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Isolation of Rhizosphere and Phyllosphere Bacteria from Cereal Samples

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Authors' contributions

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

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Original Research Article

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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.

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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, plantinteractions microbe 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 cvclina. 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, pastries; rice is a staple food and in and manv Asian countries 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 abiotic enhance nutrient uptake, mitigate 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. sheddina liaht 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 innovative strategies sustainable of for 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 vield and quality. Abjotic stress encompasses non-living factors like drought. salinity, temperature extremes and soil nutrient deficiencies, which negatively affect plant growth also 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

aives 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 medium. containing Nutrient agar Three replications were maintained for each dilution. These petri plates were incubated in an inverted position at room temperature 30 °C for 2 days [5]. The bacterial colonies exhibiting the different colour colonies were selected, purified, subcultured 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 24°C 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 °C.

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22GNVT 2Black23GNVT 3BargurPaddyBlack24BRGR 1BargurPaddyBlack25BRGR 2BlackBlack26AJHL 1AnjanhalliPaddyBlack27AJHL 2BennurPaddyBlack28BNR 1BennurPaddyBlack29BNR 2BlackBlack30BNR 3BlackBlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghum32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2VirupapurSorghumBlack	SI. No. Gangavati	Sample code	Name of place	Сгор	Soil type		
23GNV13BargurPaddyBlack24BRGR 1BargurPaddyBlack25BRGR 2BlackBlack26AJHL 1AnjanhalliPaddyBlack27AJHL 2BlackBlack28BNR 1BennurPaddyBlack29BNR 2BlackBlack30BNR 3BlackBlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati	Sample code	Name of place Gangavati	Crop Paddy	Soil type Black		
24BRGR 1BargurPadoyBlack25BRGR 2BlackBlack26AJHL 1AnjanhalliPaddyBlack27AJHL 2BennurPaddyBlack28BNR 1BennurPaddyBlack29BNR 2BackBlack30BNR 3BlackBlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22	Sample code GNVT 1 GNVT 2	Name of place Gangavati	Crop Paddy	Soil type Black Black		
25BRGR 2Hack26AJHL 1AnjanhalliPaddyBlack27AJHL 2BennurPaddyBlack28BNR 1BennurPaddyBlack29BNR 2BlackBlack30BNR 3BlackBlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23	Sample code GNVT 1 GNVT 2 GNVT 3	Name of place Gangavati	Crop Paddy	Soil type Black Black Black Black		
26AJHL 1AnjannaliiPaddyBlack27AJHL 2BennurPaddyBlack28BNR 1BennurPaddyBlack29BNR 2BlackBlack30BNR 3BlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghum32DSGR 2Black33DSGR 3Black34GRBL 1GorebalSorghum35GRBL 2Black36SNDR 1SindhanurSorghum37SNDR 2Black38SNDR 3Black39VPR 1VirupapurSorghum40VPR 2Black	SI. No. Gangavati 21 22 23 24 25	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1	Name of place Gangavati Bargur	Crop Paddy Paddy	Soil type Black Black Black Black Black		
27AJFL 2Back28BNR 1BennurPaddyBlack29BNR 2BlackBlack30BNR 3BlackBlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 25	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2	Name of place Gangavati Bargur	Crop Paddy Paddy	Soil type Black Black Black Black Black Black		
20BINR 1BernfulPaddyBlack29BNR 2BlackBlack30BNR 3BlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 26 27	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1	Name of place Gangavati Bargur Anjanhalli	Crop Paddy Paddy Paddy	Soil type Black Black Black Black Black Black Black		
29DINR 2Black30BNR 3BlackDhadesguru and SindhanurSorghumBlack31DSGR 1DhadesguruSorghum32DSGR 2Black33DSGR 3Black34GRBL 1GorebalSorghum35GRBL 2Black36SNDR 1Sindhanur37SNDR 2Black38SNDR 3Black39VPR 1VirupapurSorghum40VPR 2Black	SI. No. Gangavati 21 22 23 24 25 26 27 28	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 PND 1	Name of place Gangavati Bargur Anjanhalli	Crop Paddy Paddy Paddy Paddy	Soil type Black Black Black Black Black Black Black Black		
BlackDhadesguru and Sindhanur31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 26 27 28 20	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 1	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy	Soil type Black Black Black Black Black Black Black Black Black		
31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 20	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 2 BNR 2	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy	Soil type Black Black Black Black Black Black Black Black Black Black		
31DSGR 1DhadesguruSorghumBlack32DSGR 2BlackBlack33DSGR 3BlackBlack34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3BlackBlack39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy	Soil type Black Black Black Black Black Black Black Black Black Black Black		
32DSGR 2Black33DSGR 3Black34GRBL 1GorebalSorghum35GRBL 2Black36SNDR 1SindhanurSorghum37SNDR 2Black38SNDR 3Black39VPR 1VirupapurSorghum40VPR 2Black	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3 Iru and Sindhanur	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy	Soil type Black Black Black Black Black Black Black Black Black Black Black		
34GRBL 1GorebalSorghumBlack35GRBL 2BlackBlack36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3Black39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 22	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru	Crop Paddy Paddy Paddy Paddy Sorghum	Soil type Black Black Black Black Black Black Black Black Black Black Black Black		
35GRBL 2Black36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3Black39VPR 1VirupapurSorghumBlack40VPR 2Black	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 2 DSGR 3	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru	Crop Paddy Paddy Paddy Paddy Sorghum	Soil type Black Black Black Black Black Black Black Black Black Black Black Black Black Black		
36SNDR 1SindhanurSorghumBlack37SNDR 2BlackBlack38SNDR 3Black39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 1 DSGR 2 DSGR 3 GRB 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru	Crop Paddy Paddy Paddy Paddy Sorghum	Soil type Black Black Black Black Black Black Black Black Black Black Black Black Black Black Black		
37SNDR 2Black38SNDR 3Black39VPR 1Virupapur40VPR 2Black	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 1 DSGR 2 DSGR 3 GRBL 1 GRBL 2	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum	Soil type Black Black Black Black Black Black Black Black Black Black Black Black Black Black Black Black Black		
38SNDR 3Black39VPR 1VirupapurSorghumBlack40VPR 2BlackBlack	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 2 DSGR 3 GRBL 1 GRBL 2 SNDR 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum	Soil type Black		
39VPR 1VirupapurSorghumBlack40VPR 2Black	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36 37	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 2 DSGR 3 GRBL 1 GRBL 2 SNDR 1 SNDR 2	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum Sorghum	Soil type Black		
40 VPR 2 Black	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36 37 38	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 2 DSGR 3 GRBL 1 GRBL 2 SNDR 1 SNDR 2 SNDR 3	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum Sorghum	Soil type Black Bl		
	SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36 37 38 39	Sample code GNVT 1 GNVT 2 GNVT 3 BRGR 1 BRGR 2 AJHL 1 AJHL 2 BNR 1 BNR 2 BNR 3 ru and Sindhanur DSGR 1 DSGR 2 DSGR 3 GRBL 1 GRBL 2 SNDR 1 SNDR 2 SNDR 1 SNDR 2 SNDR 3 VPR 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum Sorghum	Soil type Black Bl		

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

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.

SI. No.	Sample code	Name of place	Crop
Raichur ta	luk		
1	UASCP 1	UAS campus	Maize
2	UASCP 2		
3	UASCP 3		
4	RPRP 1	Rampur	Sorghum
5	RPRP 2	•	3
6	YGRP 1	Yergera	Sorghum
7	YGRP 2	5	5
8	GJRLP	Gaiaral	Sorahum
9	MSLRP 1	Mansalpur	Sorahum
10	MSLRP 2		2 -
Ballari			
11	BLRP 1	Ballari	Sorahum
12	BLRP 2		2 2 3
13	RYRP 1	Ravapura	Sorahum
14	RYRP 2		3
15	HNHLP 1	Honnahalli	Maize
16	HNHLP 2		
17	SGKLP 1	Sanganakal	Maize
18	SGKLP 2		
19	IBMRP 1	Ibrampura	Maize
20	IBMRP 2		
SI. No.	Sample code	Name of place	Сгор
SI. No. Gangavati	Sample code	Name of place	Сгор
SI. No. Gangavati	Sample code GNVTP 1	Name of place Gangavati	Crop Paddy
SI. No. Gangavati 21 22	Sample code GNVTP 1 GNVTP 2	Name of place Gangavati	Crop Paddy
SI. No. Gangavati 21 22 23	Sample code GNVTP 1 GNVTP 2 GNVTP 3	Name of place Gangavati	Crop Paddy
SI. No. Gangavati 21 22 23 24	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1	Name of place Gangavati Bargur	Crop Paddy Paddy
SI. No. Gangavati 21 22 23 24 25	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2	Name of place Gangavati Bargur	Crop Paddy Paddy
SI. No. Gangavati 21 22 23 24 25 26	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1	Name of place Gangavati Bargur Anianhalli	Crop Paddy Paddy Paddy
SI. No. Gangavati 21 22 23 24 25 26 27	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2	Name of place Gangavati Bargur Anjanhalli	Crop Paddy Paddy Paddy
SI. No. Gangavati 21 22 23 24 25 26 27 28	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy
SI. No. Gangavati 21 22 23 24 25 26 27 28 29	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesqu	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 ru and Sindhanur	Name of place Gangavati Bargur Anjanhalli Bennur	Crop Paddy Paddy Paddy Paddy
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 Intu and Sindhanur DSGRP 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru	Crop Paddy Paddy Paddy Paddy Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 Tu and Sindhanur DSGRP 1 DSGRP 1 DSGRP 2	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru	Crop Paddy Paddy Paddy Paddy Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 ITU and Sindhanur DSGRP 1 DSGRP 1 DSGRP 2 DSGRP 3	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru	Crop Paddy Paddy Paddy Paddy Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 Iru and Sindhanur DSGRP 1 DSGRP 1 DSGRP 2 DSGRP 3 GRBLP 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal	Crop Paddy Paddy Paddy Paddy Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 Tru and Sindhanur DSGRP 1 DSGRP 1 DSGRP 2 DSGRP 3 GRBLP 1 GRBLP 2	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36	Sample code GNVTP 1 GNVTP 2 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 Iru and Sindhanur DSGRP 1 DSGRP 2 DSGRP 3 GRBLP 1 GRBLP 2 SNDRP 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36 37	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 ITU and Sindhanur DSGRP 1 DSGRP 2 DSGRP 3 GRBLP 1 GRBLP 2 SNDRP 1 SNDRP 2	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36 37 38	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 ru and Sindhanur DSGRP 1 DSGRP 2 DSGRP 3 GRBLP 1 GRBLP 2 SNDRP 1 SNDRP 1 SNDRP 2 SNDRP 3	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum Sorghum
SI. No. Gangavati 21 22 23 24 25 26 27 28 29 30 Dhadesgu 31 32 33 34 35 36 37 38 39	Sample code GNVTP 1 GNVTP 2 GNVTP 3 BRGRP 1 BRGRP 2 AJHLP 1 AJHLP 2 BNRP 1 BNRP 2 BNRP 3 TU and Sindhanur DSGRP 1 DSGRP 3 GRBLP 1 GRBLP 2 SNDRP 3 GRBLP 1 SNDRP 2 SNDRP 1 SNDRP 2 SNDRP 1 SNDRP 2 SNDRP 3 VPRP 1	Name of place Gangavati Bargur Anjanhalli Bennur Dhadesguru Gorebal Sindhanur Virupapur	Crop Paddy Paddy Paddy Paddy Sorghum Sorghum Sorghum

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

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°C 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 °C 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

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Plate 4. Pure cultures of the bacterial isolates

Madhaiyan et al. isolated strains of pinkpigmented facultative methylotrophic bacteria (PPFMs) from various locations within the sugarcane Co86032 2005 clone in Methylotrophic bacteria have been identified in the phyllosphere of various crop plants, including potatoes, radish, sugarcane, and pigeonpeas [11]. In 2009. Madhaivan et al. [12.13] isolated CBMB27T. an aerobic. facultativelv 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.

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