Exploring Soil Microbial Diversity: Assessing Phosphate Solubilization, Antagonism, and Antibiotic Resistance in Various Microorganisms

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ABSTRACT

This study isolates and characterizes culturable bacteria and fungi from various topsoil samples collected from different locations within Paschim Medinipur and Purba Medinipur. The main objective is to determine the microbial diversity in these soil ecosystems, laying the groundwork for their potential applications. A comprehensive survey was conducted to identify the prevalence of bacteria, fungi, and cyanobacteria. The plate count method quantified microbial populations from five randomly collected soil samples, revealing a concentration range of 1.84×10^9 to 2.88×10^9 CFU/ml. A vital aspect of this research aimed to isolate and identify cyanobacteria with potential bio-fertilizer applications for rice cultivation. Isolated cyanobacterial strains were cultured in 500 ml flasks containing 150 ml of BG-11 medium under static conditions at 25°C ± 2°C, with controlled photon flux, over 30 days.

The phenomenon of bacterial antagonism, where the growth and activity of one bacterium (bacterium A) is inhibited by another (bacterium B) due to the production of specific inhibitory compounds, was also investigated. These antagonistic interactions have significant implications, particularly in the increasing resistance of pathogenic bacteria to existing antibiotics, many of which are associated with undesirable side effects. This research highlights the adaptive strategies employed by bacteria to counteract antibiotic action.

Furthermore, phosphate-solubilizing microbial colonies were isolated using Pikovskaya's agar medium, which incorporates insoluble tricalcium phosphate (TCP). Colonies that produced clear halo zones around their growth were identified as phosphate-solubilizing microorganisms. This capability is crucial for improving soil fertility and promoting sustainable agricultural practices.

1. Introduction

Soil is the crucial interface where geology and biology meet, providing a habitat for various plant, animal, and microbial life forms (Pelczar & Chan, n.d.). This includes a diverse microcosm filled with bacteria,

fungi, algae, protozoa, viruses, earthworms, nematodes, mites, and insects. The abundance and diversity of microorganisms in soil are primarily influenced by nutrient availability, moisture content, aeration, pH levels, and temperature. Among these factors, pH is critical, significantly impacting solute concentration and absorption (Akpoveta *et al.* 2010). It is essential for farmers and gardeners, as many plant and soil organisms thrive in acidic or alkaline conditions (Kavinilavu & Pandeeswari, 2019).

The bacteria and fungi found in soil are crucial for biochemical cycles, especially in recycling organic compounds (Wall & Virginia, 1999). These microorganisms are essential in maintaining soil health, influencing above-ground ecosystems by providing plant nutrients and preserving soil structure and fertility. Fungi, in particular, substantially impact ecosystem structure and function, providing critical ecological services such as nutrient cycling, disease suppression, and water dynamics (Orgiazzi *et al.* 2012).

Using fungi in nutrient recovery has become popular in agricultural practices as an eco-friendly and cost-effective alternative to chemical fertilizers, posing significant environmental pollution risks (Suhag, 2016). Excessive use of chemical fertilizers has reduced the colonization of plant roots by symbiotic fungi, thereby diminishing the beneficial effects of soil fungi.

Cyanobacteria are well-adapted to thrive in freshwater and wetland ecosystems, including artificial wetland systems like lowland paddy fields. They exist both as free-living organisms (e.g. *Aphanocapsa, Gloeocapsa, Merismopedia*) and in symbiotic associations (e.g. Anabaena azolla) (Kulasooriya, 2012). Cyanobacteria display significant morphological diversity, ranging from unicellular forms (e.g. *Synechocystis* sp.) to complex filamentous structures and spore-forming types (Wehr *et al.* 2015). Importantly, they are major nitrogen-fixing organisms in paddy field ecosystems, with their diversity and nitrogen-supplying potential varies depending on the growth stage of rice plants and the physicochemical environment of the soil (Prasanna *et al.* 2009; Roger, 1996).

Phosphorus is a crucial growth-limiting nutrient in many ecosystems. Unlike nitrogen, there is no substantial atmospheric source of phosphorus that can be biologically converted for plant usage. Phosphorus is essential for numerous plant processes, including root development, stem and stalk robustness, flower and seed formation, crop maturity, N-fixation in legumes, and plant disease resistance (Khan *et al.* 2009). It also plays crucial biochemical roles in photosynthesis, respiration, energy storage and transfer, cell division, and cell enlargement (Amit Sagervanshi *et al.* 2012). Unfortunately, a significant portion of Indian

soils, approximately 98%, lack sufficient available phosphorus to maximize plant growth.

Microorganisms frequently exist as structured multi-species communities within various ecosystems (Stoodley *et al.* 2002). Interactions among these microorganisms, including competition for substrates and the production of metabolites, can significantly influence the development of microbial colonies (Rasmussen *et al.* 2005). Competitive substrate utilization is a primary evolutionary drive in bacterial communities (Banks & Bryers, 1991).

Microorganisms' development of resistance to antimicrobial agents poses a significant threat to public health. Some strains exhibit resistance to a single or related class of antimicrobial agents, while others, known as multidrug-resistant (MDR) strains, demonstrate resistance to multiple classes of antibiotics (Prestinaci *et al.* 2015). This resistance can render standard antibiotics ineffective, necessitating the urgent development of new and innovative antimicrobial agents. The widespread use of antibiotics in human therapy has led to the selection of pathogenic bacteria with multidrug resistance, underscoring the need for alternative strategies to combat this rising threat (Alves-Barroco *et al.* 2020).

In this study, we have various objectives. Firstly, we are focused on understanding the microbial ecology and potential applications of microorganisms found in different soil samples. We want to analyze the physiochemical properties of these soil samples, examine the microbial loads present, and establish pure cultures of the isolated microorganisms using stab and slant preservation techniques. We also aim to visualize these microorganisms using various staining procedures and isolate cyanobacteria from paddy soil and water sources.

In addition, our study aims to identify phosphate-solubilizing bacteria, investigate bacteria-bacteria antagonism dynamics, and explore the antibiotic resistance profiles of the isolated bacterial strains. Through these objectives, we hope to contribute to a deeper understanding of the microbial communities inhabiting these soil environments and the potential biotechnological implications of their interactions and properties.

2. Materials and Methods

Potassium chloride (KCl), Nutrient agar (NA) media, Crystal violet, Methylene blue, Potato dextrose agar (PDA), Lactophenol cotton blue, Sterile water, N-free BG-11 medium, Brilliant Green Agar (BGA), Bromo Cresol Purple – Deoxycholate - Citrate - Lactose - Sucrose (B.C.P.- D.C.L.S.) Agar, Pikovskaya'sagar medium, Penicillin antibiotics, Streptomycin antibiotics.

2.1.Sample Collection

We collected soil samples from various locations within Paschim Medinipur and

Purba Medinipur districts in West Bengal, including the campus of Midnapore College (Autonomous). The samples were taken from paddy fields, potato fields, forests, gardens, and playgrounds.

We obtained soil samples from a depth of 8-10 cm in most areas, except for the paddy field, where we collected mud and water samples from 3-4 cm below the field's surface. We made sure to manually remove any plant material and other debris from the samples. The collected soil samples were stored in polythene bags and kept in the laboratory for analysis.

2.2.Physiochemical Properties Analysis

The soil's physicochemical properties were analyzed by creating aqueous solutions from freshly collected samples. After filtration, 5-10 drops of KCl were added to check for colour changes. The solution was then tested using litmus paper to identify the relevant physicochemical parameters.

2.3. Microbial Analysis of Soil Sample

• Isolation and Enumeration and preservation of Microorganisms

The isolating and counting of bacteria and fungi involved a standard procedure using serial dilution and the plate technique. Initially, suspensions from the soil samples underwent serial dilution, resulting in dilutions ranging from 10^{-5} to 10^{-8}. Then, portions from these dilutions were plated onto NA for bacteria and a specific medium for fungi. The plated samples were incubated at a controlled temperature of 35°C for 24-48 hours to encourage microbial growth. After the incubation period, the developed colonies were examined and counted. This involved determining the number of colony-forming units (CFUs) per millilitre using a formula that considered both the colony count and the dilution factor.

No. of CFUs per ml of sample = No. of colonies × dilution factor

By following this thorough and systematic approach, we could accurately measure the microbial populations in the soil samples, enabling us to gather valuable insights into the abundance and diversity of bacteria and fungi in the sampled environments.

Stab and slant cultures were prepared to ensure the long-term preservation of these bacterial isolates. Stab cultures involve aseptically inoculating bacterial isolates into solid agar tubes to create a vertical stab line within the medium for storage. On the other hand, slant cultures are prepared by streaking bacterial isolates on the slanted agar surface within tubes, providing a larger surface area for bacterial growth and extended preservation. These techniques effectively maintain the viability and purity of the isolated bacterial strains over extended periods, facilitating future studies and analyses using preserved cultures.

• **Staining**

During the staining and preservation process, bacterial colonies were categorized using Gram's staining, a fundamental technique distinguishing bacteria into Gram-positive and Gram-negative types. Gram-positive bacteria retain the dark violet colour of the crystal violet stain, while gram-negative bacteria appear pink or red after counterstaining with Safranin. This staining method allows for the visual differentiation of bacterial cell wall characteristics, aiding in identifying and characterizing isolated bacteria.

2.4. Microbial Analysis of Soil Sample (Fungi)

• **Isolation of Fungi from Soil**

The process of isolating fungi from soil samples involved several steps. First, we created diluted soil suspension concentrations ranging from 10^{-2} to 10^{-4}. Then, we took 100 µl samples from each dilution and carefully spread them onto PDA media plates using aseptic techniques. The plates were then placed in a controlled incubator at 28°C for 72 hours to encourage the growth of fungi present in the soil samples.

By systematically following this method, our goal was to facilitate the growth and identification of different fungal species in the soil samples on the PDA media plates. During the incubation period, distinct fungal colonies developed, allowing for further analysis and identification of fungal isolates. This detailed and controlled approach aimed to successfully isolate and characterize diverse fungal species within the soil samples.

• **Identification of the Isolated Strain**

We used microscopic observation and staining with lactophenol cotton blue dye to identify isolated fungal strains. We placed a small portion of the fungal mat on a microscope slide and stained it by applying a few drops of lactophenol cotton blue. The stain was allowed to sit for 2-3 minutes, and then the excess stain was gently blotted out using tissue paper. After that, we delicately positioned a cover slip over the stained fungal sample. The prepared slide was then placed under a microscope at a magnification of 40X for detailed observation and examination of the fungal structures. This staining technique and microscopic analysis allowed us to visualize and assess the characteristic morphological features of the fungal isolates. It provided valuable insights for identifying and classifying the isolated fungal strains based on their unique cellular characteristics, aiding in the accurate taxonomic characterization of the fungi in the soil samples.

2.5. Isolation and Identification of Cyanobacteria

The process of isolating and identifying

cyanobacteria involved several steps. First, a 1-gram soil sample was mixed with 9 millilitres of sterile water to create the soil inoculum. Water samples from the paddy field were also used as inoculum. Then, 1 millilitre of inoculum from the soil and water samples was transferred into two conical flasks containing N-free BG-11 Medium, which provides a suitable environment for cyanobacteria growth.

After inoculation, the flasks were incubated in alternating dark and light conditions at an average temperature for 15-30 days. The cultures were monitored, kept at a consistent temperature of 25°C ± 2, and exposed to a specific photon flux to facilitate their growth in batches. At the end of the incubation period, the cyanobacterial cultures were examined under a microscope for identification. This microscopic observation allowed for visualization and assessment of the characteristic morphological features of the strains. This method aimed to successfully isolate and identify cyanobacteria in the soil and water samples, contributing to a better understanding of the microbial composition in the paddy field environment.

2.6. Isolation of Bacteria on Selective Medium

The isolation of bacteria on selective media involved the preparation and utilization of two distinct media types: Brilliant Green Agar (BGA) and Bromo Cresol Purple – Deoxycholate - Citrate - Lactose - Sucrose (B.C.P.- D.C.L.S.) Agar. The preparation of the selective media involved specific procedures tailored to each type. For the BGA plates, 58.09 grams of agar were suspended in 1 litreof distilled water, while for the B.C.P.- D.C.L.S. agar plates, 67.5 grams were suspended in 1 litre of distilled water. These carefully prepared media provided a selective environment conducive to the growth of specific bacteria.

To initiate the isolation process, 1 gram of each soil sample was mixed with 9 millilitres of distilled water, resulting in a 10^{-1} dilution. This dilution was the inoculum for isolating microorganisms on the respective selective media. Once the media solidified, the plates were inoculated with the prepared inoculum using the spread plate method to ensure even distribution of the microbial sample across the surface of the agar.

Subsequently, the inoculated plates were placed in an incubator set at 37°C and incubated for 24 hours. After the incubation period, the plates were carefully observed to assess the growth and development of bacterial colonies. This method facilitated the selective isolation and evaluation of bacteria based on their ability to thrive in the specific conditions provided by the BGA and B.C.P.- D.C.L.S. agar media, contributing to an enhanced understanding of the microbial composition within the soil samples.

2.7. Isolation of Phosphate Solubilizing Bacteria

The process of isolating phosphate-solubilizing bacteria involved a systematic procedure to identify and isolate microbes capable of solubilizing phosphate in soil samples. Initially, soil samples were collected and stored in polythene bags under aseptic conditions. These samples were then brought to the laboratory for further analysis.

To start the isolation process, 1 gram of rhizosphere soil was mixed with 9 millilitres of distilled water to create a 10^{-1} dilution. Subsequently, serial dilutions were performed to obtain dilutions up to 10^{-4}. From these dilutions, 1 millilitre of solutions from the 10^{-3} and 10^{-4} dilutions was spread on Pikovskaya's Agar Medium using the spread plate method. This specialized medium provided a conducive environment for the growth and identification of phosphate-solubilizing microbes.

The inoculated plates were then placed in an incubator set at 25°C and incubated for 5-6 days. During incubation, colonies exhibiting a phosphate-solubilizing zone around them were identified as phosphate-solubilizing bacteria (PSB). These colonies were considered significant for their ability to solubilize phosphate in the agar medium.

Individual colonies displaying the phosphate-solubilizing zone on Pikovskaya's agar plates were carefully selected and transferred into prepared stab and slant cultures for preservation and further investigation. This step ensured maintaining and properly storing the isolated phosphate-solubilizing bacteria for ongoing studies and future research endeavours.

Through this systematic approach, phosphate-solubilizing bacteria from soil samples were successfully isolated and preserved, contributing to understanding their potential role in nutrient cycling and soil health.

2.8. Determination of Bacteria-bacteria Antagonism of Different Soil Sample

A systematic method determined the antagonistic interactions between bacteria in various soil samples. The experiment began with preparing an inoculum by mixing 1 gram of each soil sample with 9 millilitres of distilled water to create a 10^{-1} dilution. This dilution was the starting point for evaluating how the bacterial strains interacted and exhibited antagonistic behaviours in the different soil samples.

Five Nutrient Agar plates were meticulously prepared and allowed to solidify for the experiment. Using the spread plate method, a bacterial lawn was evenly spread on the surface of each agar plate, ensuring the uniform distribution of the inoculum. Following this, four holes were made on each agar plate using a cork borer.

The other four diluted inoculums were then carefully poured into each punching hole of every agar plate, except for the one where the initial inoculum was spread. This setup allowed for the interaction and confrontation of bacterial strains from different soil samples and facilitated direct observation of their antagonistic behaviours under controlled laboratory conditions.

After inoculation, the plates were incubated at 37°C for 24 hours. Following the incubation period, the plates were methodically observed to evaluate the outcomes of the interactions between the bacterial strains. This direct observation provided insights into antagonistic relationships, such as inhibition zones or competitive growth, enabling the assessment of bacterial antagonism among the diverse soil samples. Through this approach, the experiment aimed to shed light on the dynamics of bacterial interactions and their implications for microbial ecology and soil health.

2.9. Isolation of Antibiotic Resistant Microorganism

The isolation of antibiotic-resistant microorganisms involved a targeted approach to identify microorganisms capable of withstanding the inhibitory effects of specific antibiotics. The experiment commenced with the preparation of inoculum, wherein 1 gram of each soil sample was mixed with 9 millilitres of distilled water to produce a 10^{-1} dilution. This dilution served as the basis for isolating antibiotic-resistant microorganisms present in the soil samples.

Five nutrient agar (NA) plates were meticulously prepared to initiate the isolation process. Each antibiotic (penicillin and streptomycin) was incorporated into the medium at a concentration of 3 µg/ml. These antibiotic concentrations enabled the selective growth of microorganisms resisting penicillin and streptomycin. Each plate was specifically labelled and visually distinguished. Subsequently, the prepared plates were allowed to solidify, forming a consistent surface for inoculation.

Following solidification, each plate was inoculated with the respective inoculum using the spread plate method, ensuring uniform distribution of the microbial samples across the agar surface. The plates were then incubated at a controlled temperature of 37°C for seven days, providing a suitable environment for the growth of microorganisms and the expression of antibiotic resistance.

After the designated incubation period, the plates were systematically observed to evaluate the growth and development of microbial colonies. This observation was critical for identifying and isolating microorganisms demonstrating resistance to the specific antibiotics in the growth medium. Through this systematic approach, the

experiment aimed to successfully isolate antibiotic-resistant microorganisms from the soil samples, thereby contributing to understanding antibiotic resistance in environmental microbiota and its implications for public health and environmental management.

Statistical analysis

The studies were conducted in triplicate, and the mean ± standard error data are reported in table. Microsoft Excel 2021 was used for data analysis and graphical representation.

3. Results

3.1. Physiochemical Property of Soil

The pH of each soil sample was carefully determined and observed, yielding insightful results as summarized in Table 1. The soil collected from the dry location exhibited alkaline characteristics, while the soil samples collected from the Potato field, Garden, and Paddy field demonstrated slightly acidic pH levels. In contrast, the soil sample obtained from the forest was acidic, reflecting the diverse pH profiles across the sampled environments.

The pH of soil plays a pivotal role in indicating the availability and balance of essential nutrients within the soil matrix. This fundamental characteristic is a key determinant of soil fertility, offering crucial insights into the potential need for soil amendments to enhance and maintain its productive capacity. This study's determination and assessment of soil pH revealed diverse pH ranges across the sampled environments. These ranges underscored the pivotal role of pH in influencing soil fertility and guiding soil management strategies.This comprehensive understanding of soil pH dynamics forms the foundation for addressing soil health and fertility concerns and facilitating sustainable agricultural and environmental stewardship initiatives.

3.2. Enumeration and Classification of Bacteria

The enumeration and classification of

Table 1. Represent the physiochemical property of soil samples.

Soil sample	pH value	Inference
Paddy field	5.5	Slightly acidic
Potato field	5.5	Slightly acidic
Forest	3	Acidic
Garden	6.8	Slightly acidic
Dry	8.5	Alkaline

bacteria revealed distinct variations in microbial load among the five different soil samples, as presented in Table 2 and depicted in Figure 1. Upon analysis, it was observed that the colony-forming units of the soil samples followed a descending order, with the paddy field soil exhibiting the highest microbial load, followed by the forest, garden, potato field, and finally, the dry soil. Notably, the differences in bacterial counts across the various soil samples were statistically insignificant.

Table 2. Total CFU count of five different soil sample

Soil sample	CFU/ml
Forest	$2.68 \pm 0.066 \times 10^9$
Potato field	$2.20 \pm 0.057 \times 10^9$
Garden	$2.52 \pm 0.057 \times 10^9$
Paddy field	$2.88 \pm 0.044 \times 10^9$
Dry	$1.84 \pm 0.043 \times 10^9$

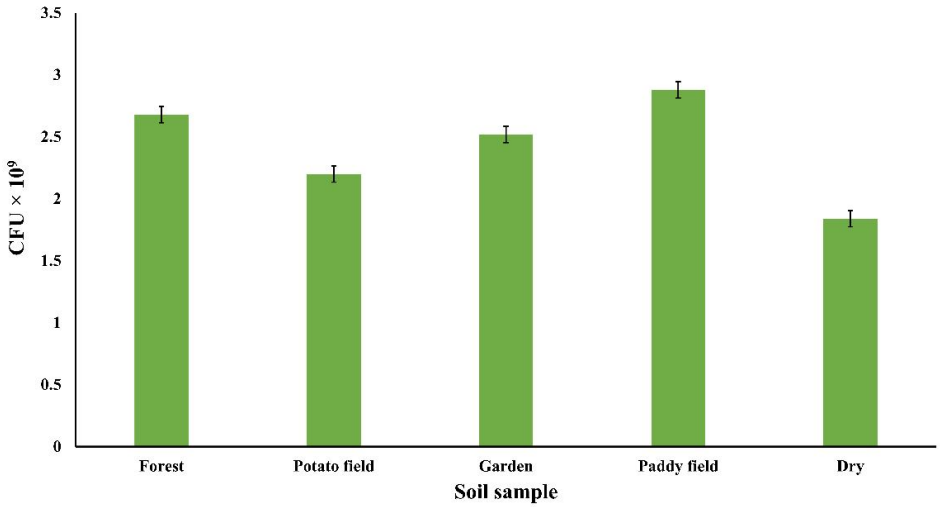


Figure 1. The total CFU count of the different soil samples.

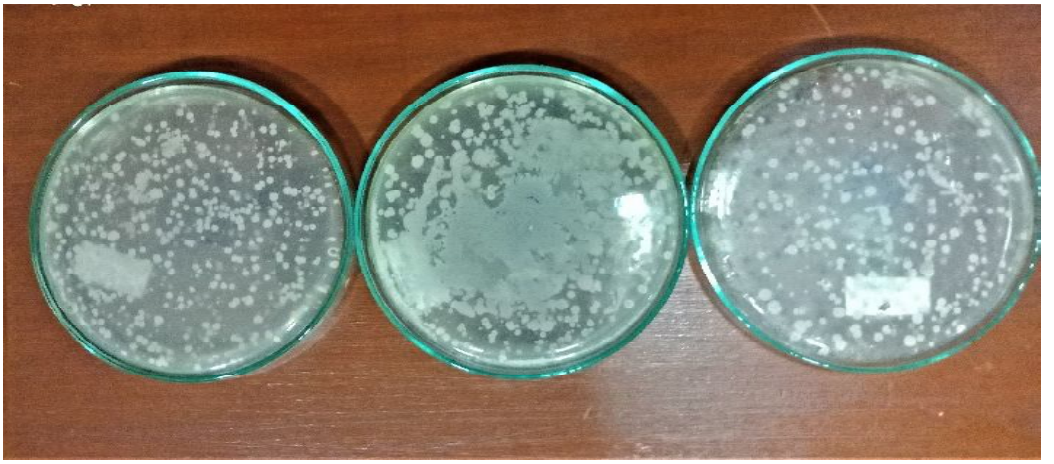


Figure 2. Nutrient agar plates with isolated bacteria from soil sample

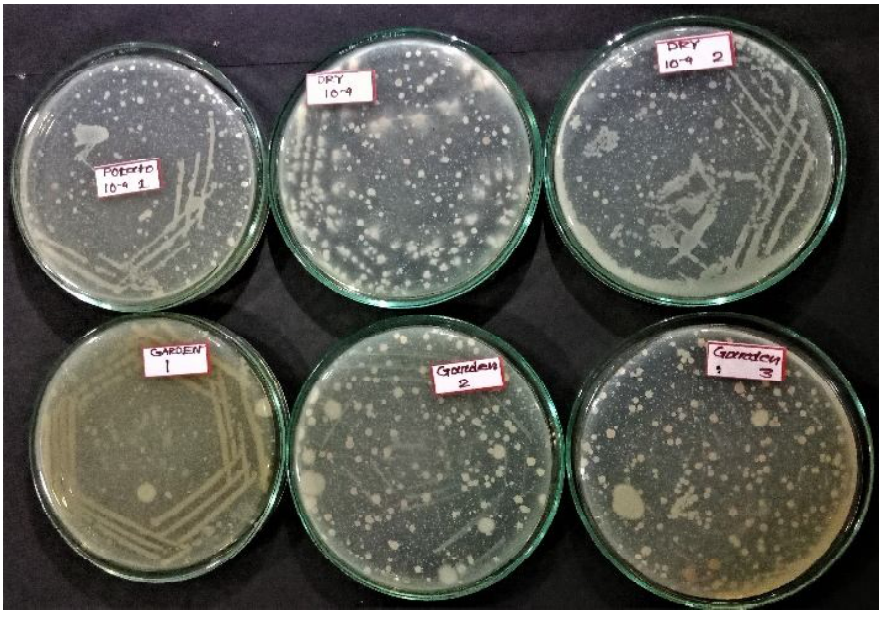


Figure 3. Streak plates for isolation of pure culture.

3.3. Morphological Identification of Bacteria

In identifying bacteria based on their structure, the Gram staining technique was used to distinguish between Gram-positive and Gram-negative bacteria by observing how they reacted to different stains. Gram-positive bacteria absorbed the Crystal violet stain and looked dark violet, while Gram-negative bacteria appeared pink or red when stained with Safranine.

Table 3. Represent the variation in bacterial types in sampling locations

Soil sample	Plate no.	Colony morphology	Gram staining	Bacterial types
Garden	Plate 1	Circular	Gram negative	Bacillus
	Plate 2	Circular	Gram positive	Bacillus
	Plate 3	Irregular	Gram positive	Cocci
Paddy field	Plate 1	Irregular	Gram positive	Bacillus
	Plate 2	Circular	Gram negative	Bacillus
	Plate 3	Irregular	Gram positive	Cocci
Forest	Plate 1	Circular	Gram positive	Bacillus
	Plate 2	Circular, Irregular	Gram negative	Bacillus
	Plate 3	Circular, Irregular	Gram positive	Cocci
Dry	Plate 1	Circular	Gram negative	Bacillus
	Plate 2	Circular	Gram negative	Cocci
	Plate 3	Circular	Gram positive	Bacillus
Potato field	Plate 1	Circular, Irregular	Gram positive	Bacillus
	Plate 2	Circular	Gram positive	Cocci
	Plate 3	Circular, Irregular	Gram negative	Bacillus

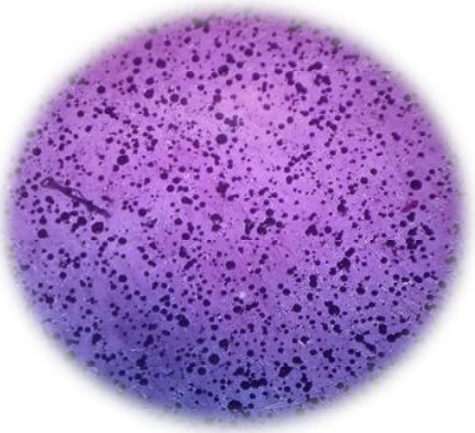


Figure 4. Microscopic image of gm-positive cocci isolated from soil sample

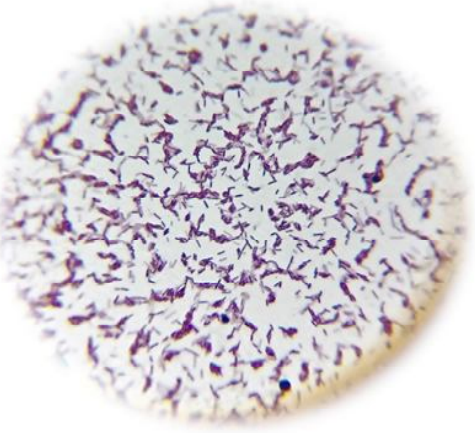


Figure 5. Microscopic image of gm-negative bacillus isolated from soil sample

3.4. Identification of Fungi

Following a meticulous incubation period, a diverse array of colonies, each with unique shapes and colours, emerged on the PDA plate. These colonies were carefully selected for further analysis, with the goal of identifying the bacteria to at least the genus level. From this selection, three colonies were successfully identified, with a high probability exceeding 90%, underscoring the thoroughness of our identification process.

Microscopic examination revealed three species among the selected colonies: *Penicillium* sp., *Aspergillus* sp., and *Rhizopus* sp. Based on the identification results, it was observed that *Aspergillus* sp. was the most prevalent species in the analyzed soil samples, indicating its significance within the studied microbial community.

Our findings have highlighted the dominance of *Aspergillus* sp. among the identified species, underscoring its significance within the studied microbial community. These results significantly contribute to a deeper understanding of the microbial diversity within the soil ecosystem, emphasizing the importance of morphological identification in understanding the structure and dynamics of the microbial community.

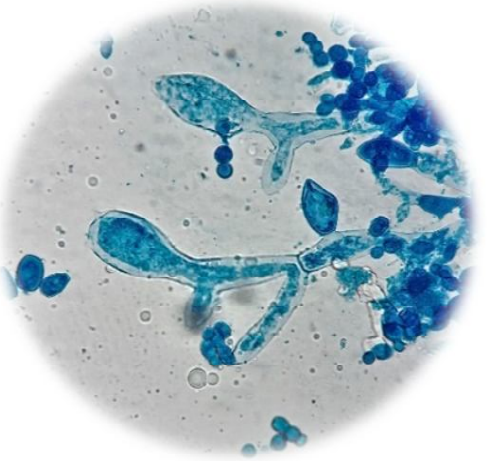
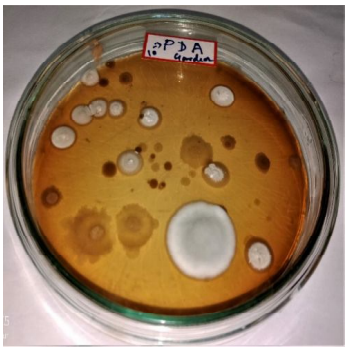


Figure 6. Microscopic image of fungi isolated from soil sample

Table 4. Represent the variation in fungal types in sampling location

Soil Sample	Plate No	Colony Colour And Morphology	Microscopic Analysis	Identification
Paddy field	Plate 1	Yellow, Filamentous		*Aspergillus* sp.
	Plate 2	Brown, dot shaped	Stained Spores	Unidentified
	Plate 3	White, Non-Filamentous		*Aspergillus* sp.
Potato field	Plate 1	Cloudy Brown, Filamentous	Septate interconnected mycelium	Unidentified
	Plate 2	Brown, Dot shaped		*Rhizopus*
	Plate 3	Cloudy Grey	Intertangled mass of mycelium	Unidentified
Garden	Plate 1	Yellow, Non-Filamentous		Basidiomycetes / Deuteromycetes
	Plate 2	Brown, Dot shaped	Only spores are stained	Unidentified
	Plate 3	Brown	Intertangled mycelia with conidia	Unidentified
Forest	Plate 1	Black		Unidentified
	Plate 2	Brown, Dot shaped	Only spores are stained	Unidentified
	Plate 3	Greenish		*Penicillium* sp.
Dry	Plate 1	White, Filamentous	Tangled mycelium (lightly stained)	Unidentified
	Plate 2	Black, Filamentous		Unidentified
	Plate 3	White, Irregular		Unidentified

A



B

Figure 7. (A) PDA plates with isolated fungi from garden soil sample. **(B)** PDA plates with isolated fungi from forest soil sample

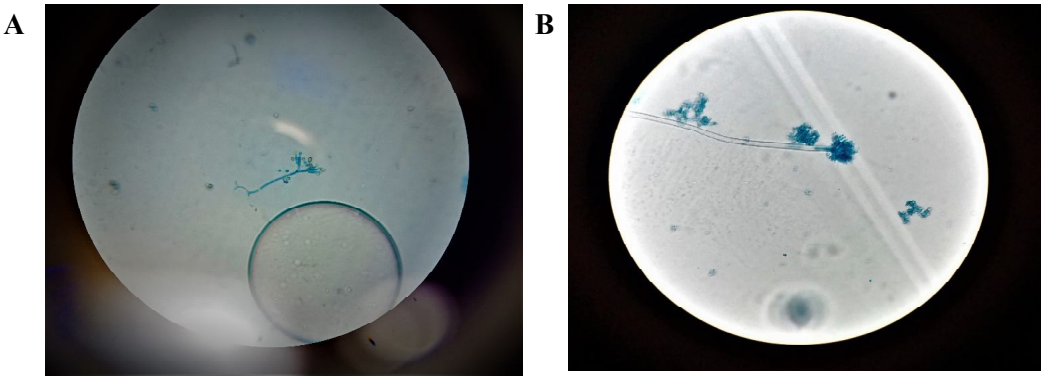


Figure 8. (A) Microscopic image of penicillin isolated from soil sample. **(B)** Microscopic image of *aspergillus* isolated from soil sample

3.5. Identification of Cyanobacteria

Our identification process for cyanobacteria was meticulous. The isolates were characterized through microscopic observation, enabling us to differentiate and classify various microbial organisms. Upon examination, we observed a mixed culture comprising green algae, blue-green algae, and phytoplankton. To isolate and identify the blue-green algae specifically, we cultured them in the specialized BG-11 media to obtain pure cultures for closer examination.

Within the pure cultures established in the BG-11 medium, distinct species of cyanobacteria were successfully identified through microscopic observation. Notably, Chlorella, Spirulina, and Ulothrix species were clearly distinguished and classified within the blue-green algae group. This microscopic analysis facilitated accurately identifying and characterizing these cyanobacterial species based on their morphological features such as cell shape, size, and arrangement and cellular structures like chloroplasts and phycobilisomes.

By isolating and identifying cyanobacteria such as Chlorella, Spirulina, and Ulothrix in the pure culture established in the BG-11 medium, we have made significant strides in understanding the diversity and composition of cyanobacterial communities within the studied environment. These findings are not just important, they are crucial, as they contribute valuable insights into the presence and distribution of cyanobacteria, shedding light on their ecological roles and potential impacts within the ecosystem.

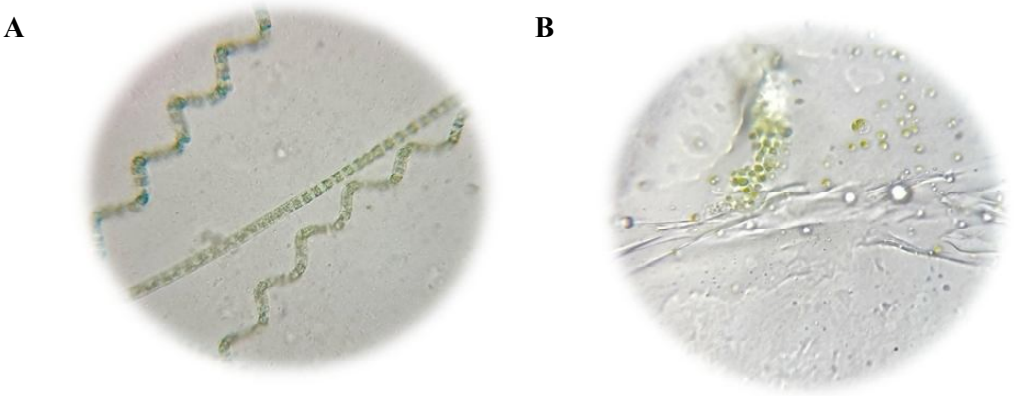


Figure 9. (A) Microscopic image of spirulina and ulothrix isolated from soil sample**. (B)** Microscopic imageof chlorella isolated from soil sample.

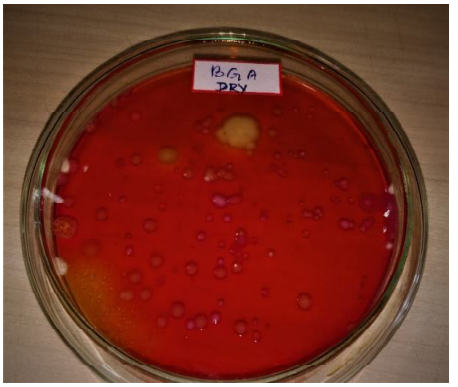
3.6. Isolation of Bacteria on Selective Medium

The results of the bacterial growth on the selected media, as shown in Table 4, revealed different patterns of microbial presence within the soil samples. Specifically, on the Blue Green Algae (BGA) medium, two types of bacteria were observed growing on the soil samples from the playground and the forest. This shows that these environments are home to diverse microbial communities with unique ecological niches and complex microbial interactions. Similarly, on the B.C.P – D.C.L.S. medium, three types of bacteria grew in the soil sample from the paddy field. This indicates rich microbial diversity in the paddy field soil, with distinct bacterial species thriving in this environment.

Table 5. Represent the bacteria isolated on selected media.

Soil sample	Selective media	Colony Colour and Morphology	Identifying Bacteria
Dry and Forest	BGA	Red to pink white, Circular and Lobate	*Salmonella sp.*
		Yellow or Yellowish green, Circular	*E. coli*
Paddy field	B.C.P – D.C.L.S.	Yellow, Circular	*E. coli*
		Blue, Lobate	*Salmonella sp. or Shigella sp.*
		White, Dot	*Proteus sp.*

A



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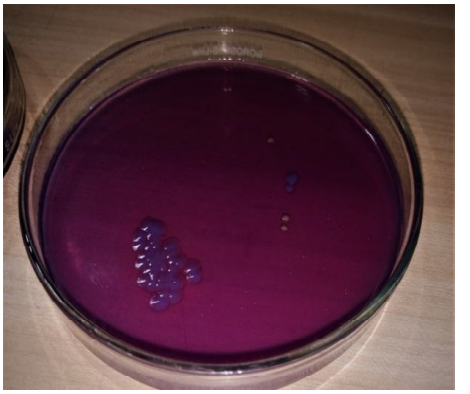

Figure 10. (A) Isolation of microorganism on selected media- BGA. **(B)** Isolation of microorganism on selected media- B.C.P-D.C.L.S.

3.7. Isolation of Phosphate Solubilizing Bacteria

The soil samples underwent a rigorous testing process for phosphate-solubilizing bacteria using Pikovskaya's agar medium. This meticulous approach led to the discovery of 13 colonies, among which four were selected for further study due to their extensive phosphate solubilization areas. These isolates demonstrated a high capacity for phosphate solubilization, as confirmed by the prominent halo zones in the agar medium. Table 6 provides a comprehensive detailed documentation of the identified phosphate-solubilizing bacteria. The successful isolation of these microbial isolates not only underscores the richness of our soil ecosystems but also instils confidence in their potential importance in soil fertility and nutrient cycling.

SI= (Colony measurement + Zone measurement) / Colony measurement

Table 6. Represent the colony and halo zone measurement of PSM

Sl. No	Isolates of PSM	Colony Measurement(cm)	Zone Measurement(cm)	Solubilization Index (SI)*
1	Colony 1	1.2	1.4	2.16
2	Colony 2	1.1	1.2	2.09
3	Colony 3	1.5	1.7	2.13
4	Colony 4	1.0	1.2	2.2

3.8. Determination of Bacteria-bacteria Antagonism of Different Soil Sample

Determining bacteria-bacteria antagonism across different soil samples unveiled intriguing insights into microbial interactions within varied ecological niches.

The study's outcomes revealed distinctive inhibition patterns among microorganisms from different soil samples. Notably, microorganisms from all soil samples were inhibited by those from the forest soil sample, indicating a potent inhibitory capacity associated with the forest microflora. Conversely, the microorganisms from the garden soil sample exhibited resistance against inhibition by microorganisms from any other soil sample, indicating a unique ecological niche with limited antagonistic interactions.

Furthermore, the results demonstrated that microorganisms from the potato and paddy fields collectively inhibited the garden soil microorganisms. Additionally, individual inhibitory effects were observed, with microorganisms from the dry soil sample specifically inhibiting those from the paddy field, while microorganisms from the potato field soil inhibited those from the dry soil sample. These findings underscore the complex and dynamic nature of microbial interactions within soil ecosystems.

Table 7. Antagonism activity of microorganisms isolated from soil sample

Microorganism from soil types	Antagonism	Activity against microorganism from soil types
Forest	✓	Paddy field, Garden, Potato field, Play ground
Paddy field	✓	Garden, Potato
Garden	×	-
Potato field	✓	Garden, Play ground
Dry	✓	Paddy field

Figure 11. Determination of bacteria-bacteria antagonism of different soil sample.

3.9. Isolation of Antibiotic Resistant Microorganism

In our study, we isolated various bacterial species from soil samples taken from the Midnapore College (Autonomous) campus and the surrounding area of Medinipur to determine their potential for antibiotic resistance. The results revealed interesting findings about the antibiotic resistance profiles of the isolated microorganisms, which are likely to capture the scientific community's interest.In particular, microorganisms from the paddy field, garden, and dry soil samples showed significant antibiotic resistance to penicillin. Conversely, microorganisms from two other soil samples showed no detectable antibiotic resistance to penicillin. Additionally, we observed a lack of antibiotic resistance to streptomycin across all soil samples tested.

These findings highlight bacterial populations' varying antibiotic resistance abilities in different soil environments. The distinct antibiotic resistance patterns exhibited by microorganisms from specific soil types underscore the diverse microbial interactions and adaptive strategies in these ecological niches. Moreover, the absence of antibiotic resistance to streptomycin suggests a nuanced response to different antibiotics within the microbial communities in the sampled soil environments.

Table 8. Represents the antibiotic resistant microorganism isolation from different soil sample.

Sl. No	Soil Samples	Isolated Microorganisms Sensitive to the Antibiotic	Isolated Microorganisms Resistant to the Antibiotic
1	Forest	Penicillin Streptomycin	_
2	Paddy field	Streptomycin	Penicillin
3	Garden	Streptomycin	Penicillin
4	Potato field	Penicillin Streptomycin	_
5	Dry	Streptomycin	Penicillin

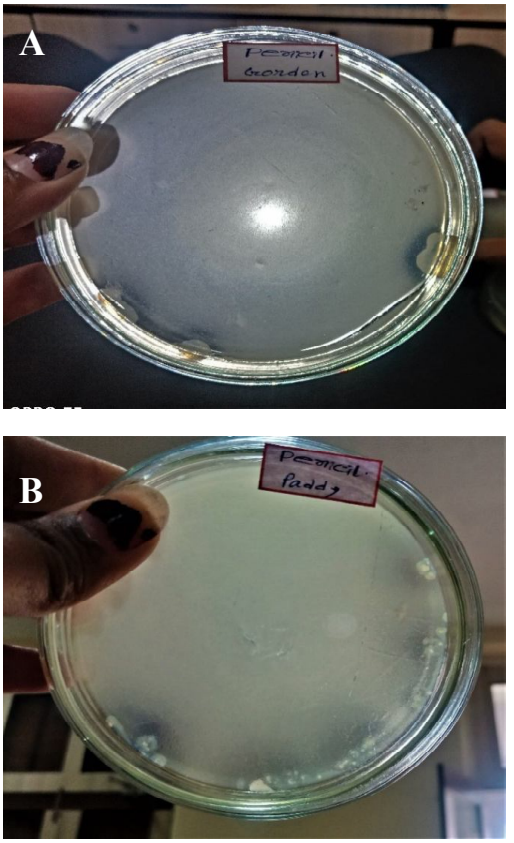

Figure 12. (A) Isolation of antibiotic resistant microorganism from garden soil sample. **(B)** Isolation of antibiotic resistant microorganism from paddy soil sample.

4. Conclusion

The results of this study, which unveil novel insights into the microbial ecology and potential biotechnological applications of microorganisms found in various soil samples, are a significant contribution to our understanding. The investigation revealed a significant microbial presence in different soil regions, with a range of microbial load from 1.84×10^9 to 2.88×10^9 CFU/ml of sample. The highest load was observed in the paddy field, followed by the forest, garden, potato field, and dry soil.

Identifying diverse microorganisms, including bacteria, fungi, and cyanobacteria, provides valuable insights into the microbial communities within these soil environments. The dominant presence of *Aspergillus* sp. among the identified species emphasizes the ecological significance of this microorganism in the sampled soils.

Additionally, the successful isolation and identification of cyanobacterial isolates, such as Chlorella, Spirulina, and Ulothrix, enriches our understanding of the cyanobacterial diversity in these environments, with potential implications for biotechnological and environmental applications.

The evaluation of soil samples for phosphate-solubilizing microbes demonstrated the presence of halo zones indicative of phosphate solubilization, highlighting the ecological role of these microbes in nutrient cycling and soil fertility.

The study's examination of bacteria-bacteria antagonism dynamics provided intriguing insights into the inhibitory relationships among microorganisms from different soil samples. The observed inhibition patterns underscore the complex interactions and competitive dynamics within

soil microbial communities, shedding light on the ecological significance of these antagonistic relationships.

Furthermore, the investigation into antibiotic resistance profiles of the isolated bacterial strains revealed significant antibiotic-resistant activity against penicillin in microorganisms from the paddy field, garden, and playground soil samples, indicating the potential presence of antibiotic-resistant bacterial strains within these environments.

In conclusion, this comprehensive study not only enhances our knowledge of microbial communities in soil environments but also underscores the potential biotechnological implications of their interactions and properties. The findings provide a valuable foundation for further research, with promising applications in agriculture, environmental remediation, and biotechnology, offering hope for future advancements in these fields.

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Reference

- Akpoveta, O. V., Osakwe, S. A., Okoh, B. E., & Otuya, B. O. Physicochemical characteristics and levels of some heavy metals in soils around metal scrap dumps in some parts of Delta State, Nigeria. *Journal of Applied Sciences and Environmental Management*, 2010; *14*(4). https://www.ajol.info/index.php/jasem/article/view/63258
- Alves-Barroco, C., Rivas-García, L., Fernandes, A. R., & Baptista, P. V. Tackling multidrug resistance in Streptococci–From novel biotherapeutic strategies to nanomedicines. *Frontiers in Microbiology*, 2020;*11*: 579916.
- Amit Sagervanshi, A. S., Priyanka Kumari, P. K., Anju Nagee, A. N., & Ashwani Kumar, A. K. *Isolation and characterization of phosphate solublizing bacteria from Anand agriculture soil.* 2012; https://www.cabidigitallibrary.org/doi/full/10.5555/20123395712
- Banks, M. K., & Bryers, J. D. Bacterial species dominance within a binary culture biofilm. *Applied and Environmental Microbiology*, 1991; *57*(7): 1974–1979. https://doi.org/10.1128/aem.
- Kavinilavu, N., & Pandeeswari, N. Studies on the Physico-Chemical properties of the soil samples collected from different locations of Dharmapuri District. *Journal of Pharmacognosy and Phytochemistry*, 2019; *8*(2S): 406–409.
- Khan, A. A., Jilani, G., Akhtar, M. S., Naqvi, S. M. S., & Rasheed, M. Phosphorus solubilizing bacteria: Occurrence, mechanisms and their role

in crop production. *J. Agric. Biol. Sci*, 2009; *1*(1): 48–58.

- Kulasooriya, S. Cyanobacteria: Pioneers of Planet Earth. *Ceylon Journal of Science (Biological Sciences)*, 2012; *40*(2): 71–88. https://doi.org/10.4038/cjsbs.v40i2.3925.
- Orgiazzi, A., Lumini, E., Nilsson, R. H., Girlanda, M., Vizzini, A., Bonfante, P., & Bianciotto, V. Unravelling soil fungal communities from different Mediterranean land-use backgrounds. *PloS One*, 2012; *7*(4): e34847.
- Pelczar, M. J., & Chan, E. C. S. (n.d.). *krieg NR. Microbiology: Concept and Application*. International edition McGraw-Hill, USA, *1993*.
- Prasanna, R., Jaiswal, P., Nayak, S., Sood, A., & Kaushik, B. D. Cyanobacterial diversity in the rhizosphere of rice and its ecological significance. *Indian Journal of Microbiology*, 2009;*49*(1): 89–97. https://doi.org/10.1007/s12088-009-0009-x.
- Prestinaci, F., Pezzotti, P., & Pantosti, A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health*, 2015;*109*(7): 309–318. https://doi.org/10.1179/2047773215Y.0000000030.
- Rasmussen, T. B., Skindersoe, M. E., Bjarnsholt, T., Phipps, R. K., Christensen, K. B., Jensen, P. O., Andersen, J. B., Koch, B., Larsen, T. O., Hentzer, M., Eberl, L., Hoiby, N., & Givskov, M. Identity and effects of quorum-sensing inhibitors produced by Penicillium species. *Microbiology*, 2005; *151*(5): 1325–1340. https://doi.org/10.1099/mic.0.27715-0
- Roger, P. A. *Biology and management of the floodwater ecosystem in rice fields*. Int. Rice Res. Inst, 1996.
- Stoodley, P., Sauer, K., Davies, D. G., & Costerton, J. W. Biofilms as Complex Differentiated Communities. *Annual Review of Microbiology*, 2002; *56*(1): 187–209. https://doi.org/10.1146/annurev.micro.56.012302.160705.
- Suhag, M. Potential of biofertilizers to replace chemical fertilizers. *Int. Adv. Res. J. Sci. Eng. Technol*, 2016;*3*(5): 163–167.
- Wall, D. H., & Virginia, R. A. Controls on soil biodiversity: Insights from extreme environments. *Applied Soil Ecology*, 1999; *13*(2): 137–150.
- Wehr, J. D., Sheath, R. G., & Kociolek, J. P. *Freshwater algae of North America: Ecology and classification*. Elsevier, 2015.