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Occurrence and Distribution of Bacteria in Non-Rhizospheric Soils of Rice Fields across Four Regions of Bangladesh

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Abstract

Non-rhizospheric soil refers to the bulk soil that lies outside the immediate influence of plant roots and their exudates, typically exhibiting lower microbial activity and reduced nutrient availability compared to the rhizosphere. This study investigates the diversity and characterization of bacterial communities in non-rhizospheric soils from rice fields across four districts of Bangladesh, including Dinajpur, Mymensingh, Netrokona, and Savar. Non-rhizospheric soil samples were collected, and bacteria were isolated using nutrient agar, enumerated as colony-forming units (CFU/g), and differentiated based on colony morphology. Bacterial loads varied significantly, with the highest count (1.04×10^{10} CFU/g) in Mymensingh and the lowest (1.64×10^9 CFU/g) in Savar. Cultural, morphological, and biochemical analyses were performed on 18 distinct bacterial isolates, which were identified as *Staphylococcus* sp., *Pseudomonas* sp., *Micrococcus* sp. *Bacillus* sp., *Alcaligenes* sp. and *Vibrio* sp., among which *Staphylococcus* sp. was found to be the predominant isolate across the sites. Furthermore, molecular identification of the bacterial isolates was performed using 16S rRNA gene sequencing, which confirmed the species of the isolated bacteria. Biocontrol assays demonstrated the ability of *Pseudomonas* sp. to inhibit foodborne *Staphylococcus* sp. Antibiotic susceptibility test revealed multidrug resistance, with all isolates resistant to most antibiotics but uniformly sensitive to amikacin. This study highlights the non-rhizospheric soil of rice fields as a reservoir of diverse bacterial communities, with *Staphylococcus* sp. showing dominance and regional variation in bacterial abundance, underscoring the ecological importance of these microbial populations.

Keywords: Non-rhizospheric soil, Rice fields, Bacterial distribution, Biocontrol, and Antibiotic susceptibility.

1. Introduction

Soil microorganisms are fundamental to ecosystem functioning and agricultural productivity, with agricultural soils being recognized as some of the most biologically diverse ecosystems due to factors such as soil type, organic matter content, pH, moisture, and temperature (Foster & Woodruff, 2010; Garbeva *et al.*, 2004; Kannan *et al.*, 2018).

Soils are typically categorized based on their proximity to plant roots and the resulting microbial interactions, distinguishing the rhizosphere from non-rhizospheric zones. While the rhizosphere is directly influenced by plant roots and root-derived exudates, creating a microhabitat that supports rich microbial communities, non-rhizospheric soil, located further from the root zone, is typically less

influenced by these root interactions, resulting in different microbial dynamics and a different array of bacterial species (Paul, 2014; Rabiul *et al.*, 2020).

Despite its less studied role, the non-rhizospheric soil harbors a variety of bacteria, such as *Arthrobacter*, *Clostridium*, *Micromonospora*, *Nocardia*, and *Paenibacillus*, which contribute to broader ecological processes, including organic matter decomposition, soil aggregation, and nutrient cycling (Nannipieri *et al.*, 2003; Jakub, 2014). These bacteria are crucial for maintaining soil health, particularly in maintaining soil structure and resistance to environmental stresses, such as nutrient leaching and erosion, which are common issues in agricultural landscapes (Paul, 2015).

In contrast to the more widely studied rhizospheric microbiota, the non-rhizospheric soil microbiota has received comparatively less attention, especially in rice field soils. While studies from other regions have highlighted diverse bacterial communities in paddy soils (Yongsuwattana *et al.*, 2023; Mwajita *et al.*, 2013), research on the non-rhizospheric bacterial populations in rice fields, particularly in Bangladesh, is limited. Moreover, there is a significant knowledge gap concerning the prevalence of beneficial bacteria with biocontrol properties and the potential for antibiotic resistance in these soils. This is a critical issue in the context of increasing agricultural intensification and the global rise in antimicrobial resistance.

The present study aims to isolate and characterize the non-rhizospheric bacterial communities from rice field soils across four districts of Bangladesh. By employing a combination of cultural, biochemical, and molecular techniques, we aim to identify dominant bacterial taxa, assess their biocontrol potential, and evaluate their antibiotic resistance profiles. This research provides valuable insights into the composition and functional roles of non-rhizospheric bacteria in rice field soils, contributing to the understanding of their potential for enhancing sustainable agricultural practices and promoting soil health in Bangladesh.

2. Materials and Methods

Sample collection

Non-rhizospheric soil samples were collected from rice fields in different regions of Bangladesh, specifically Dinajpur, Mymensingh, Netrokona, and UniversePG | www.universepg.com

Savar. The soils were collected from areas outside the immediate root zone of rice plants, transported to the laboratory under sterile conditions, and stored at 4 °C until further analysis.

Isolation and enumeration of bacteria

Bacterial isolation and enumeration were performed using the spread plate technique on nutrient agar (NA, pH 6.0) as described by Eklund and Lankford (1967). Aliquots of 0.1 mL from each serially diluted soil sample were plated and incubated at 37 °C for 24 hours. Distinct colonies were counted to determine the microbial load, expressed as colony-forming units per gram of soil (CFU/g). Representative colonies were purified through repeated streaking and preserved on NA slants at 4 °C for further analysis.

Morphological and biochemical characterization

The bacterial isolates were examined for their morphological characteristics, including colony color, shape, elevation, margin, surface texture, and optical properties, following the methods described by Eklund and Lankford, (1967). The colonies were subsequently cultured on a range of selective and differential media, such as MacConkey agar, Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB) agar, Brilliant Green Agar (BGA), Salmonella-Shigella Agar (SSA), Cetrimide Agar (CA), Thiosulfate-Citrate-Bile-Salts-Sucrose (TCBS) agar, and Xylose Lysine Deoxycholate (XLD) agar.

In addition, a series of biochemical tests, including carbohydrate fermentation, starch hydrolysis, Gram staining, catalase, indole production, Methyl Red (MR), Voges-Proskauer (VP), Simmons citrate, Motility-Indole-Urease (MIU), and Triple Sugar Iron (TSI) tests, were performed to assist in bacterial identification. The morphological, cultural, and biochemical characteristics of the isolates were analyzed and interpreted according to the guidelines provided in *Bergey's Manual of Systematic Bacteriology* (Sneath *et al.*, 1986).

Molecular identification of the bacterial isolates

Genomic DNAs were extracted from the bacterial isolates using the boiling method, and its concentration and purity were determined using a Nanodrop spectrophotometer. The 16S rRNA gene was then amplified via polymerase chain reaction (PCR) using the universal primers 8F (5'-AGAGTTTGATCMTGGCTCAG-3') and 518R (5'-

GCTACCTTGTTACGACTT-3') in a SimpliAmp™ thermal cycler (Thermo Fisher Scientific, USA). The PCR products were subsequently purified and sequenced using an ABI 3500 sequencer. Forward and reverse sequence reads were assembled into contiguous sequences (contigs) for further analysis. The obtained sequences were processed using ChromasPro software, and sequence similarity searches were performed with BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple sequence alignment was carried out using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>), and a phylogenetic tree was constructed using MEGA version 11.0 (Tamura et al., 2011), following the methodology described by Hakim et al. (2018).

Biocontrol assay

The biocontrol activity of bacterial isolates against foodborne pathogens (*Staphylococcus* sp., *Salmonella* sp., *Shigella* sp., *Bacillus* sp., *Vibrio* sp.) was tested using the agar well diffusion method. Bacterial suspensions (0.5 McFarland) were spread on Mueller-Hinton agar. Wells of 6–8 mm were made and filled with 100 µL of bacterial supernatant and incubated at 37 °C for 18–24 h. Inhibition zones were measured with sterile water as control. All tests were done in triplicate.

Antibiotic susceptibility testing

Antibiotic sensitivity of isolates was tested by the disc diffusion method on Mueller–Hinton Agar following standard guidelines. Commercial antibiotic discs included Gentamicin (10 µg), Ceftriaxone (30 µg), Kanamycin (30 µg), Cotrimoxazole (25 µg), Cefepime (30 µg), Amoxicillin (30 µg), Amikacin (30 µg), and Meropenem (10 µg). Plates were incubated at 37 °C for 24 h, and inhibition zone diameters were measured and interpreted according to reference standards.

3. Results

Microbial load determination

Bacterial counts in non-rhizospheric soils from the four districts of Bangladesh showed considerable variation. The highest bacterial load was observed in Mymensingh ($1.04 \times 10^{10} \pm 8.18$ CFU/g), followed by Dinajpur ($6.8 \times 10^9 \pm 4.58$ CFU/g), Netrokona ($4.8 \times 10^9 \pm 3.61$ CFU/g), and Savar ($1.64 \times 10^9 \pm 9.17$ CFU/g) (Table 1). These findings indicate that microbial abundance in non-rhizospheric soils is influenced by regional and environmental factors, with Mymensingh exhibiting the highest bacterial density, likely due to local variations in soil composition and nutrient availability.

Table 1: Bacterial colony forming unit (CFU) of non-rhizospheric soils from various regions per gram of soil samples.

Sample	Location	Sample Name	CFU±S.D
Non-rhizospheric soil	Dinajpur	Dinajpur non- rhizospheric soil	$6.8 \times 10^9 \pm 4.58$
	Mymensingh	Mymensingh non- rhizospheric soil	$1.04 \times 10^{10} \pm 8.18$
	Netrokona	Netrokona non- rhizospheric soil	$4.8 \times 10^9 \pm 3.61$
	Savar	Savar non- rhizospheric soil	$1.64 \times 10^9 \pm 9.17$

Morphological characteristics of bacterial isolates

Morphological examination of the bacterial colonies revealed a variety of shapes, with the majority being round, elevated, and exhibiting smooth, shiny sur-

faces. The colonies were predominantly white and opaque, characteristic of typical soil bacteria (Table 2; Fig. 1A, 1B and 1C).

Table 2: Morphological characteristics of bacterial isolates from non-rhizospheric soils.

Bacterial isolates	Shape	Margin	Elevation	Size	Texture	Appearance	Pigmentation	Optical property
DNRS1C1	Round	Entire	Raised	Medium	Smooth	Shiny	Cream white	Opaque
DNRS1C2	Irregular	Obate	Umbonate	Large	Smooth	Dry	Cream	Opaque
DNRS1C3	Rhizoid	Filamentous	Umbonate	Small	Matte	Dry	White	Translucent
DNRS1C4	Irregular	Curled	Raised	Medium	Shiny	Moist	Cream	Opaque
MNRS2C1	Round	Entire	Raised	Moderate	Moist	Shiny	Whitish	Translucent
MNRS2C2	Rhizoid	Umbonate	Raised	Moderate	Dry	Dull	White	Opaque
MNRS2C3	Irregular	Erose	Flat	Small	Moist	Dull	Cream white	Opaque
MNRS2C4	Round	Entire	Flat	Small	Moist	Shiny	Orange	Opaque

NNRS3C1	Round	Entire	Raised	Medium	Smooth	Shiny	Orange	Opaque
NNRS3C2	Round	Entire	Raised	Small	Smooth	Shiny	Red	Opaque
NNRS3C3	Round	Entire	Raised	Medium	Smooth	Shiny	White	Translucent
NNRS3C4	Irregular	Lobate	Umbonate	Medium	Smooth	Dull	Cream white	Translucent
CNRS4C1	Rhizoid	Filamentous	Flat	Large	Moist	Shiny	Cream	Opaque
CNRS4C2	Irregular	Undulate	Raised	Large	Matte	Dull	White	Opaque
CNRS4C3	Irregular	Erose	Flat	Large	Moist	Shiny	White	Opaque
CNRS4C4	Round	Entire	Flat	Medium	Dry	Shiny	White	Opaque
CNRS4C5	Round	Entire	Flat	Medium	Moist	Shiny	Yellow	Opaque
CNRS4C6	Irregular	Undulate	Raised	Medium	Moist	Shiny	White	Opaque

Growth on selective media and biochemical tests of the bacterial isolates

Bacterial growth on selective media revealed the presence of several genera, including *Staphylococcus sp.*, *Pseudomonas sp.*, *Micrococcus sp.*, *Bacillus sp.*, *Alcaligenes sp.*, and *Vibrio sp.*, as identified based on their distinct colony characteristics on specific media (Table 3; Fig. 1D, 1E, and 1F). Endospore formation was observed in two isolates, suggesting the presence of *Bacillus sp.* (Table 4; Fig. 1I). Out of the 20 isolates, 8 were Gram-positive cocci, while 10 were Gram-negative rods (Table 4; Fig. 1G and 1H). A series of biochemical tests, including carbohydrate fermen-

tation, indole, catalase, MR-VP, TSI, MIU, and Simmons citrate reactions, were conducted. Of particular note, 13 isolates exhibited starch hydrolysis. All isolates were catalase-positive, and 17 isolates were indole-negative, while fermentation activity was observed in 16 isolates. MR tests yielded positive results for 4 isolates, and VP tests yielded positive results for 14 isolates. In the Triple Sugar Iron (TSI) test, 11 isolates showed a red slant and yellow butt (K/A), whereas 7 isolates displayed a red slant and red butt (K/K). Additionally, 9 isolates were positive for Simmons citrate utilization, and motility was detected in 9 isolates (Table 4).

Table 3: Growth of Bacterial isolates on different selective and differential media.

Bacterial Isolates	MSA	EMB	Mac-Conkey	SSA	BGA	CAB	TCBS	XLD
DNRS1C1	+	-	-	-	-	-	-	-
DNRS1C2	+	+	-	-	+	-	-	-
DNRS1C3	+	-	-	-	+	-	-	-
DNRS1C4	+	-	-	-	-	-	-	-
MNRS2C1	-	-	-	-	-	-	-	-
MNRS2C2	-	-	-	-	+	+	-	-
MNRS2C3	+	-	-	-	-	-	+	-
MNRS2C4	+	-	-	-	-	-	-	-
NNRS3C1	+	+	-	-	+	-	-	-
NNRS3C2	+	-	-	-	+	-	-	-
NNRS3C3	+	-	-	-	-	-	-	-
NNRS3C4	+	-	-	-	+	-	-	-
CNRS4C1	+	-	-	-	-	-	-	-
CNRS4C2	+	-	-	-	-	-	-	-
CNRS4C3	-	-	-	-	-	-	-	-
CNRS4C4	+	-	-	-	-	-	-	-
CNRS4C5	+	-	-	-	+	-	-	-
CNRS4C6	+	-	-	-	-	-	-	-

Table 4: Biochemical characteristics of the bacterial isolates.

Isolates	Catalase test	Starch Hydrolysis test	MR test	VP test	Fermentation test	Indole test	Gram Staining	Endospore Staining	MIU			TSI				Simmons Citrate	Presumptive Organisms
									Motility	Indole	Urea	Slant	Butt	Gas	H2S		
DNRS1C1	+	+	-	+	+	-	+	NA	-	-	+	Red	Red	+	+	-	<i>Staphylococcus sp.</i>
DNRS1C2	+	+	-	-	+	-	-	NA	+	-	-	Red	Yellow	-	-	+	<i>Alcaligenes sp.</i>
DNRS1C3	+	+	-	+	+	-	-	NA	-	-	+	Red	Red	+	+	-	<i>Micrococcus sp.</i>
DNRS1C4	+	+	-	+	+	-	-	NA	+	-	-	Red	Yellow	-	-	+	<i>Micrococcus sp.</i>

MNRS2C1	+	+	-	+	+	-	+	+	+	-	-	Red	Yellow	-	-	+	<i>Bacillus</i> sp.
MNRS2C2	+	-	-	-	-	-	-	NA	+	-	-	Red	Red	-	-	+	<i>Pseudomonas</i> sp.
MNRS2C3	+	-	-	+	+	+	-	NA	+	+	-	Red	Yellow	-	-	+	<i>Vibrio</i> sp.
MNRS2C4	+	-	+	+	+	-	+	NA	-	-	+	Red	Yellow	-	-	-	<i>Staphylococcus</i> sp.
NNRS3C1	+	+	-	-	+	-	-	NA	+	-	-	Red	Yellow	-	-	+	<i>Staphylococcus</i> sp.
NNRS3C2	+	+	-	+	+	-	-	NA	-	-	+	Red	Red	+	+	-	<i>Staphylococcus</i> sp.
NNRS3C3	+	+	-	+	+	-	-	NA	-	-	+	Red	Red	+	+	-	<i>Staphylococcus</i> sp.
NNRS3C4	+	+	+	+	+	-	+	NA	-	-	+	Red	Yellow	-	-	-	<i>Staphylococcus</i> sp.
CNRS4C1	+	+	+	+	+	-	+	NA	-	-	+	Red	Yellow	-	-	-	<i>Staphylococcus</i> sp.
CNRS4C2	+	+	-	+	+	-	+	NA	-	-	+	Red	Red	+	+	-	<i>Staphylococcus</i> sp.
CNRS4C3	+	+	-	+	+	-	+	+	+	-	-	Red	Yellow	-	-	+	<i>Bacillus</i> sp.
CNRS4C4	+	-	-	+	+	-	-	NA	+	-	-	Red	Yellow	+	-	+	<i>Alcaligenes</i> sp.
CNRS4C5	+	-	-	-	-	-	-	NA	+	-	-	Red	Red	-	-	+	<i>Pseudomonas</i> sp.
CNRS4C6	+	+	+	+	+	-	+	NA	-	-	+	Red	Yellow	-	-	-	<i>Staphylococcus</i> sp.

*NA-Not Available; + indicate positive result, - indicate negative result.

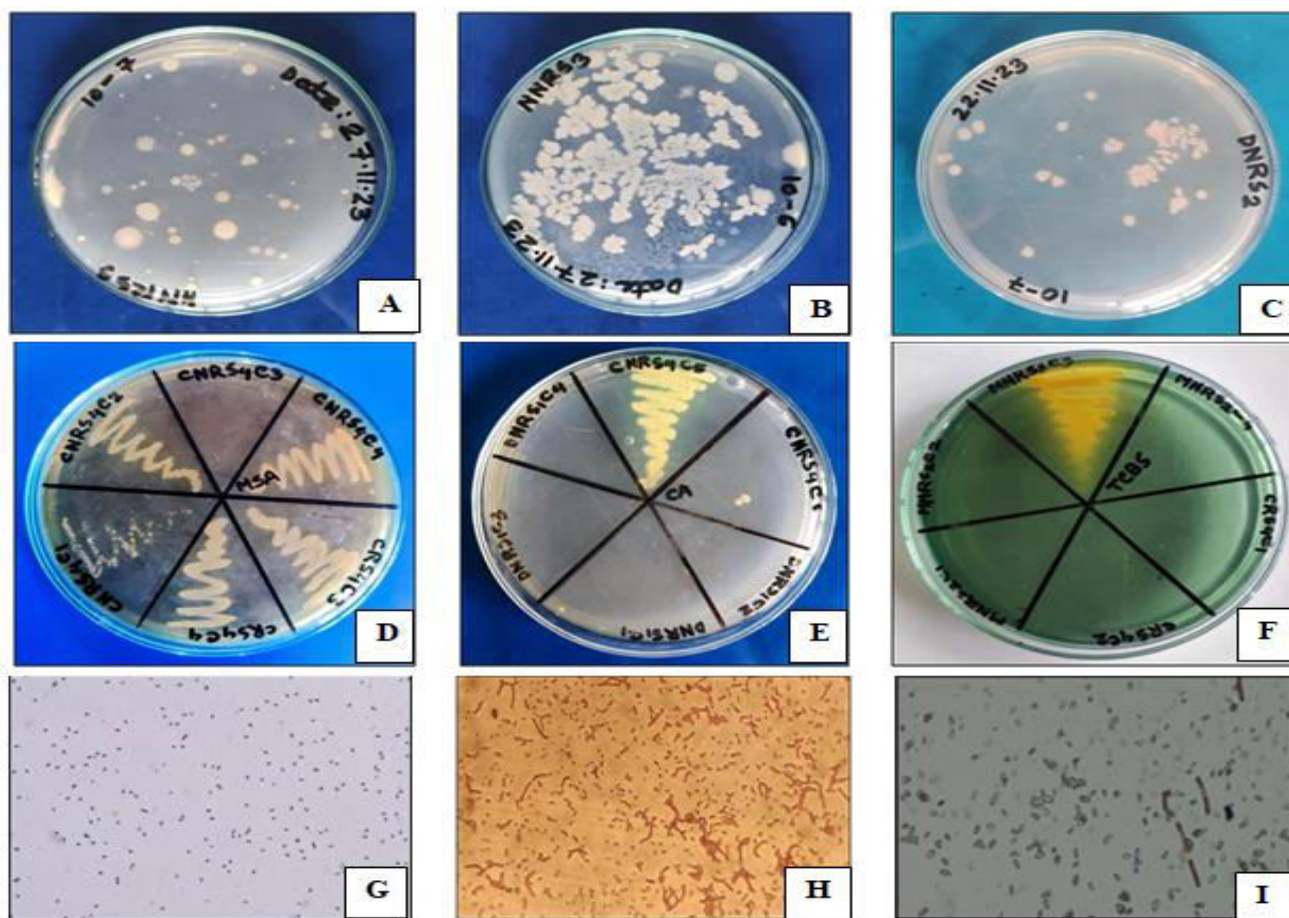


Fig. 1: (A, B, and C) Morphological characteristics of bacterial colonies isolated from non-rhizospheric soil samples. (D) Bacterial colony growth observed on MSA (Mannitol Salt Agar) medium. (E) Bacterial colony growth observed on CA (Cetrimide Agar) medium. (F) Bacterial colony growth observed on TCBS (Thiosulfate Citrate Bile Salts) medium. (G) Gram stain result of isolate DNRS1C1, showing a gram-positive reaction (violet). (H) Gram stain result of isolate MNRS2C2, showing a gram-negative reaction (pink). (I) Endospore staining result of isolate MNRS2C1.

Molecular identification of bacteria

To confirm molecular identification of the bacteria, the 16S rRNA genes of the isolates were amplified and sequenced. The PCR amplicons from the genomic DNA of the isolates were approximately 510 base pairs. BLAST similarity searches revealed high sequence similarity between the isolates and

known bacterial species. For example, isolate MNRS2C2 matching with *Pseudomonas stutzeri* strain BAB1730, and isolate CNRS4C5 corresponding to *Pseudomonas putida* strain ST2N215 species. Phylogenetic trees of the isolates were constructed using similar sequences confirmed these relationships, having 97.29%, and 93.31% identity,

respectively (Fig. 2A and 2B) (Figures of the phylogenetic trees for the other identified bacteria are not shown). Based on these analyses, the isolates

were identified and named accordingly, demonstrating their phylogenetic closeness to known species.

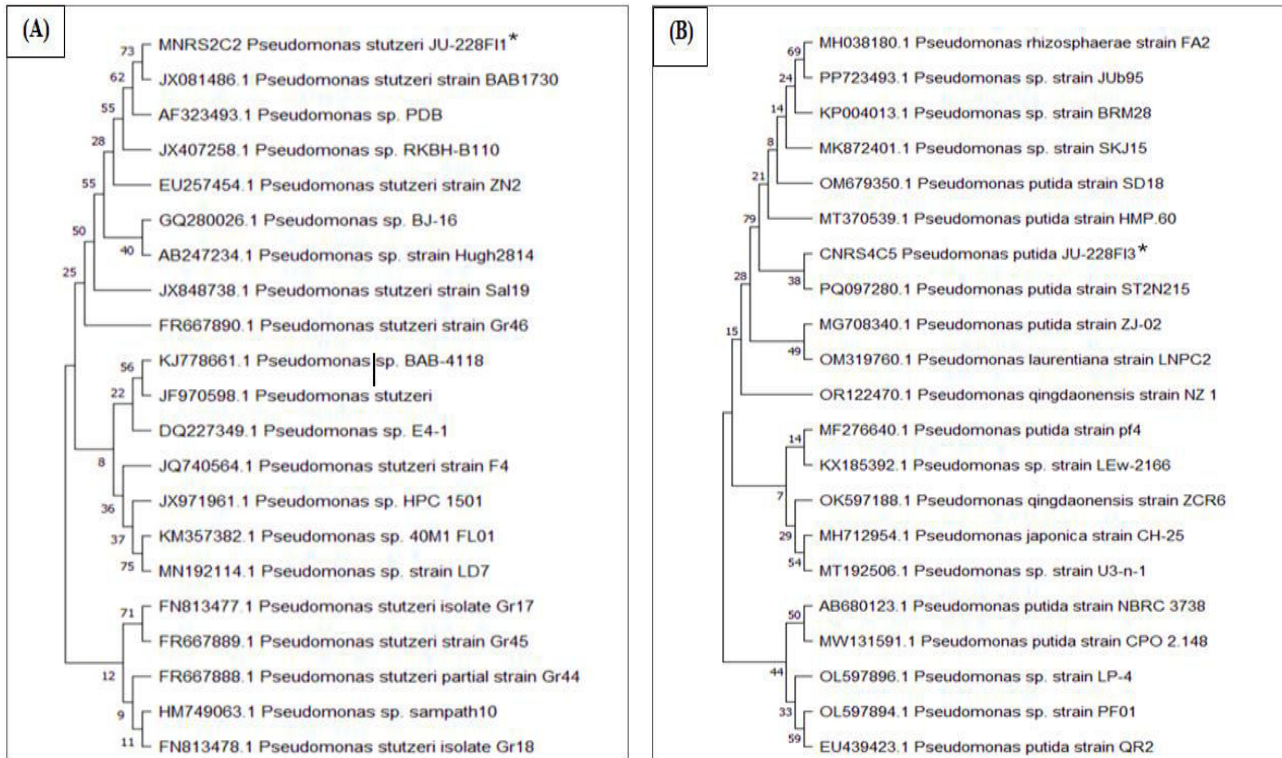


Fig. 2: Phylogenetic trees illustrating the relationships are presented as follows: (A) *Pseudomonas stutzeri* JU228FI1 compared with other *Pseudomonas* species, (B) *Pseudomonas putida* JU228FI3 compared with other *Pseudomonas* species. Asterisks (*) denote the bacteria isolated from the soil samples of this study.

Biocontrol activities of isolated bacteria

Based on cultural, morphological, biochemical characteristics, and molecular identification, several bacterial isolates were obtained from non-rhizospheric soils, including bacteria with potential for biocontrol activity. Two isolates of *Pseudomonas* sp. demonstrated significant biocontrol activity against *Staphylococcus* sp., which was recovered from dairy

food products. The culture supernatant of the isolates of *Pseudomonas* sp. produced a distinct inhibition zone in the agar well diffusion assay, confirming its ability to inhibit the growth of *Staphylococcus* sp. (Fig. 3A and 3B). This result highlights the potential of *Pseudomonas* sp. as a biocontrol agent for suppressing harmful foodborne pathogens.

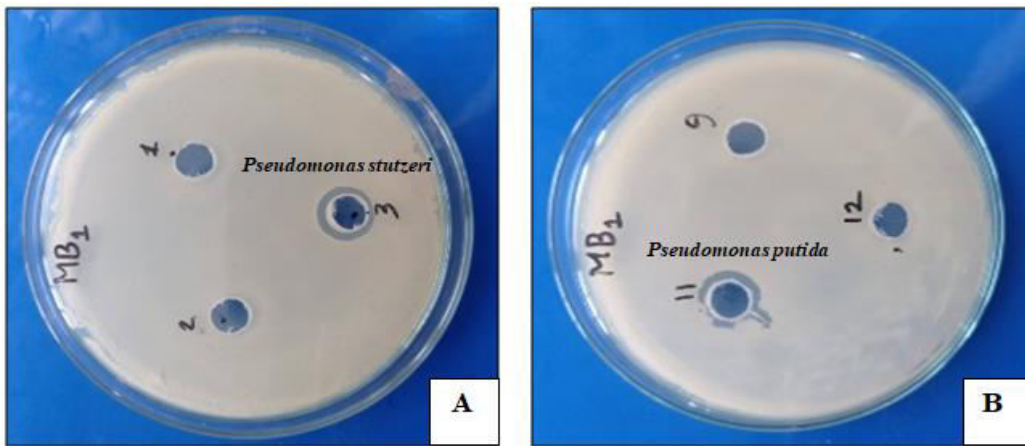


Fig. 3: (A) *Pseudomonas stutzeri* (MNR2C2) showing inhibition zone against *Staphylococcus* sp. (B) *Pseudomonas putida* (CNRS4C5) showing inhibition zone against *Staphylococcus* sp.

Antibiotic susceptibility profile of the isolated bacteria

Antibiotic susceptibility profile revealed that all isolates were sensitive to amikacin, with inhibition zones ranging from 19 to 21 mm (Table 5; Fig. 4A). Most isolates showed intermediate susceptibility to gentamicin and kanamycin, although *Pseudomonas* sp. were sensitive to both gentamicin and kanamycin

(Table 5; Fig. 4B). In contrast, all isolates exhibited resistance to ceftriaxone, cotrimoxazole, cefepime, amoxicillin, and meropenem, indicating a high level of multidrug resistance across the bacterial community. These results highlight the widespread resistance of non-rhizospheric bacteria to several commonly used antibiotics, with amikacin standing out as the most effective option.

Table 5: Antibiotic susceptibility testing of isolated bacteria against eight different antibiotics.

Bacteria	CN (10 µg)		CRO (30 µg)		K (30 µg)		SXT (25 µg)		FEP (30 µg)		AMC (30 µg)		AK (30 µg)		MEM (10 µg)	
	Inhibition Zone (mm)	Sensitivity	Inhibition Zone (mm)	Sensitivity	Inhibition Zone (mm)	Sensitivity	Inhibition Zone (mm)	Sensitivity	Inhibition Zone (mm)	Sensitivity	Inhibition Zone (mm)	Sensitivity	Inhibition Zone (mm)	Sensitivity	Inhibition Zone (mm)	Sensitivity
<i>Staphylococcus</i> sp.	16	I	NZ	R	15	I	NZ	R	NZ	R	NZ	R	19	S	NZ	R
<i>Pseudomonas</i> sp.	19	S	NZ	R	19	S	NZ	R	NZ	R	NZ	R	20	S	NZ	R
<i>Bacillus</i> sp.	16	I	NZ	R	17	I	NZ	R	NZ	R	NZ	R	20	S	NZ	R
<i>Vibrio</i> sp.	13	I	NZ	R	16	I	NZ	R	NZ	R	NZ	R	19	S	NZ	R
<i>Alcaligenes</i> sp.	17	I	NZ	R	16	I	NZ	R	NZ	R	NZ	R	21	S	NZ	R
<i>Micrococcus</i> sp.	15	I	NZ	R	14	I	NZ	R	NZ	R	NZ	R	20	S	NZ	R

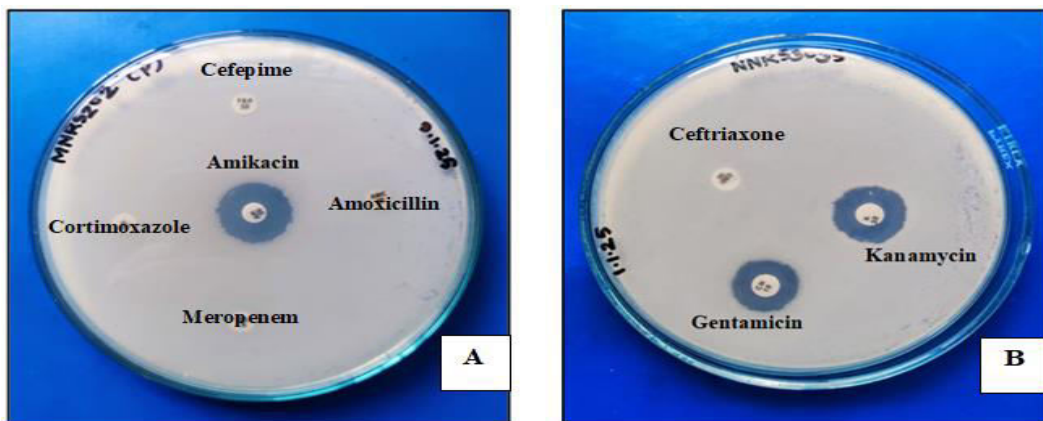


Fig. 4: Antibiotic susceptibility test of isolated bacteria against different antibiotics where clear areas around the discs indicate the zone of inhibition. (A) Isolate MNRS2C2 (*Pseudomonas stutzeri*) showed sensitivity to amikacin but resistance to cefepime, cortimoxazole, amoxicillin, and meropenem. (B) Isolate NNRS3C3 (*Staphylococcus* sp.) showed intermediate sensitivity to gentamicin and kanamycin, while resistant to ceftriaxone.

4. Discussion

The current study was conducted on 18 bacterial isolates from non-rhizospheric soils collected from rice fields in different areas across Bangladesh. The bacterial density analysis of colony-forming units (CFUs) revealed significant differences in non-rhizospheric soils across the locations. Bacterial counts ranged from 1.64×10^9 to 1.04×10^{10} CFU/g in Savar and Mymensingh, respectively. These counts were notably higher than the 8.7×10^6 to 1.8×10^7 CFU/g range reported by Singh et al. (2018) for non-

rhizospheric soils in the Allahabad district, India. Most of the bacterial isolates were Gram-negative (55.55%), while the remaining isolates were Gram-positive (44.45%). This distribution aligns with the findings of Singh et al. (2018), who also observed a predominance of Gram-negative bacteria in similar soils. The observed differences may be attributed to variations in soil types, environmental factors, agricultural practices, and sampling methods. Mannitol Salt Agar (MSA) media were used to isolate *Staphylococcus* sp. and *Micrococcus* sp., with

yellow and pink colonies. This result is consistent with Shields and Tsang, (2006) who observed similar colony color patterns on MSA, a medium known for distinguishing bacteria based on their salt tolerance and mannitol fermentation (Steubing, 1993). The isolation of *Alcaligenes* sp. was achieved using Eosin Methylene Blue (EMB) agar, a method also employed by Alkhalil, (2024) for isolating *Alcaligenes* from water samples. Additionally, *Pseudomonas* species were isolated using Cetrimide Agar Base (CAB), aligning with the work of Mokate and More, (2013), which used this medium for identifying *Pseudomonas aeruginosa* in soil and water samples. Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar was utilized to isolate *Vibrio* sp., consistent with Huq *et al.* (2013), who reported similar colony characteristics for *V. cholerae* on TCBS media. No growth was observed on MacConkey, SSA, and XLD agar media, suggesting the absence of *Salmonella* sp. in the studied soil samples.

This result contrasts with the findings of Rani *et al.* (2022), who identified *Salmonella* sp. in soil samples from Barisal, Bangladesh. Biochemical tests revealed catalase production as a common feature among the isolates, in line with Begum *et al.* (2017) who reported similar findings for soil isolates from Dhaka city. The Simmons citrate test confirmed citrate utilization, with color changes from green to blue, matching the results of Begum *et al.* (2017). Additionally, the majority of isolates were Voges-Proskauer (VP) positive, producing a red ring in the VP test. This finding contrasts with Barbaruah *et al.* (2022), where all isolates from soil samples were VP negative. *Pseudomonas* sp. exhibited biocontrol activity against *Staphylococcus* species, which aligns with the findings of Qin *et al.* (2009), who reported that *P. aeruginosa* supernatant inhibited *S. epidermidis*. In this experiment, *Staphylococcus* sp. was sensitive to Amikacin, showed intermediate susceptibility to Gentamicin and Kanamycin, and was resistant to Ceftriaxone, Cotrimoxazole, Cefepime, Amoxicillin, and Meropenem. These findings align with Bhatt *et al.* (2014) for Amikacin sensitivity and Kengne *et al.* (2020) for the susceptibility to Kanamycin, Gentamicin, and resistance to Cotrimoxazole. *Pseudomonas* sp. showed sensitivity to Amikacin, Gentamicin, and Kanamycin, consistent with Edson and Terrell (1987), who found Amikacin and Gentamicin

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effective against *P. aeruginosa*. *Bacillus* sp. was sensitive to Amikacin (Adamski *et al.*, 2023) and, like Tarale *et al.* (2015), showed intermediate sensitivity to Gentamicin and Kanamycin. *Vibrio* sp. was sensitive to Amikacin, contrary to Haque *et al.* (2023), and showed intermediate sensitivity to Gentamicin and Kanamycin, which contrasts with Okoh and Igbiosa, (2010) who reported resistance to Gentamicin. The bacterial community in the rice fields of Bangladesh showed distinct differences when compared to studies conducted in other regions. While Proteobacteria were predominant in Thai paddy fields (Yongsuwattana *et al.*, 2023), this study found *Staphylococcus* species to be most prevalent in Bangladeshi rice field soils. This variation could be attributed to differences in soil composition, environmental factors, or agricultural practices between the two regions. In conclusion, this study provides important data for understanding soil microbiota dynamics and their potential role in enhancing agricultural productivity and soil health.

5. Conclusion

This study examines the bacterial diversity in non-rhizospheric soils from rice fields in four regions of Bangladesh, revealing a complex mix of beneficial and potentially harmful bacterial communities. The prevalence of *Staphylococcus* sp. raises concerns due to its link to foodborne diseases, while *Pseudomonas* sp. shows promising biocontrol potential against dairy-associated *Staphylococcus* pathogens, suggesting a natural method for pest management. The widespread antibiotic resistance highlights the critical need for ongoing monitoring of soil microbiota to mitigate the spread of multidrug-resistant bacteria. These findings emphasize the importance of integrating microbial monitoring with biocontrol strategies in agricultural practices, particularly in resource-limited settings like Bangladesh. Such approaches can improve food safety, reduce chemical pesticide reliance, and combat the growing threat of antimicrobial resistance. This research provides a foundation for future studies aimed at using soil microbiota to promote sustainable agriculture and address public health challenges.

6. Author Contributions

The writing of the original draft of the manuscript, data collection and analysis, editing and laboratory experiments were performed by F.T.; investigation,

conceptualization, writing reviews, editing, and supervision were conducted by R.Y.S.; manuscript writing and support in laboratory experiments were contributed by N.A.; writing review and editing were carried out by M.Z.A.; writing and editing were contributed by S.N.J. All authors have read and agreed to the published version of the manuscript.

7. Ethical Clearance

The study did not involve the use of animal models or human participants. This study was conducted in compliance with the Department of Botany, Jahangirnagar University's ethical standards and guidelines. All experimental procedures were carried out in accordance with institutional and international ethical standards for laboratory-based research.

8. Acknowledgement

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9. Conflicts of Interest

This article is original work, and the authors declare that there are no conflicts of interest relevant to this article.

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