ISOLATION OF RHIZOSPHERE AND PHYLLOSPHERE BACTERIA FROM CEREAL SAMPLES

Abstract

This study presents the isolation of rhizosphere and phyllosphere bacteria from cereal samples, highlighting their importance in agricultural ecosystems. Employing surface sterilization techniques, selective media and molecular analysis, diverse bacterial communities were identified on the root and leaf surfaces of cereal crops, encompassing taxa from the Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes phyla. Functional assays revealed the ecological roles of rhizosphere and phyllosphere bacteria in nutrient cycling, disease suppression and stress tolerance, underscoring their potential applications as biofertilizers, biocontrol agents, and biostimulants for enhancing crop productivity and sustainability. This research contributes to our understanding of microbial dynamics within cereal crop ecosystems and informs strategies for optimizing agricultural practices to meet the challenges of global food security and environmental sustainability.

Keywords: Rhizosphere bacteria, Phyllosphere bacteria, Isolation, Leaf sample

Introduction

The rhizosphere and phyllosphere, representing the below-ground and above-ground habitats of plants, respectively, host diverse microbial communities that play crucial roles in plant health, growth and ecosystem functioning. In cereal crop ecosystems, understanding the composition and dynamics of rhizosphere and phyllosphere bacteria is essential for optimizing agricultural practices, enhancing crop productivity, and ensuring food security. This paper provides an introductory overview of the methods and significance of isolating rhizosphere and phyllosphere bacteria from cereal samples, shedding light on their ecological roles and potential applications in agriculture (Bulgarelli *et al*., 2013).

Cereal crops, including staples such as wheat, rice, maize, and barley, serve as fundamental sources of nutrition for a significant portion of the global population. The health and productivity of cereal crops are influenced by various biotic and abiotic factors, including soil microbiota, plant-microbe interactions and environmental conditions. Rhizosphere and phyllosphere bacteria, residing in close proximity to cereal roots and on the aerial parts of

plants, respectively, are intimately involved in nutrient cycling, disease suppression and stress tolerance mechanisms that impact plant growth and yield (Mendes *et al*., 2013)

Rhizosphere bacteria interact closely with cereal roots, forming symbiotic associations that facilitate nutrient acquisition, disease resistance and abiotic stress tolerance. Phyllosphere bacteria, on the other hand, inhabit the aerial parts of plants and contribute to plant health through mechanisms such as phytopathogen inhibition, plant growth promotion and volatile organic compound production. Understanding the interactions between rhizosphere and phyllosphere bacteria and their respective hosts is crucial for unraveling the complex networks of plant-microbe interactions that govern cereal crop ecosystems (Vorholt. 2012).

Furthermore, the isolation and characterization of rhizosphere and phyllosphere bacteria from cereal samples offer opportunities for developing sustainable agricultural practices. Beneficial rhizosphere bacteria can be utilized as biofertilizers, biocontrol agents and biostimulants to enhance soil fertility, suppress plant pathogens and improve crop productivity. Similarly, phyllosphere bacteria with plant growth-promoting traits can be harnessed to enhance nutrient uptake, mitigate abiotic stressors and promote sustainable crop production.

In summary, the isolation of rhizosphere and phyllosphere bacteria from cereal samples represents a critical step in understanding the microbial communities associated with cereal crops and their impact on plant health and productivity. By elucidating the ecological roles and potential applications of rhizosphere and phyllosphere bacteria in agriculture, this research contributes to the development of innovative strategies for sustainable crop management and ecosystem stewardship.

Materials and Method

Collection and processing of sample

Rhizosphere soil sample collection and processing

Samples were collected from rhizospheric soils of different cereal growing regions of Raichur, Ballari, Gangavati, Sindhanur and Dhadesugur. 40 (Forty) rhizospheric soil samples of cereal crop plants collected by adopting standard soil sampling methods described by Jackson (1973).

Samples of soil were taken between the roots of the crops being grown, at a depth of 0 to 10 cm. Sterilized polythene bags were used to gather soil samples. The polythene bags were carefully labelled, tied and as contaminated-free as possible. After being transported to the lab, soil samples were kept in a refrigerator at 4 °C in order to isolate effective rhizosphere isolates. A portion of the shade-dried soil samples were used for chemical analyses, while the moist soil samples were used right away for microbial investigations.

Collection of Phyllosphere sample

The isolation of phyllosphere bacteria from cereal samples is a critical step in understanding the microbial communities associated with these important agricultural crops. Phyllosphere bacteria, residing on the aerial parts of plants, play essential roles in plant health, growth promotion, and protection against pathogens.

Healthy cereal plants were collected from different areas of Raichur, Ballari, Gangavati, Sindhanur and Dhadesugur. Physiologically active leaf samples and stem samples will be collected from cereal plants. The plants were put separately into sterile bags, then transported to laboratory for isolation of phyllosphere microorganisms and stored at 4° C.

Isolation of Rhizosphere microorganisms

Microbes were isolated from collected rhizosphere samples by serial dilution plating method on Nutrient agar medium. Test tubes with 9 ml distilled water were sterilized in an autoclave for preparation of water blank. Then 1 gm of collected soil sample was weighed and transferred to the 9 ml sterile water blank which gives 10^{-1} dilution. Same procedure was repeated up to 10^{-6} and 10^{-7} dilution. Then 0.1 ml of suspension from appropriate dilution (10^{-6} and 10^{-7}) was transferred to the petri plate containing Nutrient agar medium. Three replications were maintained for each dilution. These petri plates were incubated in an inverted position at room temperature 30 ^{0}C for 2 days (Pankaj *et al*., 2012). The bacterial colonies exhibiting the different colour colonies were selected, purified, sub-cultured and stored on the slants of Nutrient agar for further morphological and biochemical studies.

Isolation of phyllosphere microorganisms:

Dilution method

From each plant, ten discs of one cm leaf bits were cut with a sterile cork borer. The discs were transferred to sterile distilled water of 100 ml and stirred for one hr. An aliquot of one ml was plated on nutrient agar medium.

Leaf imprint method

Leaf imprints on nutrient agar medium were made in order to estimate the bacterial population on the adaxial and abaxial leaf surfaces. A single, intact leaf was put on a nutrient agar plate and pressed down with the smooth side of a sterile glass rod until the entire leaf was clearly imprinted on the surface of the nutrient agar. The plates were incubated for two to five days at 24ºC in order to form colonies. According to Holland *et al*. (2000), morphological variation was used to select individual bacterial colonies.

Result and Discussion

Collection of soil samples for the isolation Rhizosphere and Phyllosphere isolates

Samples were collected from rhizosphere soils of different cereal growing regions of Raichur, Ballari, Gangavati, Sindhanur and Dhadesugur. 40 rhizospheric soil samples of cereal crop plants collected by adopting standard soil sampling methods (Table 1). Soil samples were collected in sterilized polythene bags. The Polythene bags were properly tied, labeled and at most care was taken to avoid contamination. Soil samples were transported to the laboratory and stored in refrigerator at 4 ^{o}C.

Table 1: Soil samples used for isolation of rhizosphere microbiome from different locations

Sl. No.	Sample code	Name of place	Crop	Soil type
Raichur taluk				
1	UASC 1	Main campus	Maize	Black
2	UASC 2			Black
3	UASC 3			Black
4	RPR 1	Rampur	Sorghum	Black
5	RPR 2			Black

6	YGR 1	Yergera	Sorghum	Black
7	YGR 2			Black
8	GJRL	Gajaral	Sorghum	Black
9	MSLR 1	Mansalpur	Sorghum	Black
10	MSLR 2			Black
Ballari				
11	BLR 1	Ballari	Sorghum	Black
12	BLR 2			Black
13	RYR 1	Rayapura	Sorghum	Black
14	RYR 2			Black
15	HNHL 1	Honnahalli	Sorghum	Black
16	HNHL 2		Maize	Black
17	SGKL 1	Sanganakal	Maize	Black
18	SGKL 2			Black
19	IBMR 1	Ibrampura	Maize	Black
20	IBMR 2			Black

Sl. No.	Sample code	Name of place	Crop	Soil type
Gangavati				
21	GNVT 1	Gangavati	Paddy	Black
22	GNVT 2			Black
23	GNVT 3			Black
24	BRGR 1	Bargur	Paddy	Black
25	BRGR 2			Black

26	AJHL 1	Anjanhalli	Paddy	Black
27	AJHL 2			Black
28	BNR 1	Bennur	Paddy	Black
29	BNR 2			Black
30	BNR 3			Black
Dhadesguru and Sindhanur				
31	DSGR 1	Dhadesguru	Sorghum	Black
32	DSGR 2			Black
33	DSGR 3			Black
34	GRBL 1	Gorebal	Sorghum	Black
35	GRBL 2			Black
36	SNDR 1	Sindhanur	Sorghum	Black
37	SNDR 2			Black
38	SNDR 3			Black
39	VPR 1	Virupapur	Sorghum	Black
40	VPR 2			Black

Healthy cereal plants were collected from different areas of Raichur, Ballari, Gangavati, Sindhanur and Dhadesugur. Physiologically active leaf samples and stem samples will be collected from cereal plants (Table 2). The plants were put separately into sterile bags, then transported to laboratory for isolation of phyllosphere microorganisms and stored at 4° C.

Table 2: Leaf samples used for isolation of Phyllosphere microbiome from different locations

Sl. No.	Sample code	Name of place	Crop
Raichur taluk			
1	UASCP 1	UAS campus	Maize
2	UASCP 2		
3	UASCP 3		
4	RPRP 1	Rampur	Sorghum
5	RPRP 2		
6	YGRP 1	Yergera	Sorghum
7	YGRP 2		
8	GJRLP	Gajaral	Sorghum
9	MSLRP 1	Mansalpur	Sorghum
10	MSLRP 2		
Ballari			
11	BLRP 1	Ballari	Sorghum
12	BLRP 2		
13	RYRP 1	Rayapura	Sorghum
14	RYRP 2		
15	HNHLP 1	Honnahalli	Maize
16	HNHLP 2		
17	SGKLP 1	Sanganakal	Maize
18	SGKLP 2		
19	IBMRP 1	Ibrampura	Maize
20	IBMRP 2		

Sl. No.	Sample code	Name of place	Crop
Gangavati			
21	GNVTP 1	Gangavati	Paddy
22	GNVTP 2		
23	GNVTP 3		
24	BRGRP 1	Bargur	Paddy
25	BRGRP 2		
26	AJHLP 1	Anjanhalli	Paddy
27	AJHLP 2		
28	BNRP 1	Bennur	Paddy
29	BNRP 2		
30	BNRP 3		
Dhadesguru and Sindhanur			
31	DSGRP 1	Dhadesguru	Sorghum
32	DSGRP 2		
33	DSGRP 3		
34	GRBLP 1	Gorebal	Sorghum
35	GRBLP 2		
36	SNDRP 1	Sindhanur	Sorghum
37	SNDRP 2		
38	SNDRP 3		
39	VPRP 1	Virupapur	Sorghum
40	VPRP 2		

Isolation and purification of rhizosphere and phyllopshere microbes from soil sample

Rhizosphere microbes were isolated from collected soil samples by using serial dilution plating on Nutrient Agar medium and AMS medium. The plates were kept for incubation under 30℃ for 7 days in inverted position (Plate 1).

Phyllosphere microorganisms were isolated from the collected leaf samples by using Dilution method and Leaf imprint method by plating on Nutrient Agar medium and AMS medium. The plates were kept for incubation under 30 ℃ for 7 days in inverted position (Plate 2 and 3) and pure cultures are maintained in the slants (Plate 4).

Forty rhizosphere microbes and forty phyllosphere microbes were isolated from different cereals growing regions *viz*., UAS campus, Rampur, Yergera, Gajaral, Mansalpur, Ballari, Rayapura, Honnahalli, Sanganakal, Ibrampura, Gangavati, Bargur, Anjanhalli, Bennur, Dhadesguru, Gorebal, Sindhanur and Virupapur. The locations were mentioned in the Table 1 and 2.

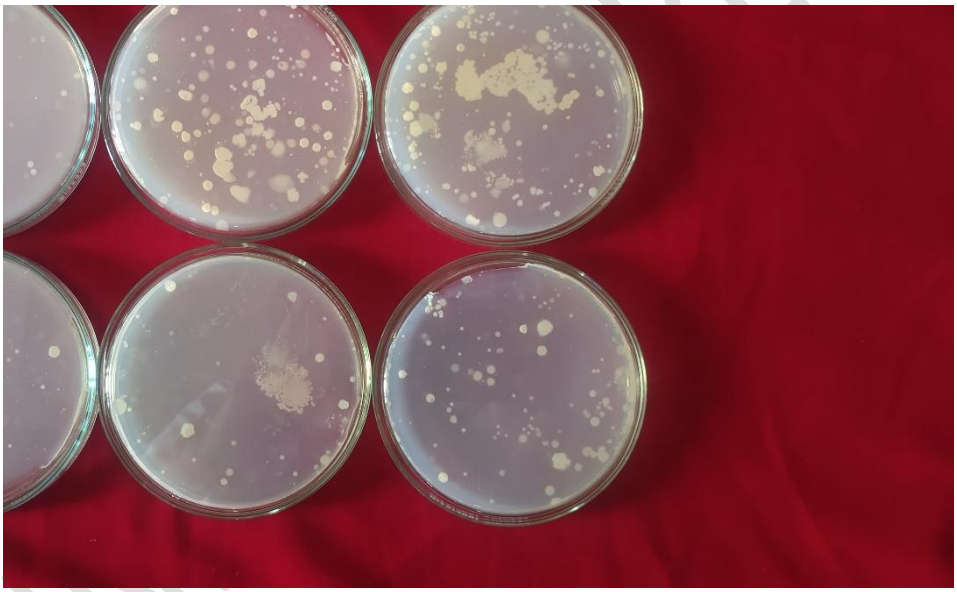

Plate 1: Isolation of Rhizosphere bacteria by serial dilution method

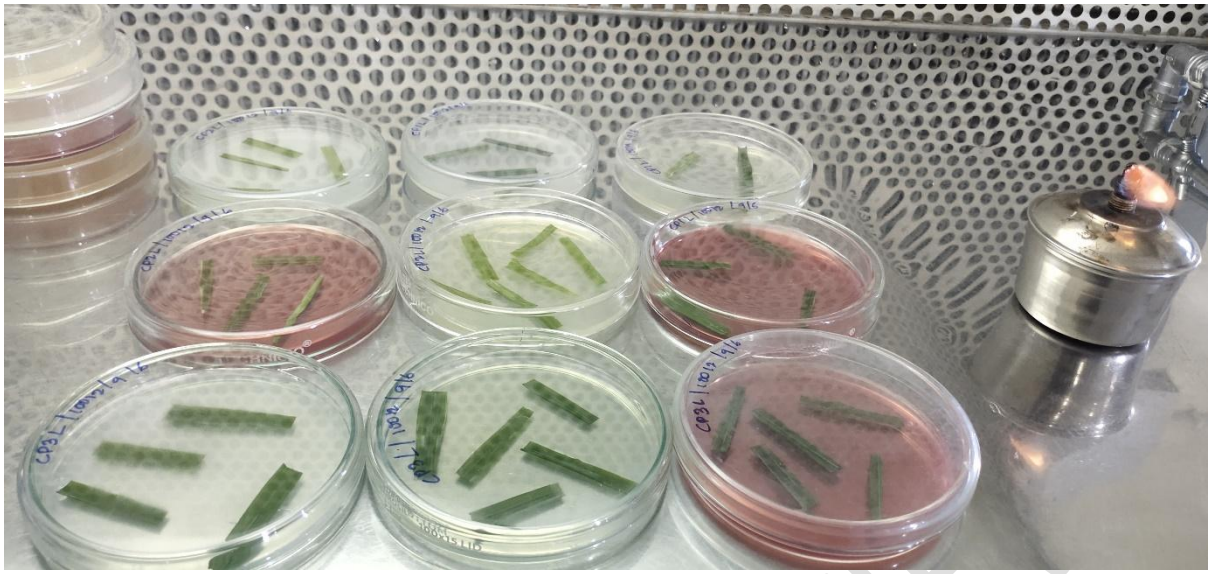

Plate 2: Leaf imprinting method for the isolation of Phyllosphere bacteria

Plate 3: Phyllosphere bacteria on surface of the media

Plate 4: Pure cultures of the bacterial isolates

The same outcomes with Based on morphological and physiological traits, 39 isolates have been classified as *Bacillus* spp. (Avasar *et al*., 2017). *Bacillus subtilis* was isolated from cotton rhizosphere soil by Gajbhiye *et al*. in 2010. There were 113 distinct bacterial strains isolated (Mazinani *et al*., 2017). A total of thirty bacteria were isolated (Kumar *et al*., 2012).

Madhaiyan *et al*. isolated strains of pink-pigmented facultative methylotrophic bacteria (PPFMs) from various locations within the sugarcane clone Co86032 in 2005. Methylotrophic bacteria have been identified in the phyllosphere of various crop plants, including potatoes, radish, sugarcane, and pigeonpeas (Meena *et al*., 2012). In 2009, Madhaiyan *et al*. isolated CBMB27T, an aerobic, facultatively methylotrophic, pink-pigmented bacterial strain, from rice (*Oryza sativa* L.) leaf tissues.

Conclusion

In conclusion, the isolation and characterization of rhizosphere and phyllosphere bacteria from cereal samples provide valuable insights into the intricate interactions between microorganisms and cereal crops. This study has demonstrated the diversity, abundance and ecological roles of bacterial communities inhabiting the root and leaf surfaces of cereal plants, highlighting their significance in agricultural ecosystems. Furthermore, the functional assays conducted in this study elucidate the potential of rhizosphere and phyllosphere bacteria as biofertilizers, biocontrol agents and biostimulants for enhancing crop productivity and resilience to biotic and abiotic stresses. By harnessing the beneficial properties of these bacteria, sustainable agricultural practices can be developed to address the challenges of global food security and environmental sustainability. Moving forward, further research is warranted to unravel the complexities of plant-microbe interactions and optimize the utilization of rhizosphere and phyllosphere bacteria for maximizing cereal crop yields while minimizing environmental impacts.

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