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Bacterial Population, Diversity and Characterization of Balagonj and Goainghat Soil Series of Moulvibazar

Shah Mohammad Kamrul Hasan[1], Md. Rayhan Kabir[2], Md. Kayes Mahmud[3], Mehedi Hasan[4], Mahmudul Hasan Chowdhury[5*]

[1,2,3,4,5*]Scientific Officer, Soil Resource Development Institute, Ministry of Agriculture, Bangladesh.

*** Correspondence:** Mahmudul Hasan Chowdhury

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ABSTRACT: Soil bacterial communities play a pivotal role in maintaining ecosystem functions, including nutrient cycling, organic matter decomposition, and soil fertility. The present study investigated the bacterial populations in agricultural soils from Balagonj and Goainghat, Bangladesh, through isolation, enumeration, and morphological characterization. Total viable counts revealed bacterial populations of 6.5×10^6 CFU/g soil in Balagonj and 5.4×10^6 CFU/g soil in Goainghat. A total of six morphologically distinct bacterial colonies were isolated from each site, exhibiting variation in colony size, shape, margin, elevation, and pigmentation. Morphological examination indicated the presence of both Gram-positive and Gram-negative bacteria, comprising rod-shaped and cocci forms arranged singly or in chains. Several isolates displayed spore-forming and capsule-forming capabilities, while acid-fast and non-acid-fast bacteria were also observed. The observed phenotypic diversity highlights the adaptability and ecological versatility of soil bacteria, reflecting their crucial role in sustaining soil health and fertility. These findings provide a foundation for further functional studies and potential applications in sustainable agriculture.

Keywords: *Soil bacteria, Diversity, Gram stain, Spore, Capsule, Acid fast bacteria*

INTRODUCTION

Bacteria are unicellular, prokaryotic organisms considered among the most ancient and evolutionary persistent forms of life on Earth. Their long evolutionary development has endowed them with remarkable adaptive abilities, enabling survival, growth, and colonization across a broad spectrum of environments, ranging from normal habitats to extreme ecological conditions. Structurally, bacterial cells are simpler than eukaryotic cells and generally measure between 0.5 and 2.0 μm in diameter. Despite this structural simplicity, bacteria display diverse morphological types, commonly grouped into cocci (spherical), bacilli (rod-shaped), and spiral forms. These microorganisms may exist as individual cells or organize into colonies, with such arrangements influenced by environmental conditions, nutrient availability, and cellular interactions [1]. Bacteria perform vital ecological functions in both terrestrial and aquatic systems. They play a key role in ecosystem productivity by decomposing organic matter and recycling essential nutrients such as carbon, nitrogen, and phosphorus [2]. Among all microorganisms, bacteria are the most widely distributed and ecologically adaptable, inhabiting diverse environments across the globe. However, their limited morphological variation creates challenges in accurate identification and classification. Therefore, relying solely on morphology is often insufficient, and comprehensive classification requires the integration of culture-based techniques with advanced analytical methods. Such approaches improve the understanding of bacterial diversity, ecological roles, physiological traits, and environmental interactions across different ecosystems [3]. Bacteria are ubiquitous in nature, occurring in nearly all environments, including soil, freshwater, marine systems, and even the atmosphere, as well as extreme habitats such as highly saline, acidic, and high-temperature regions. Their metabolic activities are essential for maintaining ecological balance and regulating biogeochemical cycles. In well-aerated soils, bacteria often interact synergistically with fungi to enhance microbial activity and nutrient cycling. In contrast, under anaerobic or oxygen-limited conditions, bacteria tend to dominate and are responsible for most biochemical transformations within the soil environment [4]. The study of bacteria generally begins with their isolation, purification, and identification from environmental

samples. Isolation techniques are used to recover bacterial populations, followed by purification processes to eliminate contaminants and obtain pure cultures [5]. These pure cultures are crucial for detailed studies of bacterial morphology, physiology, biochemical properties, and antimicrobial responses. Both selective and non-selective media are commonly used for this purpose. Standard microbiological methods, such as streak plate and pour plate techniques on solid media, are widely applied to obtain distinct bacterial colonies [6–7]. Previous studies have reported that soils in different regions of Bangladesh are often affected by heavy metal contamination, which favors the development of metal-tolerant or resistant bacterial populations. These bacteria exhibit strong adaptability under stressful environmental conditions and play important ecological roles in contaminated soils [8–11]. Native soil bacteria in Bangladesh have been shown to contribute beneficially by solubilizing phosphate, detoxifying pollutants, and regulating mineral cycles within the soil ecosystem [12–14]. Furthermore, these microbial communities are actively involved in key biochemical processes such as nitrification, which is essential for maintaining soil fertility and nutrient availability [15–17]. They also assist in the remediation of contaminated soils, suppression of pathogenic microorganisms, and stabilization of acidic soil conditions. Therefore, microbiological studies of soil ecosystems are crucial for both scientific understanding and practical applications in agriculture and environmental management. A systematic and stepwise approach to identifying soil bacterial populations is essential for understanding the complexity, dynamics, and functional diversity of soil microbial communities [13,18].

In this context, the present study aims to isolate soil bacteria, quantify colony-forming units, and evaluate colony characteristics in detail. Additionally, important morphological features, including cell shape, arrangement, and staining properties, were analyzed to better understand microbial abundance, diversity, and ecological significance in the studied soil samples.

MATERIALS AND METHODS

Sample collection

Two recently collected topsoil samples (Table 1) were aseptically obtained from agricultural lands in Bangladesh. Sampling was conducted specifically from the

upper soil layer at a depth of 0–15 cm, which is widely known for its high nutrient content and dense microbial population. Strict aseptic procedures were maintained throughout the sampling process to avoid external contamination and to preserve the native microbial communities present in the soil. Immediately after collection, the samples were transported to the laboratory in sterile thermos containers to maintain a stable temperature and reduce any potential alterations in microbial composition during transit. Upon arrival, the samples were stored under controlled laboratory conditions until further microbiological and physicochemical analyses were performed. This careful and systematic approach to sample collection, transportation, and storage ensured the accuracy, consistency, and reproducibility of the experimental findings derived from these soil samples.

Sample no.	**Series**	**Location**	**AEZ**	**Physiographic unit**	**Cropping pattern**
1	Balagonj	Akbarpur Moulvibazar	29	Northern and Eastern Piedmont Plain	Aus-T. Amam
2	Goainghat	Akbarpur Moulvibazar	22	Northern and Eastern Piedmont Plain	Citrus Fruits

Isolation of Bacteria

Bacterial isolation was carried out in accordance with standard microbiological procedures reported in earlier studies. Initially, a known quantity of soil was thoroughly mixed with a physiological saline solution (distilled water supplemented with 0.9% NaCl) to obtain a homogeneous microbial suspension. This suspension served as the primary source for subsequent analyses. The prepared soil suspension was then subjected to serial dilution in a stepwise manner to decrease microbial load, thereby facilitating the isolation of individual bacterial colonies. Appropriate dilution aliquots were aseptically inoculated onto sterile, pre-labeled Petri plates and uniformly spread over nutrient agar surfaces using the spread plate method. The inoculated plates were incubated at 37°C for 24–48 hours to support optimal bacterial growth and the development of discrete colonies. After incubation, well-

isolated and morphologically distinct colonies were carefully selected and purified through repeated streaking on fresh agar media to obtain pure (axenic) cultures. This purification step was performed multiple times to eliminate mixed populations and ensure culture uniformity and reliability. All experimental procedures were conducted in triplicate to enhance the precision and reproducibility of the results. Finally, the purified isolates were incubated again at 37°C for an additional 24–48 hours to confirm purity, ensure consistent growth, and verify their suitability for further microbiological characterization [19–20].

Viable count

The viable bacterial population present in the soil samples was determined using the standard plate count technique. For accurate and reliable estimation, only those agar plates containing 25–250 well-separated colonies were selected for enumeration. The number of viable bacteria per gram of soil was subsequently calculated by applying a standard formula that incorporates both the dilution factor and the volume of inoculum plated onto the agar surface:

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 cultured on nutrient agar plates. Detailed observations were made based on several important parameters, including colony size, color (pigmentation), shape, margin, and elevation (Figure 1). The analysis was conducted in a consistent and structured manner following the standard descriptive criteria outlined by Dubey and Maheshwari (1998) [19]. Such comprehensive morphological evaluation provides important information regarding the phenotypic variability of soil bacterial communities and serves as a fundamental basis for their identification, classification, and further comparative studies.

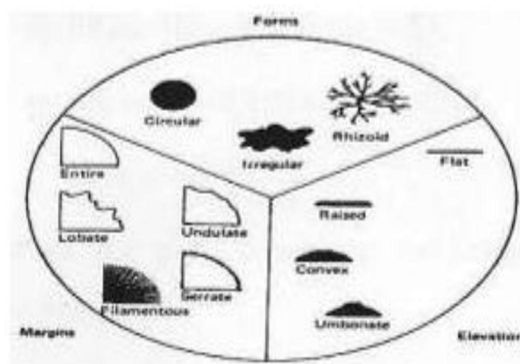

Figure 1. Colony characteristics of bacteria [21].

Staining characteristics

The cellular morphology and arrangement of the bacterial isolates were systematically investigated using a range of standard staining techniques, including simple staining, negative staining, Gram staining, capsule staining, spore staining, and acid-fast staining. Each staining method contributed specific and complementary information, enabling detailed observation of cell shape, structural features, and spatial organization. These analyses are crucial for accurate phenotypic identification, dependable taxonomic classification, and a deeper understanding of the structural diversity present within soil bacterial communities [21–22].

Simple staining

For detailed observation of bacterial morphology, thin smears were prepared on clean, sterile glass slides and subjected to heat fixation to immobilize the cells and maintain their structural integrity. The fixed smears were then stained with crystal violet, which was allowed to act for approximately 40–60 seconds to ensure proper uptake by the bacterial cells. Excess stain was gently washed off with tap water, and the slides were allowed to air-dry. Subsequently, the prepared smears were examined under a high-power microscope using oil immersion, enabling clear visualization of cellular morphology, arrangement, and other structural characteristics. This method provides essential information for accurate phenotypic characterization and preliminary identification of bacterial isolates, serving as a fundamental step in microbiological analysis [21–22].

Negative staining

Negative staining was performed using a clean, dry glass slide by placing a small drop of nigrosin stain near one end. A loopful of bacterial culture was gently mixed with the stain, and the mixture was spread across the slide with the help of another slide held at an angle of approximately 30°, producing a thin and even smears. The prepared smear was allowed to air-dry without applying heat. The slide was then examined under a microscope using oil immersion, allowing clear observation of bacterial morphology, including cell shape, size, and arrangement. Since this method avoids heat fixation, it reduces the risk of structural distortion and preserves the natural dimensions and appearance of the cells. Consequently, negative staining provides precise and dependable information for phenotypic characterization, aiding in the accurate identification and classification of bacterial isolates [21–22].

Gram stain

For Gram staining, bacterial smears were prepared on sterile glass slides and heat-fixed to secure the cells while maintaining their structural features. The fixed smears were initially stained with crystal violet for about one minute to ensure adequate penetration of the primary stain, followed by gentle rinsing with tap water to remove excess dye. Subsequently, Gram's iodine was applied for one minute, acting as a mordant to form a stable crystal violet–iodine complex within the cells, and the slides were again rinsed to eliminate excess iodine. Decolorization was then carried out using 95% ethanol applied dropwise until the runoff became clear, after which the slides were immediately rinsed with water to halt the process. The smears were then counterstained with safranin for approximately 45 seconds, followed by a final rinse and air drying. The stained slides were observed under oil immersion microscopy, allowing clear differentiation between Gram-positive and Gram-negative bacteria, as well as detailed visualization of cellular morphology, size, and arrangement. This technique provides dependable phenotypic information and is essential for accurate taxonomic identification of bacterial isolates [21–22].

Capsule stain

For the visualization of bacterial capsules, smears were prepared on sterile glass slides and air-dried without applying heat to preserve the native structure of both the

cells and their surrounding capsules. Crystal violet was then applied to the dried smears and allowed to act for 5–7 minutes to ensure adequate staining of the bacterial cells. Excess stain was carefully removed using a 20% copper sulfate solution, which functions simultaneously as a decolorizer and a background counterstain. The slides were air-dried and examined under oil immersion microscopy, enabling clear visualization of bacterial cells and their capsules, which appeared as distinct, unstained halos surrounding the stained cells. This technique is essential for the accurate identification of encapsulated bacterial strains and provides valuable information on their structural and phenotypic characteristics [21–22].

Spore stain

For the observation of bacterial endospores, smears were prepared on sterile glass slides and heat-fixed to immobilize the cells while maintaining 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 ensure effective penetration of the dye into the highly resistant spore structures. After cooling, the slides were rinsed thoroughly with tap water to remove excess stain. The smears were then counterstained with safranin for approximately 30 seconds, followed by a gentle rinse with water to provide contrast between the vegetative cells and the endospores. After air drying, the slides were examined under oil immersion microscopy, which allowed clear visualization of endospores as green-stained structures within the red-stained vegetative cells. This staining method is critical for the accurate morphological characterization and identification of spore-forming bacteria and provides important insights into their structural adaptations and survival strategies [21–22].

Acid fast stain

For the detection and characterization of acid-fast bacteria, smears were carefully prepared on sterile glass slides and heat-fixed to immobilize the cells while preserving their native structures. The fixed smears were stained with carbolfuchsin and placed on a warm hot plate for five minutes, facilitating penetration of the dye through the thick, lipid-rich cell walls typical of acid-fast organisms. After heating, the slides were cooled and gently rinsed with tap water to remove excess stain.

Decolorization was performed by applying acid-alcohol drop wise until no further dye was released, followed by a water rinse to remove residual decolorizer. The smears were then counterstained with methylene blue for about two minutes, rinsed gently, and air-dried. This procedure provided a clear contrast between acid-fast and non-acid-fast cells. Observation under oil immersion microscopy allowed definitive differentiation, with acid-fast bacteria appearing red due to carbolfuchsin retention, while non-acid-fast cells were stained blue by methylene blue. This staining method is critical for accurate morphological assessment and reliable identification of acid-fast bacteria, providing valuable information on their structural features and diagnostic characteristics [21–22].

RESULTS AND DISCUSSION

The total bacterial population was observed from both Balagonj and Goainghat 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 6.5×10^{6} CFU/g soil and 5.4×10^{6} CFU/g soil in Balagonj and Goainghat soil respectively.

A total of six distinct bacterial colonies were isolated from Balagonj soil, while six colonies were obtained from Goainghat soil. The colonies from Balagonj soil exhibited sizes ranging from pinpoint to moderate, whereas those from Goainghat soil varied from small to large, suggesting differences in growth rates and adaptability of bacterial populations under the respective soil conditions.

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

Morphological examination of Balagonj soil isolates revealed a mixed population of rod-shaped and round-shaped bacteria. The isolates included both Gram-positive and Gram-negative types, with several showing spore formation and capsule production, features likely contributing to their survival under environmental stresses. Acid-fast staining indicated that some isolates were acid-fast, while others were non-acid fast (Table 3). Similarly, bacterial isolates from Goainghat soil included both rod and cocci forms, arranged singly or in chains. These isolates also comprised Gram-positive and Gram-negative bacteria, with spore formation and capsule production observed in certain colonies (Table 5). Acid-fast staining showed the presence of acid-fast as well as non-acid-fast isolates.

Overall, these results indicate that both Balagonj and Goainghat soils harbor diverse bacterial communities with heterogeneous morphological and physiological traits. The observed variation in colony size, shape, pigmentation, arrangement, and staining characteristics highlights the complexity of soil bacterial populations and underscores their adaptability to local environmental conditions. Such diversity is essential for maintaining soil health, enhancing nutrient cycling, and supporting ecosystem functions. Furthermore, the presence of spore-forming and encapsulated bacteria suggests effective survival strategies that enable these microbial populations to persist under adverse conditions, while the detection of both Gram-positive and Gram-negative, as well as acid-fast and non-acid-fast bacteria, reflects the broad ecological versatility of soil microorganisms.

The findings of the present study were compared with those reported by Chowdhury et al. (2013) [23], who examined bacterial populations in soils from Bangladesh. Our results closely align with their observations, indicating that Bacillus species predominantly inhabit soils across various regions of the country. In addition to Bacillus, several other Gram-negative, spore-forming bacteria, such as *Enterobacter* spp., *Klebsiella* spp., and *Azospirillum* spp., were also detected [17]. Multiple investigations of Bangladeshi soils have consistently reported the widespread occurrence of diverse Bacillus species [24–27], most of which produce resilient spores. These spores enable Bacillus to survive effectively in alkaline soils and to complete their life cycles when environmental conditions become favorable. Given

that soil environments often limit opportunities for sporulating bacteria to complete their reproductive cycles, spore formation serves as a highly efficient survival mechanism, enhancing both persistence and adaptability [28]. Furthermore, the emergence of new generations of sporulating bacteria is supported by the decomposition of dead and decaying organic matter, which supplies essential nutrients and facilitates spore dispersal throughout the soil. This process ensures the long-term survival and maintenance of bacterial populations within their ecological niches [29].

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

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

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

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

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

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

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

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

CONCLUSION

The present study demonstrates that Balagonj and Goainghat agricultural soils support diverse and heterogeneous bacterial communities, exhibiting notable variation in colony size, form, margin, elevation, and pigmentation. Both Gram-positive and Gram-negative bacteria were identified, comprising rod-shaped and cocci forms arranged singly or in chains. Several isolates displayed spore-forming and capsule-forming abilities, reflecting adaptive strategies for survival under

environmental stresses, while acid-fast and non-acid-fast bacteria were also observed, indicating structural and physiological diversity. The phenotypic heterogeneity of these bacterial populations underscores their active role in maintaining soil health, facilitating nutrient cycling, promoting organic matter decomposition, and sustaining overall soil fertility. These findings emphasize the ecological importance of soil bacteria in supporting agricultural productivity and ecosystem stability, providing a strong basis for future investigations into their functional roles and potential applications in sustainable soil management.

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.