Exploring Bacterial Diversity through Isolation and Characterization of Jamalpur and Sonatala Soil Series from Jamalpur District

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ABSTRACT: Soil bacterial communities are essential contributors to nutrient cycling, organic matter decomposition, and overall soil fertility. This study aimed to isolate, enumerate, and characterize bacterial populations from agricultural soils of Jamalpur and Sonatala, Bangladesh. Topsoil samples (0–15 cm) were collected aseptically and subjected to serial dilution, followed by spread plate inoculation on nutrient agar to obtain pure bacterial cultures. Morphological characterization included assessment of colony size, form, margin, elevation, and pigmentation, while cellular features were analyzed using simple, negative, Gram, capsule, spore, and acid-fast staining techniques. A total of seven and six distinct bacterial colonies were isolated from Jamalpur and Sonatala soils, respectively. The isolates displayed diverse morphologies, including rod-shaped and cocci forms with single and chain arrangements. Both Gram-positive and Gram-negative bacteria were present, with several exhibiting spore- and capsule-forming abilities, while all isolates were non-acid fast. Colony size, pigmentation, and structural diversity

reflected metabolic heterogeneity and adaptability under varying soil conditions. The total bacterial counts were 7.5×10^7 CFU/g soil for Jamalpur and 7.4×10^7 CFU/g soil for Sonatala, indicating active and resilient microbial populations. The dominance of spore-forming Bacillus species and the presence of other Gram-negative bacteria suggest ecological significance in soil nutrient dynamics and potential applications in sustainable agriculture. These findings provide insights into microbial diversity in Bangladeshi soils and form a basis for further functional and molecular characterization.

Keywords: *Jamalpur; Sonatala; Colony; morphology; Stain; Microbial diversity; CFU*

INTRODUCTION

Bacteria are single-celled, prokaryotic microorganisms that rank among the earliest and most evolutionarily enduring forms of life on our planet. Their extensive evolutionary history has equipped them with exceptional adaptive capabilities, allowing them to survive, thrive, and colonize a remarkably wide range of environmental conditions, from moderate habitats to extreme ecosystems. Structurally, bacterial cells are relatively simple compared to eukaryotic cells, typically ranging in size from approximately 0.5 to 2.0 µm in diameter. Despite their simplicity, bacteria exhibit a variety of distinct morphological forms, which are primarily classified into coccoid (spherical), bacillary (rod-shaped), and spiral shapes. These cells may exist individually as solitary units or may group together to form complex colonies, with such arrangements being influenced by nutrient availability, environmental pressures, and intercellular interactions [1]. Bacteria fulfill crucial ecological roles in both terrestrial and aquatic ecosystems. They are central to ecosystem productivity because they participate actively in the decomposition of organic matter and facilitate the recycling of essential nutrients, including carbon, nitrogen, and phosphorus [2]. Among all microbial groups, bacteria are the most widely distributed and ecologically versatile organisms, colonizing diverse environments across the globe. However, the limited morphological diversity of bacteria presents considerable challenges for accurate identification and systematic classification. Consequently, reliance on morphology

alone is often inadequate for differentiating bacterial species. Comprehensive classification therefore requires integration of culture-based methods with complementary analytical approaches, which collectively enhance understanding of bacterial diversity. These classification frameworks are invaluable for elucidating the ecological roles, physiological characteristics, and environmental interactions of different bacterial taxa across varied ecosystems [3]. Bacteria are found ubiquitously in nature, inhabiting virtually all habitats, including soils, freshwater and marine environments, atmospheric niches, and even extreme settings such as high-salinity regions, highly acidic zones, and areas with extreme temperatures. Their metabolic activities are fundamental to maintaining environmental stability and regulating biogeochemical cycles. In well-aerated soils, bacterial populations interact synergistically with fungi to drive microbial processes and promote efficient nutrient turnover. Conversely, under oxygen-limited or anaerobic conditions, bacteria often dominate microbial communities and are primarily responsible for the majority of chemical transformations and biological activities occurring in the soil system [4]. The study of bacteria typically begins with the systematic isolation, purification, and identification of strains from environmental samples. Isolation procedures are employed to recover bacterial populations from specific sources, followed by purification methods to remove contaminating microorganisms and obtain uniform, pure cultures [5]. Establishing such pure cultures is essential, as they form the foundation for detailed investigations of bacterial morphology, physiology, biochemical activity, and antimicrobial susceptibility. To achieve this, both selective and non-selective growth media are widely used. Standard laboratory techniques—such as growth on solid agar media, streak plate methods, and pour plate approaches—effectively allow for the isolation of discrete bacterial colonies [6–7]. Several studies have indicated that soils across various regions of Bangladesh are heavily impacted by metal contamination, which promotes the selection and persistence of metal-tolerant or metal-resistant bacterial populations. These microorganisms exhibit remarkable resilience and adaptability under stressful environmental conditions, thereby fulfilling key ecological roles in contaminated habitats [8–11]. Indigenous soil bacteria in Bangladesh have been demonstrated to provide numerous beneficial functions, such as solubilizing phosphate, aiding in

detoxification of pollutants, and regulating mineral cycling within the soil ecosystem [12–14]. In addition, these microbial communities actively participate in critical biochemical processes, including nitrification, which is essential for sustaining soil fertility and nutrient availability [15–17]. Their activities further support the remediation of metal-contaminated soils, reduction of pathogenic microbes, and stabilization of acidic soil environments. Microbiological investigations of soil ecosystems are therefore essential both for advancing scientific knowledge and for developing practical solutions to environmental and agricultural challenges. The stepwise and systematic identification of soil bacterial populations is particularly critical for unraveling the complexity, dynamics, and functional diversity of microbial communities in soil [13,18].

Within this context, the present study focuses on the isolation of soil bacteria, enumeration of colony-forming units, and detailed evaluation of colony characteristics. Furthermore, key morphological features including cellular shape, arrangements, and staining responses were examined to provide insights into microbial abundance, diversity, and ecological significance within the studied soil samples.

MATERIALS AND METHODS

Sample collection

Two freshly collected topsoil samples (Table 1) were obtained aseptically from agricultural fields in Bangladesh. The soil was specifically sampled from the surface layer at a depth of 0–15 cm, a zone recognized for its high microbial density and nutrient richness. Throughout the sampling procedure, stringent aseptic techniques were strictly followed to prevent contamination from external sources and to preserve the natural microbial communities within the soil. Immediately after collection, the samples were transferred to the laboratory using sterile thermos flasks, which helped maintain a stable temperature and minimized changes in microbial composition during transport. Upon arrival, the soil samples were carefully stored under controlled laboratory conditions until they were subjected to subsequent microbiological and physicochemical analyses. This meticulous approach to sample

collection, handling, and preservation was essential to ensure the reliability, consistency, and reproducibility of the experimental results obtained from these soil samples.

Table 1. Related information of collected soil samples

Sample no.	**Series**	**Location**	**GPS reading**	AEZ	**Physiographic unit**	**Cropping pattern**
1	Jamalpur	Sadar Jamalpur	N-24°54′372″ E-89°55′086″	9	Brahmaputra-Jamuna Floodplain	Potato-Boro-T. Amam
2	Sonatala	Ramnagar Jamalpur	N-24°54′104″ E-89°54′766″	9	Brahmaputra-Jamuna Floodplain	Chilli-Boro-T. Amam

Isolation of Bacteria

Bacterial isolation was performed following well-established standard microbiological protocols as described in previous studies. Initially, a measured amount of soil was thoroughly homogenized with physiological saline solution (distilled water containing 0.9% NaCl) to create a uniform suspension of microbial cells. This homogenized mixture served as the primary material for subsequent microbiological procedures. The soil suspension was then subjected to a series of stepwise serial dilutions to reduce microbial density, facilitating the isolation of individual bacterial colonies. Aliquots from appropriate dilution levels were aseptically transferred onto sterile, labeled Petri dishes and evenly spread across the surface of nutrient agar using the spread plate technique. The inoculated plates were incubated at 37°C for 24–48 hours to promote optimal bacterial growth and allow the formation of discrete colonies. Following incubation, morphologically distinct and well-isolated colonies were carefully selected and subjected to repeated streaking on fresh agar plates to obtain pure axenic cultures. This purification process was systematically repeated to eliminate mixed microbial populations and ensure the consistency and reliability of the cultures. All isolation and purification procedures were conducted in triplicate to maintain experimental accuracy and reproducibility. Finally, the purified bacterial isolates were re-incubated at 37°C for an additional 24–48 hours to confirm culture purity, verify stable growth, and ensure suitability for further microbiological analyses [19–20].

Viable count

The viable bacterial population in the soil samples was quantified using the standard colony count method. To ensure accurate and reliable enumeration, only agar plates exhibiting between 25 and 250 well-isolated colonies were considered suitable for analysis. The total number of viable bacteria per gram of soil was then calculated using the following formula, which takes into account the dilution factor and the volume of inoculum applied to the agar surface:

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 carefully analyzed using well-isolated colonies grown on nutrient agar plates. A thorough assessment was conducted, taking into account several key parameters, including colony size, pigmentation, overall shape, margin characteristics, and elevation (Figure 1). Observations were performed systematically to maintain consistency and reproducibility, adhering to the standardized descriptive guidelines provided by Dubey and Maheshwari (1998) [19]. Such detailed morphological characterization offers valuable insights into the phenotypic diversity of bacterial populations in the soil and serves as an essential foundation for their subsequent identification, classification, and comparative analyses.

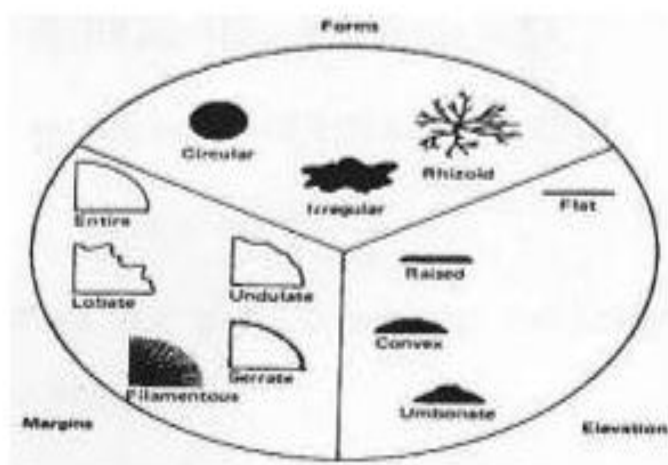

Figure 1. Colony characteristics of bacteria [21].

Staining characteristics

The morphology and spatial organization of the bacterial isolates were systematically examined using a series of established staining techniques, including simple staining, negative staining, Gram staining, capsule staining, spore staining, and acid-fast staining. Each of these staining methods provided complementary information, collectively allowing for a detailed characterization of bacterial cell shape, surface structures, and arrangement patterns. Such observations are essential for accurate phenotypic differentiation, reliable taxonomic classification, and a comprehensive understanding of the structural diversity exhibited by the soil bacterial populations [21–22].

Simple staining

For detailed examination of bacterial morphology, smears were carefully prepared on sterile glass slides and subjected to heat fixation to immobilize the cells and preserve their structural integrity. Crystal violet stain was then applied to the fixed smears and allowed to act for 40–60 seconds to ensure sufficient penetration and binding to the bacterial cells. The slides were gently rinsed with tap water to remove excess stain and subsequently air-dried. The stained smears were observed under a high-power microscope using oil immersion, which facilitated precise visualization of cellular shapes, spatial arrangements, and other structural features. This procedure provides critical data for the accurate phenotypic characterization and preliminary identification of bacterial isolates, forming an essential step in the overall bacteriological analysis [21–22].

Negative staining

Negative staining was carried out using a clean, dry glass slide, on which a small drop of nigrosin dye was placed at one end. A loopful of bacterial culture was gently mixed with the dye, and the resulting suspension was carefully spread across the slide using the edge of a second slide held at an approximate 30° angle to form a thin, uniform smear. The smear was allowed to air-dry naturally, without the application of heat. Examination under a microscope using oil immersion enabled detailed visualization of bacterial cell morphology, including cellular shape, size, and

spatial arrangement, while minimizing the structural distortions that can occur with heat fixation. By preserving the native dimensions and configuration of the cells, negative staining provides accurate and reliable information for the phenotypic characterization of bacterial isolates, contributing to their proper identification and classification [21–22].

Gram stain

For Gram staining, bacterial smears were carefully prepared on sterile glass slides and subjected to heat fixation to immobilize the cells while preserving their native morphology. The fixed smears were first treated with crystal violet for approximately one minute to allow adequate uptake of the primary dye. Excess crystal violet was gently rinsed off with tap water. The smears were then exposed to Gram's iodine for one minute, which acts as a mordant to form a stable crystal violet–iodine complex within the cells, followed by another gentle rinse to remove unbound iodine. Decolorization was performed by applying 95% ethyl alcohol dropwise until no further dye leached from the smear, after which the slides were rinsed again with tap water. To complete the procedure, the smears were counterstained with safranin for about 45 seconds, followed by a final rinse and air drying. Examination under oil immersion microscopy allowed clear differentiation between Gram-positive and Gram-negative bacteria, while also enabling detailed observation of cellular shape, size, and arrangement. This method provides reliable phenotypic characterization and plays a crucial role in the accurate taxonomic identification of bacterial isolates [21–22].

Capsule stain

For the visualization of bacterial capsules, smears were prepared on sterile glass slides and allowed to air dry naturally, avoiding heat fixation to preserve the native morphology of the cells and their surrounding capsules. Crystal violet stain was then applied to the dried smears and left in contact for 5–7 minutes to ensure thorough penetration and staining of the bacterial cells. Excess dye was gently removed using a 20% copper sulfate solution, which acts both as a decolorizing agent and as a background counterstain. The slides were then air-dried and examined under oil

immersion microscopy, allowing clear observation of the bacterial cells and their capsules, which appear as distinct, unstained halos surrounding the stained cells. This method is crucial for the accurate identification of encapsulated bacterial strains and provides important insights into their structural features and phenotypic characteristics [21–22].

Spore stain

For the visualization of bacterial endospores, smears were carefully prepared on sterile glass slides and subjected to heat fixation to immobilize the cells while preserving their structural integrity. Malachite green was applied to the fixed smears, and the slides were gently heated on a warm hot plate for 2–3 minutes to facilitate thorough penetration of the dye into the highly resistant spore structures. After heating, the slides were allowed to cool and were rinsed thoroughly with tap water to remove excess stain. The smears were then counterstained with safranin for approximately 30 seconds, followed by gentle rinsing with water to provide contrast between vegetative cells and endospores. Following air drying, the slides were examined under oil immersion microscopy, allowing clear visualization of endospores as green-stained structures within the red-stained vegetative cells. This staining technique is essential for the accurate morphological characterization and identification of spore-forming bacterial species and offers valuable insights into their structural adaptations and survival mechanisms [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 to immobilize the cells while preserving their native structural features. The fixed smears were then treated with carbolfuchsin and placed on a warm hot plate for five minutes, allowing the dye to penetrate the thick, lipid-rich cell walls characteristic of acid-fast bacteria. Following heating, the slides were cooled and gently rinsed with tap water to remove excess stain. Decolorization was performed by carefully applying acid-alcohol dropwise until no further carbolfuchsin was removed, followed by a rinse with water to eliminate residual decolorizer. The smears were

subsequently counterstained with methylene blue for approximately two minutes, then gently rinsed and air-dried, providing a clear contrast between acid-fast and non-acid-fast cells. Examination under oil immersion microscopy enabled unambiguous differentiation, with acid-fast bacteria retaining the red carbolfuchsin stain, while non-acid-fast cells appeared blue due to methylene blue uptake. This staining technique is essential for precise morphological evaluation and reliable identification of acid-fast bacterial species, offering important insights into their structural properties and diagnostic characteristics [21–22].

RESULTS AND DISCUSSION

The total bacterial population was observed from both Jamalpur and Sonatala soils, where successful isolation, purification, and characterization revealed considerable variability in bacterial colonies. The results demonstrated the presence of diverse bacterial communities in both soil samples. The colony count found 7.5×〖10〗^7 CFU/g soil and 7.4×〖10〗^7 CFU/g soil in Jamalpur and Sonatala soil respectively.

A total of seven distinct bacterial colonies were isolated from Jamalpur soil, while six distinct colonies were obtained from Sonatala soil. The colonies from Jamalpur soil exhibited size variations ranging from pinpoint to moderate, whereas those from Sonatala soil ranged from small to large, indicating differences in growth rates and adaptability of bacterial populations under different soil conditions.

In Jamalpur soil, the colonies displayed diverse forms, including circular, irregular, and rhizoid; margins were lobate, serrate, and entire; and elevations varied from flat, raised, to umbonate (Table 2). Pigmentation ranged from white, pink, to yellow, reflecting metabolic diversity among the isolates. Similarly, colonies from Sonatala soil were circular and irregular in form, with margins described as entire, lobate, or undulate, and elevations ranging from flat to raised (Table 4). Pigmentation was also variable, including white, pink, and yellow hues.

Morphological examination of Jamalpur soil isolates revealed a mixed population consisting of rod-shaped and round-shaped bacteria. These isolates comprised both Gram-positive and Gram-negative types, with several showing spore-forming ability,

particularly among rod-shaped bacteria, and some forming capsules, which likely contribute to their survival under adverse environmental conditions. All isolates were non-acid fast (Table 3). Similarly, bacterial isolates from Sonatala soil exhibited both rod and cocci forms, arranged singly or in chains. The isolates included Gram-positive and Gram-negative bacteria, with a subset displaying spore formation and capsule production (Table 5). As in Jamalpur soil, all isolates were non-acid fast.

Overall, these results indicate that both Jamalpur and Sonatala soils harbor diverse and heterogeneous bacterial communities with varied morphological and physiological traits. Such diversity is critical for maintaining soil health, supporting nutrient cycling, and ensuring overall ecosystem functioning.

The results of the present study were compared with those reported by Chowdhury et al. (2013) [23], who investigated bacterial populations in soils from Bangladesh. Our findings showed strong similarity to their observations, indicating that soils from various regions of Bangladesh are predominantly inhabited by Bacillus species. In addition to Bacillus, several other Gram-negative, spore-forming bacteria including Enterobacter spp., Klebsiella spp., and Azospirillum spp. were also identified [17]. Multiple studies on Bangladeshi soils have reported the widespread presence of diverse Bacillus species [24–27], most of which are capable of forming resilient spores. These spores enable Bacillus species to survive effectively in alkaline soils and complete their life cycles under favorable conditions. Because soil environments often offer limited opportunities for sporulating bacteria to complete their reproductive cycles, the formation of spores represents a highly effective survival strategy, enhancing their persistence and adaptability [28]. Moreover, the proliferation of new sporulating bacterial generations is supported by the breakdown of dead and decaying organic matter, which provides essential nutrients and facilitates the dispersal of spores throughout the soil. This process ensures the continued survival and maintenance of bacterial populations within their ecological niches [29].

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

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

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

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

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

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

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

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

CONCLUSION

The present study reveals that Jamalpur and Sonatala agricultural soils harbor diverse and heterogeneous bacterial communities, characterized by variation in colony size, form, margin, elevation, and pigmentation. Both Gram-positive and Gram-negative bacteria were identified, including rod-shaped and cocci forms with different spatial arrangements. Several isolates exhibited spore-forming and capsule-forming abilities, indicating adaptive strategies for survival under challenging environmental conditions, while all isolates were non-acid fast. The observed morphological and physiological diversity underscores the active and resilient nature of soil microbial populations, which play vital roles in nutrient cycling, organic matter decomposition, and the maintenance of soil fertility. These findings highlight the ecological significance of soil bacteria in sustaining agricultural productivity and environmental stability, providing a foundation for further studies aimed at their functional characterization and potential applications in sustainable agriculture.

REFERENCES

1. Stevenson, F.J., Cycles of soil: 1986, A Wiley Interscience Publication John Wiley and Sons, New York.

2. Brady, N.C. and Weil, R.R., The Nature and Properties of Soils: 2002, 13th Edition, Pearson Education (Singapore) Pte. Ltd., Indian Branch, 482 F.I.E. Patparganj, India.

3. Joklik, W.K.; Willett, H.P.; Amos, D.B. and Wilfert, C.M., Zinsser Mirobiology:1992, 20th Edition, Appleton and Lange, Norwalk, California.

4. Alexander, M., Introduction to Soil Microbiology: 1997, 2nd Edition, Sons, New York.

5. Ruangpan, L., &Tendencia, E. A. (2004). Bacterial isolation, identification and storage. In *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment* (pp. 3–11). Aquaculture Department, Southeast Asian Fisheries Development Center.

6. Salle, A. J. (1974). *Fundamental principles of bacteriology* (7th ed.). Tata McGraw-Hill Publishing Co. Ltd.

7. Mishra, R. R. (2000). *Soil microbiology* (1st ed.). CBS Publishers & Distributors.

8. Chowdhury, M. H. (2020). Isolation and characterizationof bacteria of Amjhupi and Ishurdi soil series of Bangladesh. *International Journal of Applied Chemical and Biological Sciences,* 1(4),33-39.

9. Chowdhury, M. H. (2020). Effect of heavy metal onbacterial population and diversity of Gopalpur soilseries. *International Journal of Applied Chemical and Biological Sciences*, 1(4), 20–24.

10. Marzan, L. W., Hossain, M., Mina, S. A., Akter, Y., & Chowdhury, A. M. A. (2017). Isolation and biochemical characterization of heavy metal–resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. *The Egyptian Journal of Aquatic Research, 43*(1), 65–74.

11. Salam, M. A., Hossain, M. S., Ali, M. E., Asad, M. A., & Ali, M. H. (2009). Isolation and characterization of arsenic-resistant bacteria from different environments in the south-west region of Bangladesh. *Research Journal of Environmental Sciences, 3*(1), 110–115.

12. Chowdhury, M. H. (2022). Bacterial population andcharacterization of Sonatala and Bajoa soil series of Bangladesh. *International Journal of Applied Chemical and Biological Sciences*, 3(2), 44–49.

13. Kabir, M. R., Hasan, S. M. K., Chy, M. A. H.,Miah, M. S., Billah, S. M., & Chowdhury, M. H.(2025). Assessment of soil bacterial population and characterization of Akdala and Nachol seriesof Bangladesh. *GRS J Mul Res Stud*, 2(7), 1–5.

14. Chowdhury, R., Dhar, K., Ahamed, F., Alam, S., & Uddin, M. (2017). Biodegradation of anthracene and phenanthrene by bacteria isolated from oil-contaminated soil of Bangladesh. *Chemistry and Ecology, 33*(9), 843–855.

15. Mahmud, M. K., Rahman, M. M., Chy, M. A. H.,Kabir, M. R., Nazmul, K. M., Akhter, M. K., & Chowdhury, M. H. (2025). Isolation and characterization of soil bacteria: A review. *International Journal of Modern Science and Research Technology*, 3(10), 22–33.

16. Hossain, M. A., Ahmed, S., &Hoque, S. (1997). Abundance and distribution of *Bacillus thuringiensis* in the agricultural soil of Bangladesh. *Journal of Invertebrate Pathology, 70*(3), 221–225.

17. Khan, M., & Rashid, H. (2008). Enumeration, isolation and identification of nitrogen-fixing bacterial strains at seedling stage in rhizosphere of rice grown in non-calcareous grey flood plain soil of Bangladesh. *Okayama University Scientific Achievement Repository, 13*(1), 97–101.

18. Bhuiya, Z. H., & Walker, N. (1977). Autotrophic nitrifying bacteria in acid tea soils from Bangladesh and Sri Lanka. *Journal of Applied Bacteriology, 42*(2), 253–257.

19. Dubey, R. C., &Maheshwari, D. K. (1999). *A text book of microbiology*. S. Chand & Co. Ltd.

20. Prescott, L. M., & Harley, J. P. (2002). *Laboratory exercises in microbiology* (5th ed.). McGraw-Hill.

21. Cappuccino, J. G., & Sherman, N. (1999). *Microbiology: A laboratory manual* (4th ed.). Benjamin/Cummings Publishing.

22. Shaha, S.C.,Microbiology: 2003, 1st Edition, Kobir Publications, 38/3 Banglabazar, Dhaka.

23. Chowdhury, M. H., Kibria, K. Q., Tisha, S. S., & Islam, M. S. (2013). Isolation and characterization of bacteria of Sara and Mirpur soil series of Bangladesh. *Khulna University Studies.* 11 (1&2) and 12(1&2): 36-41

24. Fakruddin, M. D., Sarker, N., Ahmed, M. M., & Noor, R. (2012). Protein profiling of Bacillus thuringiensis isolated from agro-forest soil in Bangladesh. J. Mol. Biol. Biotechnol, 20, 139-145.

25. Ahsan, M. M., Khan, M. F. R., Rahman, M. B., Chowdhury, S. M. Z. H., Parvej, M. S., Jahan, M., & Nazir, K. N. H. (2013). Investigation into Bacillus anthracis spore in soil and analysis of environmental parameters related to repeated anthrax outbreak in Sirajganj, Bangladesh. The Thai Journal of Veterinary Medicine, 43(3), 449-454.

26. Shishir, A., Roy, A., Islam, N., Rahman, A., Khan, S. N., & Hoq, M. (2014). Abundance and diversity of Bacillus thuringiensis in Bangladesh and their cry genes profile. Frontiers in Environmental Science, 2, 20.

27. Shishir, A., Akter, A., Hassan, M. H., Kibria, G., Ilias, M., Khan, S. N., & Hoq, M. M. (2012). Characterization of locally isolated Bacillus thuringiensis for the development of eco friendly biopesticides in Bangladesh. Journal of Biopesticides, 5, 216.

28. Fasanella A. Bacillus anthracis, virulence factors, PCR and interpretation of results. Virulence. 2015; 4(8): 659–660.

29. Rume, F. I., Affuso, A., Serrecchia, L., Rondinone, V., Manzulli, V., Campese, E., ... & Fasanella, A. (2016). Genotype analysis of Bacillus anthracis strains circulating Bangladesh. PLoS One, 11(4), e0153548.