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ENUMERATION, ISOLATION AND CHARACTERIZATION OF BACTERIA OF GANGACHARA AND PALASHBARI SOIL SERIES OF BANGLADESH

Md. Jaber Chowdhury[1], Jannat Ara[2], Md. Farhad Hossain[3], Manna Salwa[4], Shammi Akhter Tina[5], Joyenta Das[6], Abdul Awal[7], Mahmudul Hasan Chowdhury[8]

[1,2,3,4,5,6,7,8]Soil Resource Development Institute, Ministry of Agriculture, Bangladesh

*Corresponding Author: lipuchowdhury@gmail.com

ABSTRACT

In any condition bacteria are available in our environment. We do bacterial classification by their isolation and characterization. Series wise bacterial characterization is veru rare in Bangladesh. Enumeration, isolation and characterization of bacteria from Gangachara and Palashbari soil series was our study. Colony characterization of bacteria was done in size, pigmentation, form, margin and elevation. It has been found that Palashbari soil was to have more bacterial population than that of Amnura soil. To tell bacterial diversity, Palashbari soil had ten whereas Gangachara soil had eight types bacterial colourful colonies. From simple and negative staining, shape and arrangement were determined. Gram- negative, spore forming and capsuled bacterial abundance in soils has been proved by this study. Presence of rod shaped bacteria (bacillus) compared to round shaped (coccus) in soils is obvious.

Keywords: Soil series, bacteria, enumeration, isolation, characterization.

1. INTRODUCTION

Single celled, prokaryotic, most successful, extreme living form of the ancient life of our world is bacteria. They have very simple morphologies. Their diameter is only 0.5 to 2.0 micrometers (mm) with shapes of spherical, bacillus and spiral mainly. They are found in both individual cells and aggregated together which are called colonies (Stevenson, 1986). Bacteria play a vital role in enhancing productivity in ecosystems, both on land and in water, by facilitating nutrient cycling and organic matter decomposition. Their activities support plant growth and maintain ecological balance (Brady and Weil, 2002). Bacteria are found everywhere, making their classification and identification challenging. However, classifying bacteria is essential for better understanding and recognizing their roles and characteristics in the environment (Joklik *et. al.*, 1992). Bacteria and fungi, found in soil, water, air, and even extreme environments, dominate well-aerated soils (Alexander, 1997). The first step in bacteriological studies is isolation, which involves obtaining and purifying cultures from a specific area (Ruangpan and Tendencia, 2004). Pure bacterial cultures are essential for studying morphology, physiology, biochemical traits, and susceptibility, requiring various media. Methods like solid media, streak plates, or pour plates are used to obtain these pure cultures (Salle, 1974). Soils in many regions of Bangladesh are contaminated with heavy metals, leading to the emergence of resistant bacteria capable of surviving in extreme conditions (Sanyal *et. al.*, 2016). Certain bacteria present in the soils of Bangladesh have been reported to play a beneficial role in the environmental mineral cycle, the process of soil nitrification (Islam *et. al.*, 2007). Their presence aids in remediating heavy metal contamination, eliminating pathogenic microbes, and stabilizing acidic soils (Nahar *et. al.*, 2020). The study focused on isolating soil bacteria, estimating colonies, and analyzing their morphological characteristics to assess microbial abundance (Sarkar *et. al.*, 2012).

2. LITERATURE REVIEW

The isolation and characterization of soil bacteria are fundamental processes in microbiology, contributing to our understanding of microbial diversity, ecological functions, and potential applications in agriculture, industry, and environmental management. Recent studies have employed various methodologies to isolate and identify bacteria from diverse soil environments, leading to the discovery of novel species and insights into microbial communities. A common approach for isolating soil bacteria involves serial dilution and plating on nutrient agar media. For instance, soil samples underwent serial dilution, and aliquots were spread onto nutrient agar plates. The plates were then incubated at 37°C for 24-48 hours to allow colony formation. Distinct colonies were subsequently selected for further analysis (Islam and Hossain, 2019).

In another study, researchers collected soil samples from various locations, including river sediments and rhizosphere soils. The samples were air-dried, subjected to serial dilution, and plated on nutrient agar supplemented with cycloheximide to inhibit fungal growth. Incubation was carried out at 37°C for 24 hours, facilitating the isolation of bacterial colonies. Following isolation, bacterial colonies are typically characterized using morphological, biochemical,

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and molecular techniques. Morphological examination includes assessing colony shape, color, and Gram staining properties. Biochemical tests, such as catalase and oxidase activities, starch hydrolysis, and citrate utilization, provide further insights into the metabolic capabilities of the isolates. For example, a study identified bacterial species including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas putida* through a combination of these methods. Molecular identification often involves the extraction of genomic DNA followed by Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene using universal primers. The amplified sequences are then compared against databases such as Gen Bank to determine phylogenetic relationships. This approach was utilized in the characterization of a *Bacillus* strain exhibiting antagonistic activity against plant and fish pathogens. Isolated soil bacteria have been found to possess various beneficial properties, including nitrogen fixation, phosphate solubilization, and production of phytohormones like auxins. A study comparing microbial communities in soils treated with different fertilizers revealed that organic fertilizer-treated soils harbored higher microbial counts and greater functional diversity compared to conventionally fertilized soils. Additionally, the isolation of urease-producing bacteria from soil samples has implications for understanding nitrogen cycling and potential applications in bioremediation. Characterization of these isolates through biochemical assays and molecular techniques provides insights into their roles in soil ecosystems (Kumar and Gera, 2011). In summary, the isolation and characterization of soil bacteria involve a combination of culturing techniques, morphological and biochemical assays, and molecular methods. These approaches have led to significant discoveries regarding microbial diversity and functionality in soil ecosystems, with practical applications in agriculture and environmental management.

3. METHODOLOGY

Sample collection

Fresh top soil samples (0-15 cm) were collected from the fields of Gangachara and Rangpur Sadar of Rangpur district (Table 1) and taken aseptically into laboratory using thermo flask and kept for further study.

Table 1. General description of two soil series.

Sl. no.	Series name	AEZ	Physuigraphic unit	Location	GPS reading
1	Gangachara	3	Tista Floodplain	Gangachara, Rangpur	N-25^{0}50′051″ E-89^{0}14′292″
2	Palashbari	3	Tista Floodplain	Rangpur Sadar, Rangpur	N-25^{0}41′759″ E-89^{0}16′070″

Isolation of Bacteria

The isolation of bacteria was performed by referring to various standard methods (Prescott and Harley, 2002). The sample was prepared by mixing soil and physiological water (dw+0.9% NaCl). Serial dilution of sample was prepared and was streaked in different labeled petriplates by spread plate technique. The plates were incubated at3 7°C for 24-48 hrs. After incubation the obtained culture was picked selectively for purification of culture by streak plate technique. The process was performed in triplicates. The plate was incubated at 37°C for 24-48 hrs.

Viable count for enumeration

Viable Count of bacteria was calculated by colony count method. The plates with 25 to 250 colonies were selected for counting and calculated by following equation:

Total bacteria per gram soil = (no of colonies ×dilution factor) / (volume of sample (ml)

Characterization

The bacterial colony characteristics and morphological from both location characteristics were determined by evaluating the well-isolated colonies of nutrient agar plates. The size, pigmentation, form, margin and elevation were observed as described by Dubey and Maheshwari (1998).

Staining characteristics

The shape and arrangement of bacteria were determined by simple and negative staining, gram stain, capsule stain, spore stain and acid fast stain (Shaha, 2003)

Simple staining

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The crystal violate was poured on smear left for 40 to 60 seconds. The smear then washed with normal water to remove excess stain. After washing the slide was dried and was examined under oil immersion.

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Negative staining

On a clean dry glass slide, a drop of nigrosin dye was placed at one end. A loop ful of bacterial inoculums was placed and mixed with the drop of nigrosin. The mixture was spread with the edge of a second slide held at a 300 angle. It was placed in front of bacterial suspension to prepare a thin smear. The smear was air dried and the slide was examined under oil immersion.

Gram stain

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The crystal violate was poured on smear left for 1 minutes. The smear then washed with normal water to remove excess stain. Gram's iodine was added to the smear slide and kept for 1 minute and washed again. Ethyl alcohol (95%) was added to the smear in drop wise manner till crystal violate failed to wash. Again the smear was washed with tap water. The smear was counterstained with safranin for about 45 seconds and washed again. The slide was air dried, and was examined under oil immersion.

Capsule stain

The bacterial smear was prepared on the glass slide and air dried. The crystal violate was poured on smear and kept for 5 to 7 minutes. The smear was washed with 20% copper sulfate solution. The smear slide was air dried and the slide was examined under oil immersion.

Spore stain

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The malachite green was poured on the smear and placed on a warm hot plate for 2 to 3 minutes. The slide was removed from hot plate, cooled and washed with tap water. The safranin was poured on the smear and left for 30 seconds followed by washing with water. The smear slide was air dried and the slide was examined under oil immersion.

Acid fast stain

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The carbol fuchsin was poured on the smear and placed on a warm hot plate, allowing the preparation for 5minutes. The slide was removed from hot plate,cooled and washed with tap water. Acid alcohol was added on the slide in drop wise manner till carbol fuchsin removes and followed by washing with water. The smear was counterstained with methylene blue for 2 minutes followed by washing. The smear slide was air dried and the slide was examined under oil immersion.

Data Analysis

The observation for all the tests were made and recorded for further work.

4. RESULTS AND DISCUSSION

Enumeration

There was enumeration of bacterial population was from both Amnura and Baliadangi soil series of Bangladesh in this study. The successful isolation aw well as purification and characterization were determined. These results find variables in bacterial colonies in collected soil samples. The result was to revel that rod shaped bacteria was majorly found in both the soil with the colony count of 7.9× 〖10〗 ^7CFU/g and 8.4× 〖10〗 ^7 CFU/g soil in Gangachara soil and Palashbari soil respectively.

Isolation and Characterization

Eight distinct types of colorful bacterial colonies were from Gangachara soil and ten distinct types of colorful bacterial colonies from Palashbari soil. The bacterial colonies from both were moderate, small, large and pinpoint in size; irregular, circular and rhizoid in form; serrate, entire, undulate and lobate in margin; and flat, raised and umbonate in elevation (Table 2, Table 4). Bacteria from both the soils were variated in colour from white, pink and yellow. But most of the colonies are yellow in color. To describe morphological characteristics of isolated bacteria from Amnura soil were observed as mixed morphological type of colonies but were majorly dominated by rod shaped, spore forming, gram-nagative and non-acid fast bacteria (Table 3, Table-5). So, rod shaped, spore forming, non-acid fast and capsulated bacteria are in major positions in both soils. The result was compared with the previous study conducted by Chowdhury et al., (2013) from Bangladesh soil. Our reports shoiwed similarity to them. Bangladesh soils showed report of abundant presence of different Bacillus Sp. that are spore forming in majority (Fakruddin *et al.,* 2012).

Table 2. Colony characteristics of isolated bacteria of Gangachara soil.

Colony no.	Size	Pigmentation	Form	Margin	Elevation
1	Small	Pink	Circular	Entire	Raised
2	Moderate	White	Rhizoid	Undulate	Umbonate

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3	Pinpoint	Yellow	Circular	Entire	Flat
4	Moderate	Yellow	Circular	Serrate	Raised
5	Moderate	Yellow	Irregular	Entire	Flat
6	Pinpoint	Pink	Circular	Entire	Flat
7	Large	Yellow	Circular	Lobate	Raised
8	Moderate	Red	Irregular	Entire	Flat

Table 3. Morphological characteristics of isolated bacteria of Gangachara soil.

Colony no.	Shape	Arrangement	Gram stain	Capsule stain	Spore stain	Acid-fast
1	Round	Chain	Gram positive	Capsulated	Spore forming	Non acid fast
2	Rod	Chain	Gram negative	Capsulated	Spore forming	Acid fast
3	Rod	Single	Gram negative	Non Capsulated	Spore forming	Non acid fast
4	Round	Chain	Gram negative	Capsulated	Non Spore forming	Acid fast
5	Rod	Chain	Gram positive	Non Capsulated	Spore forming	Acid fast
6	Round	Chain	Gram negative	Capsulated	Spore forming	Non acid fast
7	Rod	Chain	Gram negative	Capsulated	Non Spore forming	Acid fast
8	Rod	Chain	Gram negative	Non Capsulated	Spore forming	Acid fast

Table 4. Colony characteristics of isolated bacteria of Palashbari soil.

Colony no.	Size	Pigmentation	Form	Margin	Elevation
1	Large	Yellow	Irregular	Entire	Flat
2	Moderate	Pink	Circular	Lobate	Raised
3	Pinpoint	Yellow	Circular	Entire	Flat
4	Small	Yellow	Irregular	Lobate	Raised
5	Moderate	Pink	Irregular	Entire	Flat
6	Small	Yellow	Circular	Entire	Flat
7	Large	Pink	Circular	Undulate	Raised
8	Small	Yellow	Irregular	Entire	Raised
9	Moderate	Yellow	Circular	Entire	Flat
10	Moderate	Yellow	Circular	Lobate	Flat

Table 5. Morphological characteristics of isolated bacteria of Palashbari soil.

Colony no.	Shape	Arrangement	Gram stain	Capsule stain	Spore stain	Acid-fast
1	Rod	Chain	Gram positive	Capsulated	Spore forming	Non acid fast
2	Rod	Single	Gram negative	Non Capsulated	Non Spore forming	Acid fast
3	Rod	Chain	Gram negative	Capsulated	Spore forming	Acid fast
4	Round	Chain	Gram positive	Non Capsulated	Spore forming	Non acid fast
5	Rod	Chain	Gram negative	Capsulated	Non Spore forming	Non acid fast
6	Rod	Chain	Gram negative	Capsulated	Spore forming	Non acid fast

7	Rod	Single	Gram positive	Capsulated	Spore forming	Acid fast
8	Round	Chain	Gram negative	Non Capsulated	Spore forming	Non acid fast
9	Rod	Chain	Gram negative	Capsulated	Non Spore forming	Acid fast
10	Rod	Single	Gram negative	Capsulated	Spore forming	Non acid fast

5. CONCLUSION AND RECOMMENDATIONS

There has been very limited research on the isolation and identification of soil microbes from Bangladesh's soil. It is crucial to accurately account for the various forms of microbes present in different soils. This understanding helps in assessing soil health and microbial diversity. Bangladesh's soils vary in types and serve different purposes, with agriculture being the primary use. These diverse soils play a significant role in the country's agricultural activities. The bacteria involved are essential for the soil's proper functioning, and exploring them is crucial for better understanding its dynamics. With a view to getting bacterial identification and classification biochemical test is mandatory here.

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