International Journal of Applied Chemical and Biological Sciences (2025), 6(1), 1-6
ISSN- 2582-788X

RESEARCH PAPER

Article Identifier: https://identifier.visnav.in/1.0001/ijacbs-24l-25002/

Isolation and Characterization of Bacteria of Amnura and Baliadangi soil series of Bangladesh

Md. Kayes Mahmud, Md. Abul Kalam Azad, Abdul Awal, Shammi Akhter Tina, Joyenta Das, Manna Salwa, Mahmudul Hasan Chowdhury *

Soil Resource Development Institute, Ministry of Agriculture, Bangladesh

* For correspondence: lipuchowdhury@gmail.com

Received on: 25 December 2024 *Published on: 26 January 2025*

ABSTRACT

Bacteria, single celled and ancient form of life, are living in any condition. Their isolation and identification are important for their classification. This study was for isolation and characterization of bacteria from Amnura and Baliadangi soil series. Isolated colonies of bacteria were evaluated in size, pigmentation, form, margin and elevation. It has been observed that Baliadangi soil was found to have more bacterial population than that of Amnura soil. From simple and negative staining, shape and arrangement were determined. Majority of the bacteria were Gram- negative, spore forming and capsuled. The presence of rod shaped bacteria (*Bacillus*) compared to round shaped (*Coccus*) in soils is a finding.

Keywords: Soil series, bacteria, isolation, characterization.

1. INTRODUCTION

Bacteria are not only single celled and prokaryotic but most successful, extreme living form of ancient life. Their morphologies are very simple as diameter is only 0.5 to 2.0 micrometers (mm) having shapes of spherical, bacillus and spiral. They are found in either individual cells or aggregated together called colonies [1]. The bacteria in ecosystem, land and water show significant role in productivity [2]. Bacteria are widely distributed which leads to difficulty in its classification and identification. It is important in order to classify bacteria for understanding and identification. The classification helps find roles and nature of bacteria in t environment [3]. Bacteria are available in soil, water, air and even on the extremist place of earth. The bacteria and fungi together dominate the adequate aerated soils [4].With a view to conducting bacteriological studies the first step isolation. Isolation is to obtain the cultures from any defined area that is followed by its purification [5]. To get pure bacterial culture from the isolated culture, various media were needed. The pure culture is needed for the morphology, physiology, biochemical characteristics, and susceptibility studies. There are various methods like solid media, streak plate or pour

plate to obtain pure cultures [6]. The soil from many regions of Bangladesh is affected by various heavy metals which lead to the resistant bacteria which make them extreme surviving [7]. The presence some bacteria in soil of Bangladesh is reported to be helpful in the mineral cycle of environment [8], nitrification of soil [9]. Their presence helps the soil in healing from heavy metal contamination, removes pathogenic microbes and maintains the acidic soils [10]. The study aimed at isolation of soil bacteria, estimation of colony, determination of colony and morphological characteristics (shape, arrangement, and staining characteristics) to find the microbial abundance.

2. MATERIALS AND METHODS

2.1. Sample collection

Fresh top soil samples (0-15 cm) were collected from the fields of Amnura (N-26004'745'', E-88030'707'') of Atwari, Panchgarh and Baliadangi (N-26013'347'', E-88026'020'') of Sadar, Thakurgaon in Bangladesh and taken aseptically into laboratory using thermo flask and kept for further study.

2.2. Isolation of Bacteria

The isolation of bacteria was performed by referring to various standard methods [11]. The sample was prepared by mixing soil and physiological water (DW+0.9% NaCl). Serial dilution of sample was prepared and was streaked in different labeled petri plates by spread plate technique. The plates were incubated at37°C for 24-48 hrs. After incubation the obtained culture was picked selectively for purification of culture by streak plate technique. The process was performed in triplicates. The plate was incubated at 37°C for 24-48 hrs.

2.3. Viable count

Viable Count of bacteria was calculated by colony count method. The plates with 25 to 250 colonies were selected for counting and calculated by following equation:

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

2.4. Characterization

The bacterial colony characteristics and morphological from both location characteristics were determined by evaluating the well-isolated colonies of nutrient agar plates. The size, pigmentation, form, margin and elevation were observed as described by Dubey and Maheshwari (1998) [12].

2.5. Staining characteristics

The shape and arrangement of bacteria were determined by simple and negative staining, gram stain, capsule stain, spore stain and acid fast stain [13].

2.5.1. Simple staining

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The crystal violate was poured on smear left for 40 to 60seconds. The smear then washed with normal water to remove excess stain. After washing the slide was dried and was examined under oil immersion.

2.5.2. Negative staining

On a clean dry glass slide, a drop of nigrosin dye was placed at one end. A loop full of bacterial inoculums was placed and mixed with the drop of nigrosin. The mixture was spread with the edge of a second slide held at a 300 angle. It was placed in front of bacterial suspension to prepare a thin smear. The smear was air dried and the slide was examined under oil immersion.

2.5.3. Gram stain

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The crystal violate was poured on smear left for 1 minutes. The smear then washed with normal water to remove excess stain. Gram's iodine was added to the smear slide and kept for 1 minute and washed again. Ethyl alcohol (95%) was added to the smear in drop wise manner till crystal violate failed to wash. Again the smear was washed with tap water. The smear was counterstained with safranin for about 45 seconds and washed again. The slide was air dried, and was examined under oil immersion.

2.5.4. Capsule stain

The bacterial smear was prepared on the glass slide and air dried. The crystal violate was poured on smear and kept for 5 to 7 minutes. The smear was washed with 20% copper sulfate solution. The smear slide was air dried and the slide was examined under oil immersion.

2.5.5. Spore stain

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The malachite green was poured on the smear and placed on a warm hot plate for 2 to 3 minutes. The slide was removed from hot plate, cooled and washed with tap water. The safranin was poured on the smear and left for 30 seconds followed by washing with water. The smear slide was air dried and the slide was examined under oil immersion.

2.5.6. Acid fast stain

The bacterial smear was prepared on the glass slide and followed by heat fixed method. The carbol fuchsin was poured on the smear and placed on a warm hot plate, allowing the preparation for 5minutes. The slide was removed from hot plate cooled and washed with tap water. Acid alcohol was added on the slide in drop wise manner till carbol fuchsin removes and followed by washing with water. The smear was counterstained with methylene blue for 2 minutes followed by washing. The smear slide was air dried and the slide was examined under oil immersion.

2.6. Data Analysis

The observation for all the tests were made and recorded for further work.

3. RESULTS AND DISCUSSION

The total bacterial count was from both Amnura and Baliadangi soil series of Bangladesh. The successful isolation, purification and characterization were determined. Results show variables in bacterial colonies in soil sample. Seven distinct types of colorful bacterial colonies were found from Amnura soil and nine distinct types of colorful bacterial colonies from Baliadangi soil. The bacteria from Amnura soil were moderate, small and pinpoint in size whereas the bacteria from

Table 1. Colony characteristics of isolated bacteria of Amnura soil.

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

Baliadangi soil were in different sizes of small, moderate, pinpoint and large. The colonies from Amnura soil were irregular, circular and rhizoid in form; serrate, entire and lobate in margin; and flat, raised and umbonate in elevation (Table 1). The colonies from Baliadangi soil were irregular and circular, undulate, entire and lobate in margin and flat to raised in elevation (Table 3).

Bacteria from both the soils were variated in colour from white, pink and yellow. The morphological characteristics of isolated bacteria from Amnura soil were observed as mixed morphological type of colonies but were majorly dominated by rod shaped, spore forming, gram-nagative and non-acid fast bacteria (Table 2). The morphological characteristics of isolated bacteria from Baliadangi soil were observed as mixed colonies of rod and round shaped, gram positive and gram negative bacteria. Spore forming, non-acid fast and capsulated bacteria are in major positions (Table 4). The result reveled that Bacillus was majorly found in both the soil with the colony count of 8.1× 〖10〗 ^7CFU/g and 9.4× 〖10〗 ^7 CFU/g soil in Amnura soil and Baliadangi soil respectively. The result was compared with the previous study conducted by Chowdhury et al., (2013)[14] from Bangladesh soil. Our reports were very much similar to them. Many reports from Bangladesh soil showed abundant presence of different Bacillus Sp. which are mostly spore forming [15].

4. CONCLUSION

Very limited works done on isolation and identification of soil microbes from Bangladesh soil we have. But there is importance to get proper accountability of various forms microbes present in different soils. The soils in Bangladesh are of variant types, having various purposes, majorly agricultural. The relevant bacteria have important roles in its proper functioning and its exploration is much more important.

Table 2. Morphological characteristics of isolated bacteria of Amnura soil.

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

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

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

Table 4. Morphological characteristics of isolated bacteria of Baliadangi soil.

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

5. ACKNOWLEDGEMENT

NA

6. CONFLICT OF INTEREST

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

7. SOURCE/S OF FUNDING

NA

8. 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 andProperties 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, *John Wiley and 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., Fundamental Principles of Bacteriology: 1974, 7th Edition, *Tata McGraw-Hill Publishing Co. Ltd.,* New Delhi.
7. Sanyal, S. K., Mou, T. J., Chakrabarty, R. P., Hoque, S., Hossain, M. A., & Sultana, M. (2016). Diversity of arsenite oxidase gene and arsenotrophic bacteria in arsenic affected Bangladesh soils. *AMB Express*, 6(1), 1-11.
8. Islam, M. T., Deora, A., Hashidoko, Y., Rahman, A., Ito, T., & Tahara, S. (2007). Isolation and identification of potential phosphate solubilizing bacteria from the rhizoplane of Oryza sativa L. cv. BR29 of Bangladesh. *Zeitschrift für Naturforschung C*, 62(1-2), 103-110.
9. Nahar, K., Ali, M. M., Khanom, A., Alam, M. K., Azad, M. A. K., & Rahman, M. M. (2020).Levels of heavy metal concentrations and their effect on net nitrification rates and nitrifying archaea/bacteria in paddy soils of Bangladesh. *Applied Soil Ecology*, 156, 103697.
10. Sarkar, A., Islam, T., Biswas, G., Alam, S., Hossain, M., & Talukder, N. (2012). Screening for phosphate solubilizing bacteria inhabiting the rhizoplane of rice grown in acidic soil in Bangladesh. *Acta*

microbiologica et. Immunological Hungarica, 59(2), 199-213.

11. Prescott, L.M. and Harley, J.P., Laboratory Exercises in Microbiology, 2002, 5th Edition, *McGraw-Hill Inc*., New York.
12. Dubey, R.C. and Maheshwari, D.K., A Text Book of Microbiology: 1999, *S. Chand and Co. Ltd.,* Ramnagar, New Delhi.
13. Shaha, S.C.,Microbiology: 2003, 1st Edition, *Kobir Publications*, 38/3 Banglabazar, Dhaka.
14. 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.
15. 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.