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Assessment of Soil Bacterial Population and Characterization of Akdala and Nachol Series of Bangladesh

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Abstract: Bacteria are ancient, single-celled organisms that exist everywhere and can thrive under diverse environmental conditions. Isolating and identifying bacteria from various sources is essential for their classification and understanding their potential to cause disease. In soil ecosystems, bacteria and fungi are the dominant microorganisms, playing key roles in driving biological and chemical transformations. This study focused on isolating and characterizing bacterial species from the Akdala and Nachol soil series in Bangladesh. Distinct bacterial colonies were assessed based on characteristics such as size, color, shape, edge, and elevation. It was noted that the Akdala soil contained a higher bacterial count than the Nachol soil. Using simple and negative staining techniques, the shape and arrangement of the bacteria were analyzed. Both soil types had a higher proportion of Gram-negative bacteria compared to Gram-positive ones. Most of the isolates were capable of forming spores and capsules. The majority were non-acid-fast and typically appeared in chain-like arrangements. Rod-shaped (bacillus) bacteria were more prevalent than spherical (coccus) types in both soil samples.

Keywords: *Soil series, bacteria, colony characteristics, isolation.*

Introduction

Bacteria are single-celled, prokaryotic microorganisms that represent some of the earliest and most enduring life forms, capable of thriving in extreme environmental conditions. They are structurally simple, usually measuring between 0.5 and 2.0 micrometers in diameter, and are typically found in three primary shapes: spherical, rod-like, and spiral. These organisms may occur as isolated cells or in groups forming colonies [1–2]. Their presence in both terrestrial and aquatic ecosystems is crucial, as they function as decomposers and significantly contribute to overall biological productivity. Despite their simplistic morphology and widespread distribution, the classification and identification of bacteria remain challenging tasks. Nevertheless, accurate identification—particularly through microbial culturing—is essential for understanding their ecological roles and behavior across different habitats [3]. Bacteria inhabit various environments, including soil, water, air, and even the most extreme locations on Earth, actively supporting ecosystem functionality. In well-aerated soils, both bacteria and fungi are predominant, whereas in oxygen-deficient soils, bacteria primarily drive biological and chemical transformations [4]. The initial step in bacteriological investigations involves isolating, purifying, and identifying bacteria. Cultures are first isolated from specific sources, then purified using appropriate media to obtain pure cultures necessary for analyzing morphological, physiological, biochemical, and antimicrobial susceptibility traits [5]. Techniques such as streak plating, pour plating, and the use of solid media are commonly employed to achieve pure bacterial cultures [6–7]. In several regions of Bangladesh, soil is contaminated with heavy metals, promoting the development of metal-resistant bacterial strains capable of surviving under extreme conditions [8–11]. Certain bacterial species found in these soils have shown potential benefits such as phosphate solubilization, detoxification, enhancement of nutrient cycling [12–14], and nitrification processes [15–17]. Additionally, these microbes aid in remediating heavy metal pollution, suppressing harmful pathogens, and stabilizing acidic soils [13, 18]. Research on soil microbiology is vital for expanding scientific understanding and addressing soil-related challenges. Identifying bacteria based on specific soil series can greatly enhance knowledge of microbial diversity and environmental dynamics. This study was undertaken to isolate and enumerate soil bacteria, and to characterize their colony and morphological features—including shape, cellular arrangement, and staining properties—to assess microbial abundance.

Materials and Methods

Sample collection

Fresh top soil samples (0-15 cm) were collected from the fields (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	Akdala	25	Barind Tract	Shibganj, Bogura	N-24^{0}58′656″ E-89^{0}20′191″
2	Nachol	26	Barind Tract	Chapainawab ganj Sadar, Chapainawab ganj	N-24^{0}35′555″ E-88^{0}16′759″

Isolation of Bacteria

Bacterial isolation was carried out following established standard procedures [19–20]. To prepare the sample, soil was mixed with physiological saline solution (distilled water containing 0.9% NaCl). A serial dilution of this mixture was then prepared, and aliquots were inoculated onto labeled petri dishes using the spread plate method. The inoculated plates were incubated at 37°C for 24 to 48 hours. After the incubation period, selected colonies were carefully picked for further purification using the streak plate technique. This entire process was performed in triplicate. The purified plates were again incubated at 37°C for 24 to 48 hours.

Viable count

The viable bacterial population was determined using the colony count method. Only petri plates containing between 25 and 250 colonies were considered appropriate for enumeration. The colony-forming units (CFU) were calculated using the following formula:

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

Characterization

The colony and morphological characteristics of bacteria from both locations were assessed by examining well-isolated colonies grown on nutrient agar plates. Observations included colony size, pigmentation, shape (form), edge (margin), and elevation, following the guidelines provided by Dubey, R.C. and Maheshwari, D.K. (1998) [19].

Staining characteristics

Bacterial shape and cellular arrangement were identified using various staining techniques, including simple staining, negative staining, Gram staining, capsule staining, spore staining, and acid-fast staining [21–22].

Simple staining

A bacterial smear was prepared on a clean glass slide and subsequently heat-fixed. Crystal violet stain was applied to the smear and allowed to sit for 40 to 60 seconds. The slide was then gently rinsed with tap water to remove excess stain. After drying, the slide was examined under a microscope using oil immersion.

Negative staining

A drop of nigrosin dye was placed at one end of a clean, dry glass slide. A loopful of bacterial culture was then added to the dye and mixed thoroughly. Using the edge of a second slide held at an approximate 30° angle, the mixture was spread across the surface to form a thin, even smear. The smear was allowed to air dry, and the slide was subsequently examined under oil immersion.

Gram stain

A bacterial smear was prepared on a glass slide and heat-fixed. Crystal violet stain was applied to the smear and left for 1 minute. The slide was then rinsed gently with water to remove excess stain. Gram's iodine was added and allowed to sit for 1 minute before washing the slide again. Next, 95% ethyl alcohol was added dropwise until the crystal violet no longer washed off. The smear was rinsed once more with tap water, then counterstained with safranin for approximately 45 seconds. After a final wash, the slide was air-dried and examined under oil immersion.

Capsule stain

A bacterial smear was prepared on a glass slide and allowed to air dry. Crystal violet stain was applied to the smear and left for 5 to 7 minutes. The slide was then rinsed with 20% copper sulfate solution. After air drying, the smear was examined under oil immersion.

Spore stain

A bacterial smear was prepared on a glass slide and heat-fixed. Malachite green stain was applied to the smear, which was then placed on a warm hot plate for 2 to 3 minutes. After heating, the slide was removed, allowed to cool, and rinsed with tap water. Safranin was then applied to the smear and left for 30 seconds before being washed off with water. The slide was air-dried and examined under oil immersion.

Acid fast stain

A bacterial smear was prepared on a glass slide and heat-fixed. Carbol fuchsin stain was applied to the smear, and the slide was placed on a warm hot plate for 5 minutes. After heating, the slide was removed, cooled, and rinsed with tap water. Acid alcohol was then added dropwise until the carbol fuchsin was washed off, followed by another rinse with water. The smear was counterstained with methylene blue for 2 minutes and washed again. Finally, the slide was air-dried and examined under oil immersion.

Results and Discussion

The total bacterial count was determined for both Akdala and Nachol soil samples, with successful isolation, purification, and characterization carried out. The results revealed variation in bacterial colony types between the two soils. From Nachol soil, approximately five distinct colorful bacterial colony types were identified, whereas six distinct colorful colony types were found in Akdala soil. The bacterial colonies in Akdala soil were generally moderate to small, including pinpoint sizes, while those from Ishurdi soil varied from small to medium and large. Colonies from Akdala soil exhibited diverse forms such as irregular, circular, and rhizoid, with margins described as serrate, undulate, entire, and lobate, and elevations ranging from flat to raised and umbonate (Table 2). In contrast, Nachol soil colonies were irregular, rhizoid, and circular in form, with margins that were undulate, serrate, entire, or lobate, and elevations between flat and raised (Table 3). The bacterial colonies from both soils displayed colors including white, pink, red, and yellow. Morphological analysis of bacteria

isolated from Akdala soil revealed mixed colony types, predominantly consisting of rod-shaped, spore-forming, Gram-negative, and non-acid-fast bacteria (Table 4). The isolates from were encapsulated (Table 5). The study found Bacillus species to be the dominant bacteria in both soils, with colony counts of 8.5 × 10^7 CFU/g in Akdala soil and 8.1 × 10^7 CFU/g in Nachol soil. These findings align closely with previous research by Chowdhury et al. (2013) [23], which also reported Bacillus as a dominant genus in Bangladesh soils. Other identified Gram-negative, spore-forming bacteria included Enterobacter spp., Klebsiella spp., Bacillus spp., and Azospirillum spp. [17]. Numerous reports confirm the abundant presence of Bacillus species in Bangladeshi Nachol soil showed mixed colonies composed of both rod- and round-shaped Gram-positive and Gram-negative bacteria. All isolates were spore formers, most were non-acid-fast, and many soils [24–27], most of which are spore-formers. The spores of Bacillus can better survive in alkaline soils and complete their life cycles. Due to limited opportunities for sporulating bacteria to finish their reproductive cycle in soil, their high pathogenicity is considered a strategy to enhance survival chances [28]. Many new generations of sporulating bacteria are neutralized by feeding on dead and decaying matter, which aids their survival and dispersal in the soil. This cycle ensures the continuation of the species within the environmental population [29–30].

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

Colony no.	Size	Pigmentation	Form	Margin	Elevation
1	Pinpoint	White	Rhizoid	Undulate	Flat
2	Moderate	Red	Circular	Entire	Raised
3	Small	Red	Circular	Serrate	Raised
4	Small	Pink	Irregular	Lobate	Umbonate
5	Small	White	Rhizoid	Entire	Flat
6	Moderate	Yellow	Irregular	Lobate	Flat

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

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

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

Colony no.	Size	Pigmentation	Form	Margin	Elevation
1	Small	Yellow	Circular	Entire	Raised
2	Pinpoint	Yellow	Rhizoid	Undulate	Flat
3	Moderate	White	Rhizoid	Entire	Flat
4	Small	Red	Circular	Serrate	Raised
5	Pinpoint	Pink	Irregular	Lobate	Raised

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

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

3	Rod	Chain	Gram positive	Capsulated	Spore forming	Non acid fast
4	Rod	Chain	Gram negative	Capsulated	Spore forming	Acid fast
5	Rod	Chain	Gram negative	Capsulated	Spore forming	Non acid fast

Conclusion

Research on isolating and identifying soil microbes from Bangladesh soils is very limited. There is a significant gap in knowledge regarding the microbial diversity in these soils. Accurate identification and quantification of various microbial forms present in different soil types is essential. Understanding the microbial composition is crucial for soil health and management. Bangladesh soils vary greatly in type and serve multiple purposes. They are predominantly used for agriculture and include many marshy areas. The bacteria associated with these soils have vital roles in maintaining soil functions. Therefore, exploring these microbial communities is highly important and necessary.

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Conflict Of Interest

The authors have declared that there is no conflict of interest.

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