Journal of Advances in Biology & Biotechnology

Volume 27, Issue 3, Page 200-207, 2024; Article no.JABB.114415
ISSN: 2394-1081

Isolation of Rhizosphere and Phyllosphere Bacteria from Cereal Samples

Chaitra Lakkannavar [a*], Mahadeva Swamy [a], Yallappa M [a], Ramesh Y M [b] and Satyanarayan Rao [b]

[a] Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur, India.
[b] Department of Agronomy, University of Agricultural Sciences, Raichur, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2024/v27i3734

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/114415

Original Research Article

Received: 08/01/2024
Accepted: 12/03/2024
Published: 15/03/2024

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. A total of 40 rhizosphere and phyllosphere bacteria were isolated from different cereal crops 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; biofertilizers; environmental sustainability; microbial dynamics; cereal crop; bacteroidetes.

**Corresponding author: E-mail: chaitranm96@gmail.com;*

1. INTRODUCTION

The rhizosphere and phyllosphere, repress enting 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.

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 [1].

In January 2022, global consumption of wheat, rice, maize (corn), and barley varies annually based on factors such as population growth, dietary trends, economic conditions, and weather patterns. Historically, these four grains have been staples in the human diet and are consumed in various forms across the globe. Wheat is commonly used to make bread, pasta, and pastries; rice is a staple food in many Asian countries and is also consumed worldwide; maize is used for food, animal feed, and industrial purposes and barley is often used in brewing beer and for animal feed.

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 [2].

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 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 [3].

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.

Biotic stress refers to the impact of living organisms such as pests, diseases and weeds on plants, which can reduce crop yield and quality. Abiotic stress encompasses non-living factors like drought, salinity, temperature extremes and soil nutrient deficiencies, which also negatively affect plant growth and productivity. Both biotic and abiotic stresses pose significant challenges to global agriculture, leading to economic losses and food insecurity. Plant breeding and biotechnology efforts aim to develop crops with improved resilience to these stressors through traits such as pest resistance, drought tolerance, and nutrient efficiency. Sustainable farming practices and advanced technologies further mitigate the impact of biotic and abiotic stresses, ensuring food security in the face of environmental challenges.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Sample

2.1.1 Rhizosphere soil sample collection and processing

Samples were collected from rhizospheric soils of different cereal growing regions of Raichur, Ballari, Gangavati, Sindhanur and Dhadesugur. Forty rhizospheric soil samples of cereal crop plants collected by adopting standard soil sampling methods described by Jackson [4].

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.

2.1.2 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.

2.1.3 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 [5]. 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.

2.2 Isolation of Phyllosphere Microorganisms

2.2.1 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.

2.2.2 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. [6], morphological variation was used to select individual bacterial colonies.

3. RESULTS AND DISCUSSION

3.1 Collection of soil samples for the isolation Rhizosphere and Phyllosphere isolates

Forty rhizospheric soil samples of cereal crop plants are collected by adopting standard soil sampling methods an the details of the soil sample collection is depicted in 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		Black
18	SGKL 2		Maize	Black
19	IBMR 1	Ibrampura		Black
20	IBMR 2		Maize	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		

3.2 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).

Forty 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. After incubation at 30 ℃ for 7 days in inverted position, the isolates were seen on the plates (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.

Based on morphological and physiological traits, 39 isolates have been classified as *Bacillus* spp. [7]. *Bacillus subtilis* was isolated from cotton rhizosphere soil by Gajbhiye et al. in [8]. Mazinani et al. [9], isolated 113 distinct bacterial strains. A total of thirty bacteria were isolated [10].

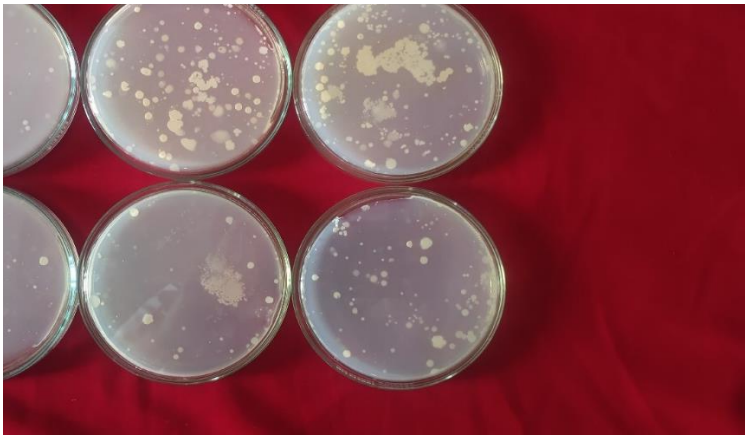

Plate 1. Isolation of Rhizosphere bacteria by serial dilution method

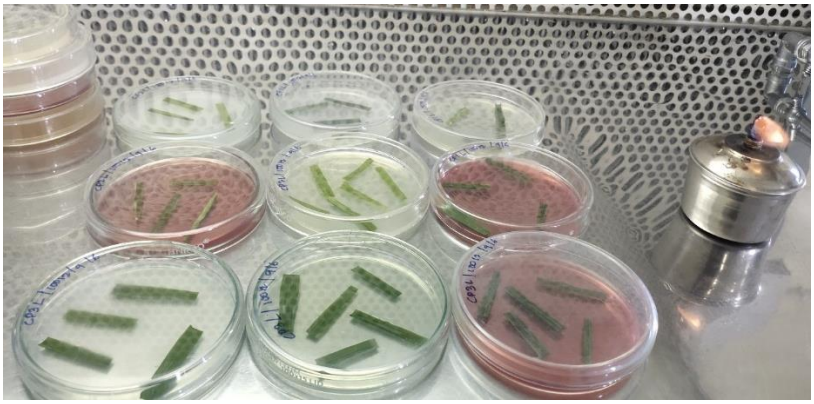

Plate 2. Leaf imprinting method for the isolation of Phyllosphere bacteria

Plate 3. Phyllosphere bacteria on surface of the media

Plate 4. Pure cultures of the bacterial isolates

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 [11]. In 2009, Madhaiyan et al. [12,13] isolated CBMB27T, an aerobic, facultatively methylotrophic, pink-pigmented bacterial strain, from rice (*Oryza sativa* L.) leaf tissues.

4. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mendes R, Garbeva P. Raaijmakers JM. The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews. 2013;37(5):634-663.
2. Vorholt JA. Microbial life in the phyllosphere. Nature Reviews Microbiology. 2012;10(12):828-840.
3. Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology. 2013;64:807-838.
4. Jackson ML. Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi; 1973.
5. Pankaj Kumar, Dubey RC, Maheshwari DK. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Asain J. Boil. Life Sci. 2012;2(2):56- 67.
6. Holland M, Davis R, Moffitt S, Olaughlin K, Peach D, Sussan S, Wimbrow L. and Tayman B, Using 'Leaf Prints' to investigate a common bacterium. *American*. Biol. Teach. 2000; 62:128- 131.
7. Avsar C, Koyuncu H, Aras ES. Isolation and molecular characterization of *Bacillus* spp. isolated from soil for production of industrial enzymes. Biol. Chem. Res. 2017; 3(9):72-86.
8. Gajbhiye A, Rai AR, Meshram SU, Dongre AB. Isolation, evaluation and characterization of *Bacillus subtilis* from cotton rhizospheric soil with biocontrol activity against *Fusarium oxysporum*. World. J. Microb Biot. 2010;26(7):1187-1194.

9. Mazinani Z, Zamani M, Sardari S. Isolation and identification of phyllospheric bacteria possessing antimicrobial activity from *Astragalus obtusifolius*, *Prosopis juliflora*, *Xanthium strumarium* and *Hippocrepis unisiliqousa*. Avicenna J. Med. Biotechnol. 2017;9(1):31.
10. Kumar A, Kumar A, Devi S, Patil S, Payal C, Negi S. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: An in vitro study. Recent Res. Sci. Technol. 2012;4 (1):1-5.
11. Meena KK, Kumar M, Kalyuzhnaya MG, Yandigeri MS, Singh DP, Saxena AK, Arora DK. Epiphytic pink-pigmented methylotrophic bacteria enhance germination and seedling growth of wheat (*Triticum aestivum*) by producing phytohormone. Antonie Van Leeuwenhoek. 2012101: 777-786.
12. Madhaiyan M, Poonguzhali S, Lee HS, Hari K, Sundaram P, Sa TM. Pink-pigmented facultative methylotrophic bacteria accelerate germination, growth and yield of sugarcane clone Co86032 (*Saccharum officinarum* L.). Biol. Fertil soils. 2005;41:350-358.
13. Madhaiyan M, Suresh Reddy BV, Anandam R, Senthilkumar M, Poonguzhali S, Sundaram S. P. Plant growth promoting *Methylobacterium* induces defense responses in groundnut (*Arachis hypogaea* L.) compared with root pathogen. Curr. Microbiol. 2006;53:270–276.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/114415