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Isolation and Characterization of soil bacteria from Kaunia and Jamun series of Bangladesh

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ABSTRACT: Soil bacteria are essential for maintaining soil fertility and ecosystem functioning; however, information on the culturable bacterial diversity of many Bangladeshi soil series remains limited. This study aimed to isolate, enumerate, and characterize culturable bacteria from the Kaunia and Jamun soil series of Bangladesh. Soil samples were collected from agricultural fields in Mithapukur, Rangpur, and bacterial

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isolates were obtained using serial dilution and spread plate techniques. Bacterial populations were quantified as colony-forming units (CFU), while colony morphology and staining characteristics were used for phenotypic characterization. Kaunia soil contained a slightly higher bacterial population (4.6×10^8 CFU g^{-1} soil) than Jamun soil (4.4×10^8 CFU g^{-1} soil). Nine and seven distinct bacterial colonies were isolated from Kaunia and Jamun soils, respectively. The isolates showed considerable variation in colony morphology and included both Gram-positive and Gram-negative bacteria, with several exhibiting spore- and capsule-forming abilities. These findings provide baseline information on the culturable bacterial diversity of the Kaunia and Jamun soil series and support future molecular and functional studies for sustainable agricultural applications.

Keywords: *Soil bacteria; Kaunia soil series; Jamun soil series; Colony, morphology; Bacterial characterization.*

Introduction

Bacteria are single-celled prokaryotic microorganisms recognized as one of the oldest and most evolutionarily successful forms of life on Earth. Their extensive evolutionary history has enabled them to develop exceptional adaptive capabilities, allowing survival and proliferation across a broad spectrum of environmental conditions. Bacterial cells possess a relatively simple structural organization and generally range from 0.5 to 2.0 µm in diameter. Although structurally simple, they display considerable morphological diversity and are commonly classified into three major forms: coccoid (spherical), bacillary (rod-shaped), and spiral. Depending on environmental conditions and nutrient availability, bacterial cells may exist individually or aggregate to form colonies [1]. Bacteria are indispensable components of both terrestrial and aquatic ecosystems, where they contribute significantly to ecological stability and productivity. They are actively involved in the decomposition of organic matter and play a fundamental role in the biogeochemical cycling of essential elements, including carbon, nitrogen, and phosphorus, thereby sustaining nutrient availability within ecosystems [2]. Among all microbial groups, bacteria are the most abundant and ecologically diverse organisms. However, their relatively limited morphological variability makes accurate identification and taxonomic classification challenging when based solely on phenotypic characteristics. Consequently, comprehensive characterization of bacterial diversity requires an integrated classification

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approach that combines culture-dependent techniques with complementary analytical and molecular methods.These classification frameworks provide valuable insights into the ecological functions, physiological characteristics, and environmental interactions of different bacterial taxa across diverse ecosystems [3]. Bacteria are universally distributed and inhabit nearly every natural environment, including soil, freshwater and marine ecosystems, the atmosphere, and extreme habitats characterized by high salinity, extreme temperatures, or acidic conditions. Through their diverse metabolic activities, bacterial communities play an indispensable role in maintaining environmental balance and regulating major biogeochemical cycles. In aerobic soils, bacteria interact synergistically with fungi to facilitate organic matter decomposition and nutrient cycling. Conversely, in oxygen-deficient or anaerobic soils, bacteria become the dominant microbial group, carrying out most of the biochemical transformations and essential biological processes that govern soil functioning [4]. The foundation of bacteriological investigations lies in the systematic isolation, purification, and identification of bacterial strains. Isolation techniques are employed to recover bacterial populations from specific environmental or biological sources, followed by purification procedures to eliminate contaminating microorganisms and establish homogeneous cultures [5]. The development of a pure bacterial culture is a prerequisite for accurate characterization, as it enables detailed investigations of bacterial morphology, physiological properties, biochemical capabilities, and susceptibility to antimicrobial agents. Such pure cultures also provide a dependable basis for taxonomic studies, ecological assessments, and a wide range of microbiological and biotechnological applications. The isolation of pure bacterial cultures from mixed microbial communities is commonly achieved using both selective and non-selective culture media. Standard microbiological techniques, including streak plate, pour plate, and cultivation on solid agar media, are widely employed to obtain well-isolated bacterial colonies suitable for further characterization [6,7]. Numerous investigations have demonstrated that soils in different regions of Bangladesh are increasingly contaminated with heavy metals, creating selective pressure that favors the proliferation of metal-tolerant and metal-resistant bacterial populations. These indigenous bacteria possess remarkable adaptive capabilities, enabling them to survive under adverse environmental conditions while contributing to the ecological functioning of contaminated ecosystems [8–11]. Studies have further revealed that native soil bacteria in Bangladesh perform several

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essential ecosystem services, such as phosphate solubilization, facilitation of environmental detoxification, and regulation of mineral nutrient cycling within the soil environment [12–14]. Moreover, they are actively involved in key biochemical processes, particularly nitrification, which plays a fundamental role in sustaining soil fertility and ensuring the continuous availability of plant nutrients [15–17]. Beyond nutrient transformation, these bacterial communities contribute to the bioremediation of heavy metal-contaminated soils, suppress the proliferation of pathogenic microorganisms, and help stabilize acidic soil conditions. Consequently, microbiological investigations of soil ecosystems are crucial for improving our understanding of microbial diversity and ecological processes, while also supporting the development of sustainable strategies for environmental management and agricultural productivity. A systematic and sequential approach to the identification of soil bacterial populations is therefore essential for revealing the composition, diversity, and dynamic interactions of complex soil microbial communities [13,18]. Against this background, the present study focuses on the isolation and characterization of soil bacterial populations, the determination of colony-forming units (CFUs), and the assessment of colony morphology. In addition, detailed morphological characterization, including cell shape, cellular arrangement, and staining characteristics, was performed to evaluate the abundance, diversity, and structural variation of bacterial communities present in the soil samples.

Materials and Methods

Sample collection

Two fresh topsoil samples (Table 1) were collected aseptically from agricultural fields in Bangladesh for microbiological analysis. Sampling was carried out from the surface soil layer (0–15 cm depth), which is generally recognized as the most biologically active zone due to its high microbial abundance and nutrient content. Throughout the sampling procedure, sterile techniques were strictly followed to prevent external contamination and preserve the native microbial communities. Immediately after collection, the samples were placed in sterile thermos flasks and transported promptly to the laboratory under controlled temperature conditions to minimize changes in microbial composition during transit. Upon arrival, the soil samples were stored under appropriate laboratory conditions until microbiological and physicochemical analyses were performed. The standardized sampling, transportation, and storage procedures ensured the integrity of the samples and

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enhanced the accuracy, reliability, and reproducibility of the subsequent experimental analyses.

Table 1. Related information of collected soil samples.

Sample no.	Series	Location	GPS reading	Texture	Cropping pattern
01	kaunia	Mithapukur, Rangpur	N-25°31′17″ E-89°19′27″	Loamy	Rice-Fallow- Rice
02	Jamun	Mithapukur, Rangpur	N-25°34′52″ E-89°10′40″	Loamy	Maize-Fallow- Rice

Isolation of bacteria

Bacterial isolation was performed following established standard microbiological protocols described in previous studies [19,20]. Briefly, a measured amount of each soil sample was suspended in sterile physiological saline (0.9% NaCl prepared with distilled water) and thoroughly mixed to obtain a uniform microbial suspension. The resulting suspension served as the initial inoculum for subsequent microbiological analyses. To reduce the microbial load and facilitate the recovery of discrete bacterial colonies, the suspension was subjected to a series of tenfold serial dilutions. Appropriate dilutions were selected, and aliquots were aseptically inoculated onto pre-labeled agar plates using the spread plate technique to ensure uniform distribution of bacterial cells across the medium. The inoculated plates were incubated at 37°C for 24–48 h, allowing sufficient time for bacterial growth and colony development. Following incubation, morphologically distinct and well-isolated colonies were carefully picked and purified by repeated streaking on fresh agar plates to obtain axenic (pure) cultures. This purification step was repeated until homogeneous cultures free from microbial contamination were achieved. All isolation and purification procedures were carried out in triplicate to improve the reliability and reproducibility of the experimental results. Finally, the purified isolates were re-incubated at 37°C for an additional 24–48 h to confirm culture purity and ensure stable bacterial growth before further characterization [19,20].

Viable count

The viable bacterial population was quantified using the standard plate count technique. To ensure accurate and reliable enumeration, only agar plates containing 25–250 well-isolated

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colonies were selected for counting. The viable bacterial density was expressed as colony-forming units (CFU) per gram of soil, which was calculated using the following equation:

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

Characterization

The morphological features of bacterial colonies isolated from both sampling sites were evaluated using well-separated colonies grown on nutrient agar plates. Colony characterization was performed based on standard phenotypic attributes, including colony size, color (pigmentation), shape, margin, and elevation. All observations were recorded systematically according to the standardized morphological criteria described by Dubey and Maheshwari (1998) to ensure consistency and reproducibility of the results [19]. The assessment of these colony characteristics provided valuable information on the phenotypic variability of the bacterial isolates and served as a preliminary basis for their identification and taxonomic characterization.

Staining characteristics

The cellular morphology and arrangement of the bacterial isolates were examined using a series of standard staining techniques, including simple staining, negative staining, Gram staining, capsule staining, endospore staining, and acid-fast staining. These complementary staining methods facilitated detailed observation of bacterial cell morphology, cellular arrangement, and structural features, providing comprehensive phenotypic information. The resulting microscopic characteristics served as essential criteria for phenotypic characterization, taxonomic identification, and differentiation of the bacterial isolates, while also contributing to a better understanding of the structural diversity of soil bacterial communities [21,22].

Simple staining

For microscopic examination of bacterial morphology, thin smears of each isolate were prepared on sterile glass slides and heat-fixed to preserve cellular morphology and firmly attach the cells to the slide surface. The fixed smears were stained with crystal violet for 40–60 s, followed by gentle rinsing with tap water to remove excess stain. After air-drying, the stained preparations were examined under a light microscope using the oil immersion

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objective. Microscopic observations focused on cell shape, cellular arrangement, and other morphological characteristics. These observations provided essential phenotypic information for the preliminary identification and characterization of the bacterial isolates [21,22].

Negative staining

Negative staining was carried out using a clean, grease-free glass slide. A small drop of nigrosin was placed near one end of the slide, and a loopful of bacterial culture was mixed gently with the stain. Using the edge of a second sterile slide held at an angle of approximately 30°, the suspension was spread evenly across the slide to produce a thin smear. The preparation was allowed to air dry without heat fixation and subsequently examined under a light microscope using the oil immersion objective. This staining technique preserved the natural size and morphology of the bacterial cells by eliminating the need for heat fixation, thereby minimizing structural distortion. As a result, negative staining facilitated accurate observation of cell shape, size, and cellular arrangement, providing valuable phenotypic information for the characterization and preliminary identification of the bacterial isolates [21,22].

Gram stain

Gram staining was performed following the standard differential staining procedure to distinguish Gram-positive and Gram-negative bacterial isolates. Thin bacterial smears were prepared on sterile glass slides, air-dried, and heat-fixed to ensure proper cell adhesion while preserving cellular morphology. The fixed smears were stained with crystal violet for approximately 1 min, rinsed gently with tap water, and subsequently treated with Gram's iodine for 1 min to facilitate the formation of the crystal violet–iodine complex. After rinsing, the smears were decolorized by carefully applying 95% ethanol until the excess stain was completely removed, followed by immediate washing with tap water to terminate the decolorization process. The slides were then counterstained with safranin for approximately 45 s, rinsed thoroughly, and allowed to air dry. Finally, the stained preparations were examined under a light microscope using the oil immersion objective. This differential staining technique enabled clear discrimination between Gram-positive and Gram-negative bacteria while providing detailed information on cell morphology, size,

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and cellular arrangement, thereby supporting the phenotypic characterization and taxonomic identification of the bacterial isolates [21,22].

Capsule stain

Capsule staining was performed to visualize the extracellular capsules surrounding bacterial cells. Thin smears were prepared on sterile glass slides and allowed to air dry without heat fixation to preserve the integrity of both the bacterial cells and their capsules. The dried smears were stained with crystal violet for 5–7 min, after which excess stain was gently removed using 20% copper sulfate solution, which simultaneously acted as a decolorizing agent and a counterstain. The slides were then air-dried and examined under a light microscope using the oil immersion objective. Encapsulated bacteria were identified by the presence of distinct, unstained halos surrounding the stained cells, indicating the capsule layer. This staining method provided valuable information on capsule production and contributed to the phenotypic characterization and identification of the bacterial isolates [21,22].

Spore stain

Endospore staining was carried out using the Schaeffer–Fulton staining method to visualize bacterial endospores. Thin bacterial smears were prepared on sterile glass slides, air-dried, and heat-fixed to ensure proper cell attachment and preservation of cellular morphology. The smears were flooded with malachite green and heated gently over a hot plate for 2–3 min to facilitate penetration of the stain into the resistant endospore structures. After cooling, the slides were rinsed thoroughly with tap water to remove excess primary stain from the vegetative cells. The smears were then counterstained with safranin for approximately 30 s, followed by gentle washing with water and air drying. The stained preparations were examined under a light microscope using the oil immersion objective. Endospores appeared as green-stained structures, whereas vegetative cells were observed in red, allowing clear differentiation between the two cell types. This staining technique is widely used for the morphological characterization and identification of spore-forming bacteria and provides important information regarding their structural adaptations and taxonomic classification [21,22].

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Acid fast stain

Acid-fast staining was performed to detect and differentiate acid-fast bacterial isolates using the Ziehl–Neelsen staining technique. Thin bacterial smears were prepared on sterile glass slides, air-dried, and heat-fixed to ensure proper cell adhesion and preservation of cellular morphology. The fixed smears were flooded with carbolfuchsin and heated gently on a hot plate for approximately 5 min to promote dye penetration through the waxy, mycolic acid-rich cell wall of acid-fast bacteria. After cooling, the slides were rinsed with tap water to remove excess stain. Decolorization was carried out by carefully applying acid-alcohol until no further red dye was released, followed by washing with water to stop the decolorization process. The smears were then counterstained with methylene blue for approximately 2 min, rinsed gently, and allowed to air dry. Finally, the stained preparations were examined under a light microscope using the oil immersion objective. Acid-fast bacteria retained the red carbolfuchsin stain, whereas non-acid-fast cells appeared blue due to the methylene blue counterstain. This differential staining method provided reliable morphological and diagnosticinformation for the identification and characterization of acid-fast bacterial isolates and supported their taxonomic differentiation [21,22].

Statistical analysis

All microbiological experiments, including bacterial isolation, viable cell enumeration, and morphological characterization, were performed in triplicate to ensure the accuracy, reliability, and reproducibility of the experimental findings. Colony-forming unit (CFU) data were recorded and expressed as mean values. Descriptive statistical analyses were employed to summarize the bacterial population characteristics of the two soil samples. Total viable bacterial populations were calculated using the standard CFU equation and reported as CFU g^{-1} soil. Differences in bacterial abundance and colony morphology between the Soil samples were evaluated through comparative analysis of their mean CFU values and observed colony characteristics.

Results and Discussion

The total bacterial population was observed from both Kaunia and Jamun soils, where successful isolation, purification, and characterization revealed considerable variability in bacterial colonies. The results demonstrated the presence of diverse bacterial communities

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in both soil samples. The colony count found 4.6×(10)^8 CFU/g soil and 4.4×(10)^8 CFU/g soil in Kaunia and Jamun soil respectively. A total of nine distinct types of bacterial colonies were identified from Kaunia soil, whereas seven distinct colonies were observed from Jamun soil.

The bacterial colonies isolated from Kaunia soil exhibited considerable variation in colony size, ranging from pinpoint to moderate-sized colonies, whereas those isolated from Jamun soil varied from pinpoint to moderate-sized colonies with small colonies also being observed. This variation in colony size suggests differences in the growth characteristics of bacterial isolates present in the two soil types. In Kaunia soil, the colonies were observed to be circular, irregular, and rhizoid in form; entire, serrate, and lobate in margin; and flat, raised, and umbonate in elevation (Table 2). Similarly, the colonies isolated from Jamun soil exhibited circular, irregular, and rhizoid forms with entire, serrate, and lobate margins, while their elevation varied from flat, raised, and umbonate (Table 4). Colony pigmentation in both soils included white, pink, and yellow, indicating the presence of metabolically diverse bacterial populations.

The morphological characteristics of bacterial isolates from Kaunia soil revealed a heterogeneous population consisting of both rod-shaped and round-shaped bacteria arranged either singly or in chains. The isolates included both Gram-positive and Gram-negative bacteria, with several isolates exhibiting spore-forming ability, particularly among the rod-shaped bacteria (Figure 1). Capsule formation was observed in some isolates, which may enhance bacterial survival under unfavorable environmental conditions. The isolates showed variation in acid-fast staining, with both acid-fast and non-acid-fast bacteria being present. Likewise, bacterial isolates from Jamun soil also comprised both rod-shaped and round-shaped bacteria with single and chain arrangements. The isolates included both Gram-positive and Gram-negative bacteria, and several isolates exhibited spore-forming ability as well as capsule formation (Figure 2). Similar to Kaunia soil, both acid-fast and non-acid-fast bacterial isolates were recorded.

The comparative morphological analysis (Figure 3) revealed that rod-shaped bacteria predominated in both Kaunia and Jamun soils, accounting for the majority of the isolates. Single-cell arrangement was slightly more frequent than chain arrangement in both soils. Gram-negative bacteria constituted the dominant group in both locations, whereas Gram-

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positive isolates were comparatively fewer. Acid-fast bacteria were more abundant among Kaunia isolates, while non-acid-fast bacteria predominated slightly in Jamun soil. Similarly, spore-forming bacteria were relatively more frequent in Kaunia, whereas non-spore-forming isolates were slightly more common in Jamun. Capsule-forming bacteria were dominant in both soils, with Kaunia exhibiting a higher frequency than Jamun. Overall, the observed morphological similarities suggest that both soils harbor comparable bacterial groups, although differences in Gram reaction, acid-fastness, spore formation, and capsule production indicate variations in the phenotypic diversity of their bacterial communities.

Overall, the results indicate that both Kaunia and Jamun soils harbor diverse and heterogeneous bacterial communities with considerable variation in colony morphology and cellular characteristics. Such diversity reflects the ecological complexity of the soil microbial populations and may contribute to important soil functions, including nutrient cycling and plant growth promotion. The findings are consistent with previous reports on the morphological diversity of soil bacteria isolated from different agroecological regions of Bangladesh [23].

The findings of the present study are in close agreement with those reported in previous investigations, suggesting that Bacillus spp. constitute one of the dominant bacterial groups in soils from different regions of Bangladesh. In addition to Bacillus spp., several other Gram-negative and spore-forming bacteria, including Enterobacter spp., Klebsiella spp., and Azospirillum spp., have also been identified from Bangladeshi soils [17]. Numerous studies have consistently reported the widespread occurrence of Bacillus species in Bangladesh, with most isolates possessing the ability to form endospores [24–27]. Endospore formation provides these bacteria with a significant ecological advantage by enabling them to tolerate unfavorable environmental conditions, particularly in alkaline soils, and to resume active growth when conditions become favorable. Since soil-dwelling sporulating bacteria often encounter limited opportunities to complete their reproductive cycle, spore formation serves as an important survival strategy that enhances their persistence in the soil ecosystem [28]. Moreover, these bacteria actively participate in the decomposition of organic matter, facilitating nutrient recycling and promoting their

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dispersal throughout the soil. This ecological adaptation contributes to the long-term maintenance and continuity of their populations within the soil environment [29].

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

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

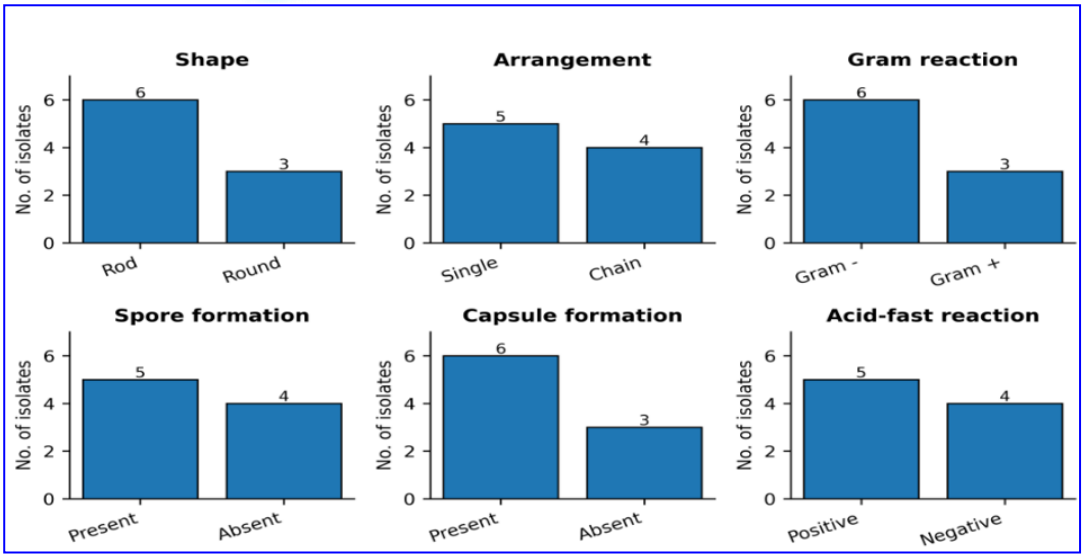

Figure 1. Morphological characteristics of isolated bacteria of Kaunia soil.

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Table 3. Colony characteristics of isolated bacteria of Jamun soil.

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

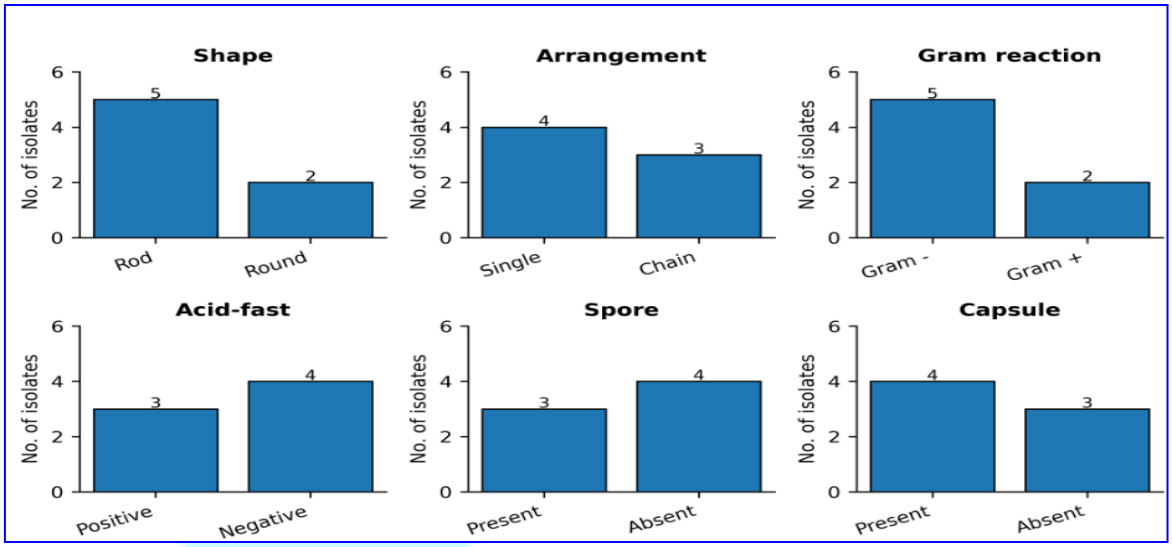

Figure 2. Morphological characteristics of isolated bacteria of Jamun soil.

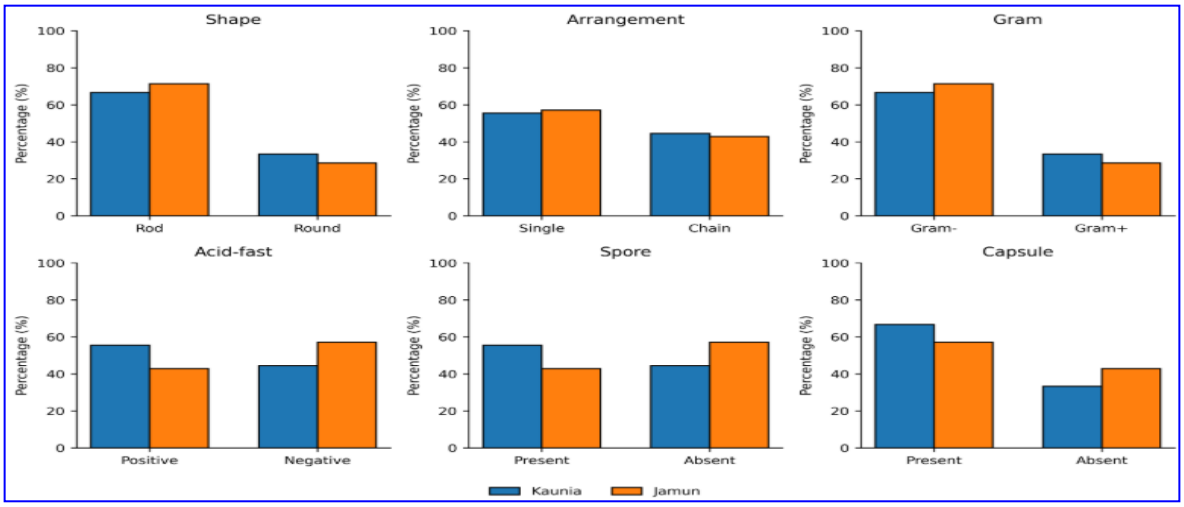

Figure 3. Comperative Morphological characteristics of isolated bacteria between two series.

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Limitation

Despite providing important baseline information on the isolation and characterization of bacterial communities associated with the Kaunia and Jamun soil series of Bangladesh, this study has several limitations that should be acknowledged. The research relied primarily on culture-dependent methods, which recover only a small fraction of the microorganisms naturally inhabiting soil. As a considerable proportion of soil bacteria remain unculturable under standard laboratory conditions, the bacterial diversity observed in this study may not accurately reflect the complete microbial community. In addition, bacterial identification was based mainly on colony morphology and conventional staining techniques. Although these phenotypic methods are appropriate for preliminary characterization, they lack the taxonomic resolution required for reliable species-level identification. The absence of molecular approaches, particularly 16S rRNA gene sequencing, therefore restricts the accuracy and taxonomic confidence of the bacterial identification reported in this investigation.

Conclusion

The present study successfully isolated and characterized culturable bacterial populations from the Kaunia and Jamun soil series of Bangladesh, revealing diverse colony morphology and cellular characteristics. Both soils contained abundant bacterial populations, with Kaunia soil showing a slightly higher bacterial count than Jamun soil. The isolates included both Gram-positive and Gram-negative bacteria with rod- and round-shaped forms, and several exhibited spore-forming and capsule-forming abilities, indicating their adaptability to varying soil environments. Overall, the findings demonstrate that the Kaunia and Jamun soils harbor diverse bacterial communities that are likely to contribute to nutrient cycling and soil fertility. This study provides baseline information on the culturable bacterial diversity of these soils. Further molecular identification and functional characterization are recommended to accurately identify the isolates and explore their potential applications in sustainable agriculture.

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I/we hereby declare that artificial intelligence (AI) tools were used solely to assist in the preparation of this manuscript, including language refinement, grammatical correction, structural organization, and editorial improvement. The study design, experimental work,

data collection, data analysis, interpretation of the results, and the conclusions presented in this manuscript are entirely my/our original work. I/we accept full responsibility for the accuracy, integrity, originality, and authenticity of the content submitted for publication.

Disclosure Statement

The author declares that there are no conflicts of interest, financial or otherwise, that could have influenced the design, conduct, interpretation, or publication of this study.

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