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Assessment of Bacterial Isolation and Characterization of Chhiata and Silmondi Soil Series of Madhupur Tract

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ABSTRACT: This study investigated the bacterial diversity in two agricultural soils of Bangladesh, Chhiata and Silmondi. Soil samples were collected aseptically from the top 0–15 cm layer and subjected to isolation, purification, and enumeration of bacterial populations. Colony morphology, cellular characteristics, and staining properties were systematically examined to assess microbial diversity. The bacterial counts were 7.6×10^6 CFU/g soil for Chhiata and 6.5×10^6 CFU/g soil for Silmondi, reflecting active microbial communities. The isolates displayed considerable phenotypic heterogeneity, with variations in colony size, pigmentation, form, margin, and elevation. Morphological analysis revealed both Gram-positive and Gram-negative bacteria, including rod-shaped and cocci forms with single and chain arrangements. Several isolates exhibited spore- and capsule-forming abilities, indicative of adaptive survival strategies under environmental stress. The findings corroborate previous studies from Bangladesh,

highlighting the dominance of Bacillus spp., along with other Gram-negative, spore-forming bacteria such as Enterobacter spp., Klebsiella spp., and Azospirillum spp. This study underscores the rich microbial diversity of Bangladeshi soils and emphasizes the ecological importance of soil bacteria in nutrient cycling, soil fertility, and sustainable agriculture.

Keywords: *Bacillus spp., Gram-positive, Gram-negative, Colony, Morphology, Spore, Capsule*

INTRODUCTION

Bacteria are unicellular, prokaryotic microorganisms that are recognized as some of the most ancient and evolutionarily enduring life forms on Earth. Owing to their extensive evolutionary history, they have acquired extraordinary adaptive capabilities, enabling them to inhabit and persist in a wide variety of environmental conditions, ranging from moderate to extreme. In terms of structure, bacterial cells are relatively simple and typically range in size from approximately 0.5 to 2.0 μm in diameter. Despite this apparent simplicity, bacteria display considerable morphological diversity, which is generally classified into three principal forms: coccoid (spherical), bacillary (rod-shaped), and spiral configurations. Depending on environmental conditions and the availability of nutrients, bacterial cells may exist independently as single units or aggregate to form colonies and complex groupings [1]. Bacteria play indispensable roles in maintaining ecological balance in both terrestrial and aquatic ecosystems. They are key drivers of ecosystem productivity, primarily through their involvement in the decomposition of organic matter and the recycling of essential nutrients such as carbon, nitrogen, and phosphorus. These processes are fundamental to sustaining life and ensuring the continuity of biogeochemical cycles [2]. Among all groups of microorganisms, bacteria are the most widely distributed and exhibit remarkable ecological versatility. However, their relatively limited morphological differentiation makes accurate identification and taxonomic classification challenging. Consequently, reliance solely on morphological traits is often inadequate. To overcome these limitations, systematic classification approaches that integrate culture-based techniques along with advanced analytical methods are essential for a more comprehensive understanding of bacterial

diversity. Such frameworks provide valuable insights into the ecological roles, physiological characteristics, and environmental interactions of diverse bacterial taxa [3]. Bacteria are ubiquitous in nature, occurring in nearly all conceivable habitats, including soil, water, air, and even extreme environments such as highly saline regions, acidic conditions, and areas with extreme temperatures. Through their diverse metabolic activities, bacteria contribute significantly to environmental stability and the regulation of global biogeochemical cycles. In well-aerated soils, bacterial populations interact synergistically with fungi to facilitate microbial processes and nutrient cycling. Conversely, in oxygen-deficient or anaerobic soil environments, bacteria tend to dominate the microbial community and are primarily responsible for most biochemical transformations and biological activities within the soil ecosystem [4]. The foundation of bacteriological research lies in the systematic processes of isolation, purification, and identification of bacterial strains. Isolation techniques are employed to recover bacterial populations from specific sources, followed by purification procedures to eliminate contaminants and obtain homogeneous cultures [5]. Establishing a pure culture is critically important, as it ensures reliability in subsequent analyses of bacterial morphology, physiological behavior, biochemical properties, and responses to antimicrobial agents. To achieve pure cultures from mixed microbial populations, both selective and non-selective media are commonly utilized. Standard laboratory methods, including growth on solid agar media, streak plate techniques, and pour plate methods, are widely used for the effective isolation of distinct bacterial colonies [6–7]. Numerous studies have indicated that soils in different regions of Bangladesh are significantly impacted by heavy metal contamination, which promotes the selection and predominance of metal-tolerant and metal-resistant bacterial communities. These bacteria exhibit strong resilience and adaptability under stressful environmental conditions, thereby playing crucial ecological roles in contaminated ecosystems [8–11]. Indigenous soil bacteria in Bangladesh have been reported to perform several beneficial functions, including phosphate solubilization, participation in environmental detoxification, and regulation of mineral cycling processes within soil systems [12–14]. Furthermore, these microbial communities are actively involved in essential biochemical processes such as nitrification, which is vital for maintaining soil fertility and ensuring nutrient

availability for plant growth [15–17]. In addition, they contribute to the remediation of metal-contaminated soils, suppression of pathogenic microorganisms, and stabilization of acidic soil conditions.

Microbiological investigations of soil ecosystems are essential for advancing scientific knowledge and addressing environmental and agricultural challenges. The systematic and stepwise identification of soil bacterial populations is particularly important for understanding the complexity, diversity, and dynamic nature of soil microbial communities [13, 18]. In this context, the present study focuses on the isolation of soil bacteria, enumeration of colony-forming units (CFUs), and assessment of colony morphology. Additionally, important morphological characteristics, including cell shape, arrangement patterns, and staining properties, were examined to evaluate microbial diversity and abundance in soil samples.

MATERIALS AND METHODS

Sample collection

Two freshly collected topsoil samples (Table 1) were aseptically obtained from selected agricultural fields in Bangladesh. The sampling was deliberately carried out from the surface soil layer at a depth of 0–15 cm, as this region is widely recognized for possessing the highest levels of microbial activity, organic matter content, and nutrient availability. To preserve the native characteristics of the soil microbiota, stringent aseptic techniques were followed throughout the entire sampling procedure, thereby minimizing the risk of external contamination and ensuring that the indigenous microbial populations remained undisturbed. Immediately after collection, the soil samples were carefully transferred into sterile thermos flasks, which helped maintain a relatively stable temperature and prevented significant physicochemical or biological changes during transportation. This step was crucial to limit microbial fluctuations and preserve the original state of the samples. Upon arrival at the laboratory, the samples were handled with great care and stored under controlled environmental conditions until further analyses were performed. Subsequent microbiological and physicochemical examinations were conducted using these well-preserved samples. The meticulous procedures followed during

sampling, transportation, and storage played a vital role in maintaining sample integrity, thereby ensuring the accuracy, consistency, and reproducibility of the experimental results obtained from the study.

Table 1. Related information of collected soil samples

Sample no.	**Series**	**Location**	**GPS reading**	AEZ	**Physiographic unit**	**Cropping pattern**
1	Chhiata	Yugitola Gazipur	N-23°58′911″ E-90°24′388″	28	Madhupur Tract	Aus-T. Amam
2	Silmondi	Madhupur Tangail	N-24°37′060″ E-90°05′385″	28	Madhupur Tract	Pineapple-Papaya-Turmeric

Isolation of Bacteria

Bacterial isolation was performed following established and widely accepted standard microbiological protocols as described in previous studies. Initially, the collected soil samples were thoroughly homogenized by suspending a measured amount of soil in a physiological saline solution (distilled water containing 0.9% NaCl). This step ensured the even dispersion of microbial cells and facilitated the preparation of a uniform microbial suspension, which served as the primary source for subsequent analyses. The homogenized suspension provided a representative distribution of the soil microbiota for accurate isolation procedures. Following homogenization, the soil suspension was subjected to a systematic serial dilution process. This stepwise dilution was essential to progressively reduce the microbial load, thereby increasing the likelihood of obtaining discrete and well-isolated bacterial colonies. From appropriate dilution levels, aliquots were aseptically transferred onto pre-labeled sterile Petri dishes and evenly spread across the surface of solidified agar media using the spread plate technique. This method allowed for uniform distribution of microbial cells and supported the development of distinct colonies. The inoculated plates were then incubated under controlled conditions at

37°C for a period of 24–48 hours, which is considered optimal for the growth of a wide range of mesophilic bacteria. Following incubation, clearly distinguishable and well-separated colonies were carefully observed and selected based on their morphological characteristics. These selected colonies were subsequently subjected to repeated streaking on fresh agar plates in order to obtain pure (axenic) cultures. This purification process was carried out meticulously to eliminate any mixed microbial populations and to ensure the consistency and uniformity of the isolates. To enhance the reliability of the experimental outcomes, all isolation and purification procedures were conducted in triplicate. The purified bacterial isolates were then re-incubated at 37°C for an additional 24–48 hours to confirm their purity, stability, and consistent growth patterns. This rigorous and systematic approach ensured the accuracy, reproducibility, and validity of the bacterial isolation process and the resulting cultures [19–20].

Viable count

The viable bacterial population was quantified using the standard colony count (plate count) method, which is widely regarded as a reliable approach for estimating culturable microorganisms. For precise and statistically acceptable enumeration, only those agar plates containing between 25 and 250 well-isolated and clearly distinguishable colonies were selected, as this range minimizes counting errors and ensures accuracy. Plates with colony numbers below or above this threshold were excluded to avoid underestimation or overcrowding effects. Each visible colony was considered to have originated from a single viable bacterial cell or a cluster of cells, commonly referred to as a colony-forming unit (CFU). Based on this assumption, the total viable bacterial population present in the soil samples was calculated and expressed as CFU per gram of soil. The estimation was performed using the following standard formula:

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

Characterization

The morphological characteristics of bacterial colonies obtained from both sampling sites were systematically examined using well-isolated colonies grown on nutrient

agar plates. A detailed and comprehensive evaluation was carried out by considering several important parameters, including colony size, pigmentation, overall shape, margin characteristics, and elevation patterns (Figure 1). Each of these features was carefully observed, as they provide essential preliminary information about the identity and diversity of the bacterial isolates. The observations were conducted in a consistent and organized manner to ensure accuracy and reproducibility across all samples. Standardized descriptive criteria, as outlined by Dubey and Maheshwari (1998) [19], were strictly followed during the assessment to maintain uniformity in morphological characterization. Special attention was given to distinguishing subtle variations in colony appearance, which can be indicative of different bacterial taxa. These detailed morphological analyses offer valuable insights into the phenotypic diversity of bacterial communities present in the soil samples. Furthermore, such characterization serves as a fundamental step for subsequent identification, differentiation, and taxonomic classification of bacterial isolates, thereby contributing to a better understanding of soil microbial diversity and structure.

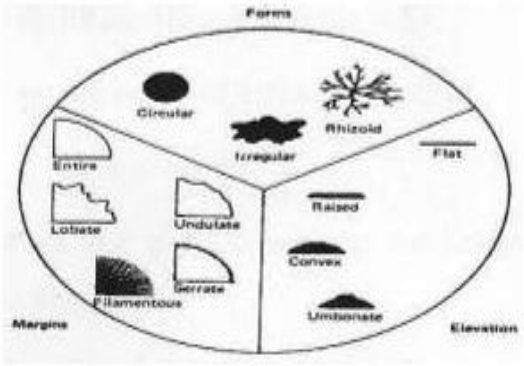

Figure 1. Colony characteristics of bacteria [21].

Staining characteristics

The morphology and spatial arrangement of the bacterial isolates were systematically examined using a series of well-established staining techniques, including simple staining, negative staining, Gram staining, capsule staining, spore staining, and acid-fast staining. Each of these methods provided distinct yet complementary information, allowing for a thorough and multidimensional characterization of the bacterial cells. Simple staining facilitated the observation of basic cell shape and arrangement, while negative staining enhanced the visualization of cell outlines and

size by providing a contrasting background. Gram staining played a crucial role in differentiating bacteria based on cell wall composition, categorizing them into Gram-positive and Gram-negative groups. Capsule staining enabled the detection of extracellular protective layers, whereas spore staining helped identify endospore-forming bacteria. Additionally, acid-fast staining was employed to detect bacteria with waxy cell wall components, particularly those resistant to conventional staining techniques. Together, these staining approaches offered a comprehensive understanding of bacterial morphology, including cell shape, structural features, and spatial arrangement patterns. Such detailed observations are essential for accurate phenotypic differentiation and reliable taxonomic classification. Moreover, they contribute significantly to a deeper understanding of the structural diversity and complexity of soil bacterial communities, thereby supporting further microbiological and ecological investigations [21–22].

Simple staining

For the detailed examination of bacterial morphology, smears were carefully and aseptically prepared on clean, sterile glass slides. These smears were then subjected to heat fixation, a crucial step that serves to immobilize the bacterial cells, enhance their adherence to the slide surface, and preserve their structural integrity during subsequent staining procedures. Proper heat fixation also helps prevent distortion or loss of cellular components during washing steps. Following fixation, crystal violet stain was applied to the smear and allowed to remain in contact with the bacterial cells for approximately 40–60 seconds, ensuring sufficient penetration and uniform staining. After staining, the slides were gently rinsed with tap water to remove excess dye, thereby improving clarity and contrast. The slides were then allowed to air-dry under clean conditions to avoid contamination or disruption of the stained smear. The prepared and stained smears were subsequently examined under a high-power microscope using oil immersion, which provides enhanced resolution and magnification for detailed visualization. This allowed for precise observation of bacterial cell shape, size, arrangement patterns, and other structural characteristics. Such a method is fundamental for accurate phenotypic characterization and serves as an important preliminary step in the identification and differentiation of bacterial isolates [21–22].

Negative staining

Negative staining was carried out using a clean and dry glass slide, where a small drop of nigrosin dye was initially placed near one end of the slide. A loopful of bacterial culture was then aseptically transferred and gently mixed with the dye to form a uniform suspension. Using the edge of a second sterile slide held at an angle of approximately 30°, the mixture was carefully spread across the surface to produce a thin and even smear. Unlike other staining methods, the prepared smear was not subjected to heat fixation; instead, it was allowed to air dry naturally. This step is particularly important, as it prevents cell shrinkage and distortion, thereby preserving the natural morphology of the bacterial cells. Once dried, the slide was examined under a microscope using oil immersion to achieve high-resolution visualization. This technique allowed for clear observation of bacterial cell morphology, including size, shape, and spatial arrangement, against a darkly stained background. Since the cells themselves remain unstained while the background is colored, negative staining enhances contrast without altering cellular structure. As a result, it provides more accurate and reliable information for phenotypic characterization, especially in studies where maintaining the true size and configuration of bacterial cells is essential [21–22].

Gram stain

For Gram staining, bacterial smears were carefully and aseptically prepared on clean, sterile glass slides and subsequently subjected to heat fixation. This step ensured proper adhesion of the bacterial cells to the slide surface while preserving their structural integrity and native morphology during the staining procedure. The fixed smears were first flooded with crystal violet, the primary stain, and allowed to act for approximately one minute to facilitate adequate penetration into the bacterial cells. Excess stain was then gently rinsed off with tap water. Following this, Gram's iodine was applied for about one minute; this reagent acts as a mordant, forming a stable crystal violet–iodine complex within the cell wall. The slides were again rinsed to remove any unbound iodine. Decolorization, a critical step in the Gram staining process, was performed using 95% ethyl alcohol applied dropwise until no more color leached from the smear. This step differentiates bacteria based on the structural

differences in their cell walls. Immediately after decolorization, the slides were rinsed with water to halt the process and prevent over-decolorization. The smears were then counterstained with safranin for approximately 45 seconds, providing a contrasting color to the decolorized cells. A final gentle rinse was performed, and the slides were allowed to air dry under clean conditions. The stained preparations were examined under a microscope using oil immersion, which enabled clear differentiation between Gram-positive bacteria (retaining the purple color) and Gram-negative bacteria (appearing pink or red). In addition to differentiation, this method allowed detailed observation of cellular morphology, including shape, size, and arrangement patterns. Overall, Gram staining serves as a fundamental and reliable technique for phenotypic characterization and plays a crucial role in the preliminary taxonomic identification of bacterial isolates [21–22].

Capsule stain

For the visualization of bacterial capsules, smears were carefully prepared on clean, sterile glass slides and allowed to air dry naturally without applying heat fixation. This precaution was essential to preserve the native morphology of the bacterial cells as well as the integrity of the delicate capsule structures, which can be easily distorted or destroyed by heat. After drying, the smears were flooded with crystal violet stain and left undisturbed for approximately 5–7 minutes to ensure sufficient staining of the bacterial cells. Following this, the excess stain was gently removed using a 20% copper sulfate solution. This reagent served a dual purpose: it acted as a mild decolorizing agent to remove excess crystal violet and simultaneously functioned as a counterstain, enhancing the contrast between the cells, capsules, and background. The slides were then allowed to air dry under clean conditions and subsequently examined under a microscope using oil immersion. This method enabled clear visualization of bacterial cells along with their capsules, which appeared as distinct, transparent halos surrounding the deeply stained cells against a lightly colored background. This staining technique is particularly important for the identification of encapsulated bacteria, as the presence of a capsule is a significant structural and functional feature. Moreover, it provides valuable insights into bacterial virulence, protection mechanisms, and overall phenotypic characteristics,

thereby contributing to more accurate identification and classification of bacterial isolates [21–22].

Spore stain

For the visualization of endospores, bacterial smears were carefully prepared on sterile glass slides and subjected to heat fixation to immobilize the cells and preserve their structural integrity. Following fixation, malachite green was applied to the smears, and the slides were gently heated on a warm hot plate for 2–3 minutes. This heating step ensured deep penetration of the dye into the highly resistant spore structures, which are otherwise difficult to stain due to their robust protective layers. After heating, the slides were allowed to cool and then thoroughly rinsed with tap water to remove excess dye from the vegetative cells and background. To enhance contrast, the smears were subsequently counterstained with safranin for approximately 30 seconds, followed by a gentle rinse with water. This counterstaining step allowed the differentiation of vegetative cells, which took up the red safranin stain, from the endospores, which retained the green malachite dye. Finally, the air-dried slides were examined under oil immersion microscopy, providing a detailed view of the bacterial cells. Endospores appeared as distinct green structures embedded within the red-stained vegetative cells, enabling clear identification. This staining technique is essential for accurate morphological characterization and identification of spore-forming bacterial species. It also offers important insights into their structural adaptations, resilience, and survival mechanisms under adverse environmental conditions [21–22].

Acid fast stain

For the detection and characterization of acid-fast bacteria, bacterial smears were meticulously prepared on sterile glass slides and subjected to heat fixation. This step served to immobilize the cells while preserving their native structural features, ensuring that delicate cell wall components remained intact during staining. The fixed smears were then treated with carbolfuchsin and placed on a warm hot plate for approximately five minutes. Gentle heating facilitated the penetration of the dye into the thick, lipid-rich cell walls of acid-fast bacteria, which are resistant to conventional staining methods. Following this, the slides were allowed to cool and

were gently rinsed with tap water to remove excess stain. Decolorization was carried out by carefully applying acid-alcohol dropwise until no further carbolfuchsin was released from the smear. A subsequent rinse with water removed any residual decolorizing agent. To provide contrast and allow visualization of non-acid-fast cells, the smears were counterstained with methylene blue for about two minutes, followed by a gentle rinse and air drying. The prepared slides were then examined under oil immersion microscopy. Acid-fast bacteria appeared as red-stained cells due to the retention of carbolfuchsin, whereas non-acid-fast bacteria were visualized in blue, stained by methylene blue. This technique is essential for accurate morphological assessment and reliable identification of acid-fast bacterial species. It also provides valuable insights into the structural and diagnostic features of these microorganisms, which are often of significant clinical and environmental importance [21–22].

RESULTS AND DISCUSSION

The overall bacterial populations were assessed in both Chhiata and Silmondi soils, and the processes of isolation, purification, and characterization revealed significant heterogeneity among the bacterial colonies. These findings indicate the existence of diverse and varied bacterial communities within each of the soil samples. The colony count found 7.6×〖10〗^6 CFU/g soil and 6.5×〖10〗^6 CFU/g soil in Chhiata and Silmondi soil respectively.

The present study involved the isolation and characterization of bacterial populations from two distinct agricultural soils in Bangladesh: Chhiata and Silmondi. The isolates were examined for colony morphology, cellular characteristics, and staining properties to assess microbial diversity and phenotypic traits.

Colony Characteristics

Chhiata Soil: The colony characteristics of eight bacterial isolates from Chhiata soil are summarized in Table 2. The colony sizes varied from pinpoint to moderate, with pigmentation ranging from white, yellow, to pink. Colony forms included circular, rhizoid, and irregular shapes, while margin types were serrate, lobate, and entire. Elevation patterns included flat, raised, and umbonate forms. Specifically, isolate 3 exhibited a small, yellow, circular colony with an entire margin and umbonate

elevation, whereas isolate 2 formed a moderate pink rhizoid colony with serrate margins and umbonate elevation. These variations suggest considerable phenotypic heterogeneity among the bacterial population in Chhiata soil.

Silmondi Soil: Five bacterial isolates from Silmondi soil were characterized for colony morphology (Table 4). Colony sizes ranged from small to large, with pigmentation primarily white, yellow, and pink. Colony forms were predominantly circular, except isolate 4, which exhibited an irregular form. Margins were undulate, lobate, or entire, and elevations included flat and raised types. For instance, isolate 1 formed a large yellow circular colony with entire margins and raised elevation, whereas isolate 2 presented a medium pink circular colony with undulate margins and flat elevation. The observed differences in colony morphology between the two soils reflect the influence of soil microenvironment and nutrient availability on bacterial growth and phenotype.

Morphological Characteristics

Chhiata Soil: The bacterial isolates from Chhiata soil displayed a range of cellular shapes and arrangements (Table 3). Rod-shaped bacteria were observed in isolates 1,2,3,4, 7, and 8, whereas the remaining isolates were round. Chain arrangements were common among most isolates, though isolates 3 and 7 were observed as single cells. Gram staining revealed both Gram-positive and Gram-negative bacteria, with a predominance of Gram-negaive forms. Spore formation was observed in five isolates, while capsule formation was detected in four isolates. Most isolates were positive for acid-fast staining. These findings indicate that the Chhiata soil harbors a diverse bacterial population, comprising both spore-forming and capsule-forming bacteria, which may play distinct ecological roles in nutrient cycling and soil fertility.

Silmondi Soil: The five bacterial isolates from Silmondi soil also displayed diverse morphological characteristics (Table 5). Round cells predominated (isolates 1 and 5), while rod-shaped cells were observed in isolates 2,3 and 4. Single-cell arrangements were seen in isolates 1 and 2, whereas chain formations were observed in isolates 3, 4, and 5. Gram staining results indicated that most isolates were Gram-negative. Spore formation was present in isolates 3 and 4, and capsule formation was observed

in isolates 1 and 2. Most isolates were also positive for acid-fast staining. Compared to Chhiata soil, Silmondi soil exhibited fewer isolates, with a slightly higher proportion of chain-forming rod-shaped bacteria, suggesting variations in microbial composition likely influenced by soil physicochemical properties and management practices.

The results of the present study were compared with those of a previous investigation by Chowdhury et al., 2013 [23], conducted on soils from Bangladesh, and showed a strong similarity. This confirms that bacterial communities across different regions of Bangladesh are predominantly composed of *Bacillus* species. In addition to *Bacillus*, other Gram-negative, spore-forming bacteria such as *Enterobacter* spp., *Klebsiella* spp., and *Azospirillum* spp. have also been identified [17]. Numerous studies on Bangladeshi soils have reported a high abundance of various *Bacillus* species [24–27], which are largely spore-forming. The spores of *Bacillus* exhibit enhanced survival in alkaline soils, allowing the bacteria to complete their life cycle under challenging conditions. Because opportunities for soil bacteria to complete their reproductive cycle are often limited, the ability to form spores represents a key survival strategy, contributing to their persistence and ecological success [28]. Even when many newly formed sporulating bacteria are neutralized by decomposition or predation, survival is facilitated through utilization of dead and decaying organic matter, enabling these bacteria to maintain population continuity and disperse effectively within the soil environment [29].

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

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

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

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

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

Colony no.	Size	Pigmentation	Form	Margin	Elevation
1	Large	Yellow	Circular	Entire	Raised
2	Medium	Pink	Circular	Undulate	Flat
3	Small	White	Circular	Undulate	Flat
4	Small	Pink	Irregular	Lobate	Raised
5	Large	Yellow	Irregular	Entire	Raised

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

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

CONCLUSION

The present study successfully isolated, quantified, and characterized bacterial populations from Chhiata and Silmondi agricultural soils, revealing considerable diversity in both colony morphology and cellular traits. Variations in colony size, pigmentation, form, margin, and elevation reflect the heterogeneity of bacterial communities in these soils. Both sites harbored Gram-positive and Gram-negative bacteria, including rod-shaped and cocci forms with different arrangements. Several isolates exhibited spore- and capsule-forming abilities, indicating important survival strategies under environmental stress. Acid-fast bacteria were largely absent, suggesting that the populations are predominantly non-mycobacterial. The relatively high bacterial counts in both soils indicate active microbial ecosystems that contribute to nutrient cycling, organic matter decomposition, and soil fertility. The

prevalence of spore-forming bacteria, particularly Bacillus spp., highlights their ecological resilience and potential applications in agriculture, including as biofertilizers. In summary, this study emphasizes the considerable microbial diversity within Bangladeshi soils and highlights the crucial role of soil bacteria in supporting agricultural productivity and maintaining ecological balance. Further molecular and biochemical investigations are suggested to enable precise identification of these microorganisms and to evaluate their functional potential for sustainable agricultural applications.

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