ISSN 2582-788X RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF
APPLIED CHEMICAL AND BIOLOGICAL SCIENCES

Isolation and characterization of bacteria of Amjhupi and Ishurdi soil series of Bangladesh

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ABSTRACT

Bacteria are single celled, ancient life form, omnipresent and living in all conditions. Its isolation and identification from different resources is very much important for its classification and knowledge of its pathogenicity. In soils the bacteria and fungi dominated by its presences and account for almost all the biological and chemical changes in environments. In this study, bacteria from Amjhupi and Ishurdi, Bangladesh soil series were isolated and characterized. Well-isolated colonies of bacteria were evaluated in size, pigmentation, form, margin and elevation. It has been observed that Ishurdi soil was found to have more bacterial population than that of Amjhupi soil. From simple and negative staining, shape and arrangement were determined. The ratio of Gram- positive bacteria to Gram- negative bacteria was evaluated for both soils. Majority of the bacteria were spore and capsule former. The bacteria were non-acid fast and were arranged in chain form in both soils. The presence of rod shaped bacteria (bacillus) compared to round shaped (coccus) in soils is conspicuous.

Keywords: Soil series, bacteria, colony characteristics, isolation.

1. INTRODUCTION

Bacteria are single celled, prokaryotic, most successful, extreme living form of ancient life. The bacterial morphology is very simple as their diameter is only 0.5 to 2.0 micrometers (mm) with basic shapes of spherical, bacillus and spiral. They can be found in either individual cells or aggregated together as clumps called colonies [1]. The bacteria both in ecosystem, land and water have a significant role in productivity and as decomposers [2]. Among all the microorganisms, bacteria's are widely distributed with simple in morphology which leads to difficulty in its classification and identification. It is important to classify bacteria for its better understanding and for this the identification from microbial culture is very important. The classification helps in finding the different roles and nature of various bacteria in the existing environment [3]. Bacteria are found in soil, water, air and even on the extremist place of earth contributing to its functioning and existence. The bacteria and fungi together dominate the adequate aerated soils, whereas in scanty oxygen soils the bacteria alone account for almost all the chemical and biological changes [4].

To conduct any bacteriological studies the first step is to isolate, purify and identify the bacteria. Isolation is done to obtain the cultures from any defined area which is followed by its purification [5]. To obtain pure bacterial culture from the isolated culture various media were used. The pure culture is necessary 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, 7].

The soil from many regions of Bangladesh is heavily affected by various heavy metals which lead to these metal resistant bacteria which make them extreme surviving [8-11]. The presence some bacteria in soil of Bangladesh is reported to be helpful in phosphate solubilizing, decontamination removal and maintaining the mineral cycle of environment [12-14], nitrification of soil [15-17]. Their presence also helps the soil in healing from heavy metal contamination, removes pathogenic microbes and maintains the acidic soils [13, 18].

The soil research needed for the development of knowledge and it helps in resolving soil related problems. Series wise identification of soil bacteria will go a long way to understand the dynamic soil environment. The study aimed at isolation of soil bacteria, estimation of colony, determination of colony and morphological characteristics (shape, arrangement, 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 Amjhupi (N-23017.778', E-89016.963') and Ishurdi (N-23013.323', E-89013.753') of Jashore district 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 [19-20]. 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 petriplates by spread plate technique. The plates were incubated at 37°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, R.C. and Maheshwari, D.K (1998) [19].

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 [21-22].

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 60 seconds. 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 loopful of bacterial inoculum 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 5 minutes. 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.

3. RESULTS AND DISCUSSION

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

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

The total bacterial count was observed from both Amjhupi and Ishurdi soil. The successful isolation, purification and characterization were evaluated. The results show variables in bacterial colonies in soil sample. About five distinct types of colorful bacterial colonies were found from Amjhupi soil and five distinct types of colorful bacterial colonies from Ishurdi soil. The bacteria from Amjhupi soil were moderate, small and pinpoint in size whereas the bacteria from Ishurdi soil were in different sizes of small, medium and large. The colonies from Amjhupi 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 Ishurdi soil were irregular and circular, undulate, entire and lobate in margin and flat to raised in elevation (Table 2). Bacteria from both the soil were variated in colour from white, pink and yellow. The morphological characteristics of isolated bacteria from Amjhupi soil were observed as mixed morphological type of colonies but were majorly dominated by rod shaped, spore forming, gram positive and non-acid fast bacteria (Table 3). The morphological characteristics of isolated bacteria from Ishurdi soil were observed as mixed colonies of rod and round shaped gram positive and gram negative bacteria. All the colonies were spore forming, non-acid fast bacteria and capsulated (Table 4). The result reveled that Bacillus was majorly found in both the soil with the colony count of

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

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

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

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

Table 4. Morphological characteristics of isolated bacteria of Ishurdi 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	Single	Gram positive	Non acid-fast	Spore forming	Capsule forming
3	Rod	Single	Gram positive	Non acid-fast	Spore forming	Capsule forming
4	Rod	Chain	Gram negative	Non acid-fast	Spore forming	Capsule forming

9.6×⟦10⟧^7CFU/g and 7.4×⟦10⟧^7 CFU/g soil in Ishurdi soil and Amjhupi soil respectively.

The result was compared with the previous study conducted by Chowdhury et al., 2013 [23] from Bangladesh soil. Our reports were very much similar to them. It proves that the bacterial colony from different regions of Bangladesh dominantly contains Bacillus sp. Many other gram negative, spore forming bacteria as Enterobacter spp., Klebsiella spp., Bacillus spp. and Azospirllum spp. [17] are also identified. Many reports from Bangladesh soil stated that there is abundant presence of different Bacillus Sp. [24-27] which are mostly spore forming. The spores of Bacilli can survive better in soils which are alkaline and can proceed with its life cycle. The sporulating bacterium of soil has few opportunities to complete its reproductive cycle; therefore, their extraordinary pathogenicity is one of the effective strategies to increase the probability of survival [28]. Although many new sporulating bacterial generations are neutralized by feeding on dead and decaying matter which helps them in survival and spreads into the soil. This process ensures the continuation of the species within the environmental density [29].

4. CONCLUSION

There are very limited works done on isolation and identification of soil microbes from Bangladesh soil. It is important to get proper accountability of various forms microbes present in different soils. The soil in Bangladesh is of variant type, serving various purposes, majorly agricultural and marshy. The associated bacteria play important roles in its proper functioning and its exploration is much needed.

5. ACKNOWLEDGEMENT

The author is grateful to Ms. Sadia Shafin Tisha and Prof. Khandoker Qudrata Kibria of Soil Water and Environment Discipline from Khulna University, Bangladesh for providing necessary facilities to carry out my research work successfully.

6. CONFLICT OF INTEREST

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

7. SOURCE/S OF FUNDING

No source of funding.

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