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Bacterial population and characterization of Sonatala and Bajoa soil series of Bangladesh

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ABSTRACT

Being single celled, ancient life form, omnipresent and living in all conditions, bacterial population and characterization from different resources are very much important for its classification and identification. In soils the bacteria are to account for almost all the biological and chemical changes in environments. To determine bacterial population with characterization soil samples from two soil series, Sonatala and Bajoa, were collected. The bacterial population was counted by colony count method where 5.4×10^7 CFU/g soil in Sonatala soil and 7.7×10^7 CFU/g soil in Bajoa soil were available. Moreover, with intensive care not only colony characteristics but morphological characteristics were studied. Size, pigmentation, form, margin and elevation of well-isolated colonies of nutrient agar plates were observed. Shape and arrangement were determined from simple and negative stain. For determination of staining characteristics Gram stain, capsule stain, spore stain and acid fast stain methods were followed. Most of the bacteria of these soils were found as rod shaped. Gram- positive as well as spore forming bacteria were prominent where Gram-negative, non-spore forming, capsulated, non- capsulated, acid fast and non-acid fast bacteria were found.

Keywords: enumeration, bacterial population, soil series, colony characteristic, morphological characteristic

1. INTRODUCTION

Bacteria are an ancient form of life. Without distinct nucleus we find them as very small with single celled and prokaryotic. It is a very simple system to study the morphological characteristics of bacteria. Most bacteria having only 0.5 to 2.0 micrometers in diameter are with a few basic shapes: spherical, bacillus and spiral in the environment as either individual cells or aggregated together as clumps [1]. The maintenance of both life and the ecosystem, not only on land but also in the water closely related with bacteria that have a vital role in productivity and as decomposers [2]. Among the microorganisms, it is hard to classify and hardest to identify the bacteria even though they are widely distributed, the simplest in morphology, the smallest in size. In order to identify bacteria from microbial culture is very much important for classification. The various performances of different bacteria in our environment can be known and bacteria availability in nature can be understood with the classification [3]. It has been reported that the presence of definite type bacteria in soil of Bangladesh is helpful in phosphate solubilizing, decontamination removal and maintaining the mineral cycle of environment [4-6], soil's nitrification [7-8]. Their

presence helps the soil to control heavy metal pollution, remove pathogenic microbes and maintain the acidic soils [9]. Unknown bacterial species are studied and compared with the known species by identification of bacteria which involves a comparative process. It is an urge with a view to isolating the pure culture by different techniques before proceeding to the identification of an unknown bacterial species. As research in soil microbiology in Bangladesh is unfortunately very much limited, development of this branch compared with other branches of soil science is a minimum one. Series wise identification of soil bacteria will ensure a very important way to know the soil dynamic environment. Thus, the start of some works in soil microbiology is being felt.

The main objectives of this study were to enumerate soil bacterial population, examine their colony and morphological characteristics (shape, arrangement, staining characteristics).

2. METHOD AND MATERIALS

2.1. Sample collection

Soil sample of Sonatala series (N: 24º 75.041′, E: 90º 50.701′) was collected from Bangladesh Agricultural University field laboratory, Mymensingh and Bajoa series (N: 23º 26.045′, E: 88º 57.535′) was collected from Noldanga under Jhenidah sadar upazila of Jhenidah district, Bangladesh.

2.2. Soil preparation

The top (0-15 cm) soil sample was collected from the field and taken into laboratory using thermo flask. Fresh soil samples were used for this study.

2.3. Isolation of bacteria

At first sample was prepared by soil and physiological water (dw + 0.9% NaCl solution) as described by Dubey and Maheshwari (1999) [10]. Serial dilution of sample was performed as described by Joklik *et al,*. (1992) [3]. Nutrient agar media and equipment's used in bacterial culture were sterilized by exposure to steam at 121 °C temperature and 15 lbs (pound per square inch) of pressure [11]. Then petriplate for bacterial culture was prepared, from isolated colony obtained by spread plate technique pure culture was prepared by streak plate technique [11]. Three replications were adopted for spread plate technique and streak plate technique.

2.4. Enumeration of bacterial population

Enumeration was made by colony count method as described by Prescott and Harley (2002) [11]. The plates with 25 to 250 colonies were selected for counting. The following formula was used.

$$T = \frac{\text{no. of colonies} \times \text{dilution factor}}{V_s}$$

Where:
T= Total bacteria per gram soil
V_s= Volume of sample(ml)

2.5. Characterization

Colony characteristics and morphological characteristics were determined. Well-isolated colonies of nutrient agar plates were evaluated in size, pigmentation, form, margin and elevation [12]. Shape and arrangement were determined by simple and negative staining as described by Shaha (2003) [13]. Staining characteristics were determined by Gram stain, capsule stain, spore stain and acid fast stain as described by Cappuccino and Sherman (1999) [12].

2.6. Simple staining

Heat fixed bacterial smear was prepared on a glass slide. The smear was flooded with crystal violate for 20 to 60 seconds. Then the smear was washed with tap water to remove excess stain. After drying, the slide was examined under oil immersion.

2.7. Negative staining

A drop of nigrosin was placed close to one end of a clean slide. Using sterile technique, a loopful of inoculum from the culture was placed and mixed in the drop of nigrosin. The mixture was pushed with the edge of a second slide held at a 300 angle and placed in front of bacterial suspension to form a thin smear. After air drying, the slide was examined under oil immersion.

2.8. Gram stain

Heat fixed bacterial smear was prepared on a glass slide. The smear was flooded with crystal violate and kept for 1 minute. Then the smear was washed with tap water. After that the smear was flooded with Gram' s iodine and kept for 1 minute. The smear was washed with tap water. Ethyl alcohol 95% was added drop by drop till crystal violates failed to wash from smear. Again the smear was washed with tap water. After that the smear was counterstained with safranin for 45 seconds. Again the smear was washed with tap water. After air drying, the slide was examined under oil immersion.

2.9. Capsule stain

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

2.10. Spore stain

Heat fixed bacterial smear was prepared on a glass slide. The smear was flooded with malachite green and placed on a warm hot plate, allowing the preparation to steam for 2 to 3 minutes. The stain was prevented from boiling. The slide was removed from hot plate, cooled and washed with tap water. The smear was counterstained with safranin for 30 seconds. Then the smear was washed with tap water. After air drying, the slide was examined under oil immersion.

2.11. Acid fast stain

Heat fixed bacterial smear was prepared on a glass slide. The smear was flooded with carbol fuchsin and placed on a warm hot plate, allowing the preparation to steam for 5 minutes. The stain was prevented from boiling. The slide was removed from hot plate, cooled and washed with tap water. Acid alcohol was added drop by drop till carbol fuchsin failed to wash from smear. The smear was washed with tap water. Then the smear was counterstained with methylene blue for 2 minutes. Then the smear was washed with tap water. After air drying, the slide was examined under oil immersion.

2.12. Data Analysis

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

3. RESULTS AND DISCUSSION

The study observed distinct colorful colonies of bacteria. We had the successful isolation, purification and characterization. The result shows variable information's on bacterial colonies in soil sample.

3.1. Sonatala Soil

The total bacterial number was 5.4×10^7 CFU/g soil. There were eight types of distinct colorful colonies of bacteria. Table 1 represents the colony characteristics of isolated bacteria. The colonies were found as small, moderate and large in size; irregular, circular and rhizoid in form; serrate, filamentus, undulate, entire and lobate in margin; white, pink and yellow in color; and flat, raised and umbonate in elevation (Table 1). In another observation, examination of morphological characteristics, rod shaped, chain, spore forming, gram positive, capsulated, non-capsulated and non-acid fast bacteria were prominent (Table 2).

3.2. Bajoa Soil

Table 1. Colony characteristics of isolated bacteria

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

Table 2. Morphological characteristics of isolated bacteria

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

Table 3. Colony characteristics of isolated bacteria

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

The total bacterial number was 7.7×10^7 CFU/g soil. Eleven types of distinct colorful colonies of bacteria were observed. Table 3 presents the colony characteristics of isolated bacteria. The colonies were small, moderate and large in size; irregular, circular and rhizoid in form; serrate, filamentus, undulate, entire and lobate in margin; white, pink and yellow in color; and flat, raised and umbonate in elevation (Table 3). Rod shaped, chain, gram positive, spore forming and non-acid fast bacteria were found prominent as morphological characteristics were determined (Table 4).

Khan & Rashid, (2008) also examined bacterial colonies that have those morphological characteristics [14]. A study shows that rod shaped bacteria are dominantly present in the soils of different regions of Bangladesh [15]. We have many reports to state that different rod shaped bacteria having spore forming morphology are abundant in Bangladesh soils [16-19]. Our result shows similarity with the previous study conducted from Bangladesh soil [20-22]. This study also found the abundance of rod shaped with chain arrangement and spore forming bacteria as major.

Table 4. Morphological characteristics of isolated bacteria

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

4. CONCLUSION

As very limited works done on isolation and identification of soil microbes from Bangladesh soil, it is an urge to get proper accountability of various forms microbes present in different soils. The soils in Bangladesh being variant types are serving various purposes majorly in agricultural sectors. The associated bacteria playing important roles in its proper functioning need exploration exactly.

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6. CONFLICT OF INTEREST

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

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