methods, different stories? Biol. Fertil. Soils. 51:959-971.

- Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. Van Themaat, and P. Schulze-Lefert. 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant. Biol. 64:807-838.
- Hernandez Castillo, F. D., A. M. B. Padilla, G. G. Morales, M. C. Siller, R. R. Herrera, C. N. A. Gonzales, and F. C. Reyes. 2011. In vitro antagonist action of Trichoderma strains against Sclerotinia sclerotiorum and Sclerotium cepivorum. Am. J. Agric. Biol. Sci. 6:410-417.
- Coico, R. 2006. Gram staining. Curr. Protoc. Microbiol. 1: A-3 C.
- Cook, R.J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. Annu. Rev. Phytopathol. 31:53-80.
- Compant, S., M. C. Cambon, C. Vacher, B. Mitter, A. Samad and A. Sessitsch. 2021. The plant endosphere world– bacterial life within plants. Environ. Microbiol. 23:1812-1829.
- Cruz-Martínez, K., K. B. Suttle, E. L. Brodie, M. E. Power, G. L. Andersen, and J. F. Banfield. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to longterm changes in rainfall than overlying grassland. ISME. J. 3:738-744.
- Flaishman, M. A., Z. Eyal, A. Zilberstein, C. Voisard, and D. Haas. 1996. Suppression of Septoria tritici blotch and leaf rust of wheat by recombinant cyanide-producing strains of

Pseudomonas putida. Mol. Plant. Microbe. Interact. 9:642-645.

- Forster, J.C. 1995. Soil sampling, handling, storage and analysis. In: Methods in applied soil microbiology and biochemistry. Academic Press. pp. 49-121.
- Griffiths, R. I., B. C. Thomson, P. James, T. Bell, M. Bailey, and A.S. Whiteley. 2011. The bacterial biogeography of British soils. Environ. Microbiol. 13:1642-1654.
- Hallmann, J. and G. Berg. 2006. Spectrum and population dynamics of bacterial root endophytes. In: Schulz, B. J. E., Boyle, C. J. C., Sieber, T. N. (eds) Microbial Root Endophytes. Soil Biology, 9. Springer, Berlin, Heidelberg. 15-31.
- Hallmann, J., J. W. Kloepper, R. Rodriguez-Kabana, and R. A. Sikora. 1995. Endophytic rhizobacteria as antagonists of Meloidogyne incognita on cucumber. Phytopathology. 85:136.
- Hallmann, J., A. Quadt-Hallmann, W. F. Mahaffee, and J.W. Kloepper. 1997.Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43:895-914.
- Han, J., L. Sun, X. Dong, Z. Cai, X. Sun, H. Yang, Y. Wang, and W. Song. 2005. Characterization of a novel plant growth-promoting bacteria strain Delftia tsuruhatensis HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst. Appl. Microbiol. 28:66-76.
- Hartmann, A., M. Rothballer, and M. Schmid. 2008. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil. 312:7-14.
- Hardoim, P. R., L. S. Van Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A.

Campisano, M. Döring, and A. Sessitsch. 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. 79:293-320.

- Hiltner, L. 1904. Uber nevere Erfahrungen und Probleme auf dem Gebiet der Boden Bakteriologie und unter besonderer Beurchsichtigung der Grundungung und Broche. Arbeit. Deut. Landw. Ges. Berlin. 98:59-78.
- Holt, J. G., N. R. Krieg, and P. H. Sneath. 1994. Bergey's manual of determinative bacterology.
- Hoster, F., J. E. Schmitz, and R. Daniel, 2005. Enrichment of chitinolytic microorganisms: isolation and characterization of a chitinase exhibiting antifungal activity against phytopathogenic fungi from a novel Streptomyces strain. Appl. Microbiol. Biotechnol. 66:434-442.
- Jagessar, R. C., A. Mars, and G. Gomes. 2008. Selective Antimicrobial properties of Phyllanthus acidus leaf extract against Candida albicans, Escherichia coli and Staphylococcus aureus using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method. Nature and science. 6:24-38.
- Kaiser, C., M. R. Kilburn, P. L. Clode, L. Fuchslueger, M. Koranda, J.B. Cliff, Z. M. Solaiman, and D. V. Murphy. 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New Phytol. 205:1537-1551.
- Khokhar, L.K. 1990. Bakanae and foot rot of rice in Punjab, Pakistan. Int. Rice Res. News. 3:15.

- Kusari, S. and M. Spiteller. 2012. Metabolomics of endophytic fungi producing associated plant secondary metabolites: progress, challenges and opportunities. J. Metabolomics. 10:241-266.
- Kusari, S., M. Lamshöft, P. Kusari, S. Gottfried, S. Zühlke, K. Louven, and M. Spiteller. 2014. Endophytes are hidden producers of maytansine in Putterlickia roots. J. Nat. Prod. 77:12, 2577-2584. Lauber, C.L., M. Hamady, R. Knight, and N. Fierer. 2009. Soil pH as a predictor of soil bacterial community structure at the continental scale: a pyrosequencingassessment. Appl. Environ. based Microbiol. 75: 5111-5120.
- Li, W. K. and Z. B. Hu. 2005. Endophytes and natural medicines. Chin. J. Nat. Med. 3:193-199.
- Lim, H. S., Y. S. Kim, and S. D. Kim. 1991. Pseudomonas stutzeri YPL-1 genetic transformation and antifungal mechanism against Fusarium solani, an agent of plant root rot. Appl. Environ. Microbiol. 57:510-516.
- Loaces, I., L. Ferrando, and A. Fernández Scavino. 2011. Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microb. Ecol. 61:606-618.
- Majeed, A., Z. Muhammad, and H. Ahmad. 2018. Plant growth promoting bacteria: role in soil improvement, abiotic and biotic stress management of crops. Plant Cell Rep. 37:1599-1609.
- Mano, H. and Morisaki, H., 2008. Endophytic bacteria in the rice plant. Microbes Environ. 23:109-117.
- Malhadas, C., R. Malheiro, J. A. Pereira, P.G. de Pinho, and P. Baptista. 2017. Antimicrobial activity of endophytic

fungi from olive tree leaves. World J. Microbiol. Biotechnol. 33:1-12.

- Marchesi, J. R., T. Sato, A. J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, and W.G. Wade. 1998. Design and evaluation of useful bacteriumspecific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64:795-799.
- Maurhofer, M., C. Reimmann, P. Schmidli-Sacherer, S. Heeb, D. Haas, and G. Défago. 1998. Salicylic acid biosynthetic genes expressed in Pseudomonas fluorescens strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology. 88:678-684.
- Mazhar, H.R., M.F. Ullah, M. Atiq, N.A. Rajput, S. Ali, M. Usman, U. Ahmad, A. Nawaz, and S. Iqbal. 2021. Determination of antibacterial activity of phytochemicals towards *Xanthomonas citri* pv. *citri* causing citrus canker. Agricultural Sciences Journal, 3(2):1-12.
- Mendes, R., P. Garbeva, and J. M. Raaijmakers. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. 37:634-663.
- Mercado-Blanco, J. and B. J. J. Lugtenberg. 2014. Biotechnological applications of bacterial endophytes. Curr. Biotechnol. 3:60-75.
- Mew, T.W. and A.M. Rosales. 1992. Control of Rhizoctonia sheath blight and other diseases of rice by seed bacterization. In Biological control of plant diseases. Springer, Boston. MA. 113-123.

- Mingma, R., W. Pathom-aree, S. Trakulnaleamsai, A. Thamchaipenet, and K. Duangmal. 2014. Isolation of rhizospheric and roots endophytic actinomycetes from Leguminosae plant and their activities to inhibit soybean pathogen, Xanthomonas campestris pv. glycine. World J. Microbiol. Biotechnol. 30:271-280.
- Mukhopadhyay, K., N. K. Garrison, D. M. Hinton, C. W. Bacon, G.S. Khush, H.D. Peck, and N. Datta. 1996. Identification and characterization of bacterial endophytes of rice. Mycopathologia. 134:151-159.
- Nawaz, A., A. U. Rehman, A. Rehman, S. Ahmad, K. M. Siddique, and M. Farooq. 2022. Increasing sustainability for rice production systems. J. Cereal Sci.103:103400.
- Nguyen, M. T., S. L. Ranamukhaarachchi, and D. B. Hannaway. 2011. Efficacy of antagonist strains of Bacillus megaterium, Enterobacter cloacae, Pichia guilliermondii and Candida ethanolica against bacterial wilt disease of tomato. J. Phytol. 3:2
- O'Callaghan, M. 2016. Microbial inoculation of seed for improved crop performance: issues and opportunities. Appl. Microbiol. Biotechnol. 100:5729-5746.
- Raaijmakers, J. M., M. Vlami, and J. T. De Souza. 2002. Antibiotic production by bacterial biocontrol agents. Antonie van leeuwenhoek. 81:537-547.
- Rajaniemi, T. K. and V. J. Allison. 2009. Abiotic conditions and plant cover differentially affect microbial biomass and community composition on dune gradients. Soil Biol. Biochem. 41:102-109.
- Ramesh, R. and G. S. Phadke. 2012. Rhizosphere and endophytic bacteria

for the suppression of eggplant wilt caused by Ralstonia solanacearum. J. Crop Prot. 37:35-41.

- Ranjbariyan, A. R., M. Shams-Ghahfarokhi,
 S. Kalantari, and M. Razzaghi-Abyaneh. 2011. Molecular identification of antagonistic bacteria from Tehran soils and evaluation of their inhibitory activities toward pathogenic fungi. Iran. J. Microbiol. 3:140.
- Rao, R. M., G. N. Reddy, and J. Sreeramulu. 2011. Synthesis of some new pyrazolo-pyrazole derivatives containing indoles with antimicrobial activity. Der Pharma Chem. 3:301-309.
- Rasool, M., A. Akhter, and M. S. Haider. 2021. Molecular and biochemical insight into biochar and Bacillus subtilis induced defense in tomatoes against *Alternaria solani*. Sci. Hortic. 285:110203.
- Reinhold-Hurek, B. and T. Hurek. 2011. Living inside plants: bacterial endophytes. Curr. Opin. Plant Biol. 14:435-443.
- Rosenblueth, M. and E. Martínez-Romero. 2006. Bacterial endophytes and their interactions with hosts. Mol. Plant Microbe Interact. 19:827-837.
- Ryan, R. P., K. Germaine, A. Franks, D. J. Ryan, and D. N. Dowling. 2008. Bacterial endophytes: recent developments and applications. FEMS Microbiol. Lett. 278:1-9.
- Santoyo, G., G. Moreno-Hagelsieb, M. del Carmen Orozco-Mosqueda, and B. R. Glick. 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183:92-99.
- Sessitsch, A., A. Weilharter, M. H. Gerzabek, H. Kirchmann, and E. Kandeler. 2001. Microbial population structures in soil particle size fractions of a long-

term fertilizer field experiment. Appl. Environ. Microbiol. 67:4215-4224.

- Shobha, G. and B. S. Kumudini. 2012. Antagonistic effect of the newly isolated PGPR Bacillus spp. on Fusarium oxysporum. Int. J. Appl. Sci. Eng. Res. 1:463-474.
- Singh, A. K. and J. N. Srivastava. 2015. Sheath blight disease of paddy and their management. In Recent advances in the diagnosis and management of plant diseases. Springer, New Delhi. pp. 91-99
- Srivastava, R., B. N. Johri, and A. Sharma. 1999. Colonization of Wheat (Triticum aestivum L.) Root by Fluorescent Pseudomonads (GRP~ 3 and PRS~ 9). Indian J. Microbiol. 39:205-210.
- Strobel, G., B. Daisy, U. Castillo, and J. Harper. 2004. Natural products from endophytic microorganisms. J. Nat. Prod. 67:257-268.
- Tripathi, A. K., V. Prajapati, N. Verma, J. R.
 Bahl, R. P. Bansal, S. P. S. Khanuja, and S. Kumar. 2002. Bioactivities of the leaf essential oil of Curcuma longa (var. ch-66) on three species of stored-product beetles (Coleoptera).
 J. Econ. Entomol. 95:183-189.
- Velazhahan, R., R. Samiyappan, and P. Vidhyasekaran. 1999. Relationship between antagonistic activities of Pseudomonas fluorescens isolates against Rhizoctonia solarii and their production of lytic enzymes/Beziehungen zwischen antagonistischer Aktivität von Pseudomonas fluorescens-Isolaten gegen Rhizoctonia sotaní und ihrer Produktion lytischer Enzyme. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. JPDP 244-250.
- Verma, S. C., J. K. Ladha, and A. K. Tripathi. 2001. Evaluation of plant growth

promoting and colonization ability of endophytic diazotrophs from deep water rice. J. Biotechnol. 91:127-141.

- Vidhyasekaran, P. and M. Muthamilan. 1999. Evaluation of a powder formulation of Pseudomonas fluorescens Pf1 for control of rice sheath blight. Biocontrol Sci. Technol. 9:67-74.
- Voisard, C., C. Keel, D. Haas, and G. Dèfago. 1989. Cyanide production by Pseudomonas fluorescens helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J. 8:351-358.