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ISOLATION AND CHARACTERIZATION OF BACTERIA OF *SARA* AND *MIRPUR* SOIL SERIES OF BANGLADESH

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Abstract: Soil samples from two soil series, *Mirpur* and *Sara* were taken for isolation and characterization of bacteria. It has been observed that Sara soil series had more bacterial population than Mirpur soil. Moreover, diversified types of colony were found in Sara soil than that of Mirpur soil. Most of the bacteria of both soils were spore forming. Bacteria with capsule were very rare and all of the bacteria were non acid fast. The ratio of Gram- positive to Gram- negative bacteria was 1:1 for both soils. There were no round shaped bacteria (coccus) in Sara soil and only one round shaped bacteria (coccus) has been found in Mirpur soil.

Keywords: Soil series, isolation of bacteria, colony characteristics and morphological characteristics

Introduction

Bacteria are the most successful and ancient form of life. They are very small, single celled and prokaryotic that minces without distinct nucleus. Morphology of bacteria is very simple. Most bacteria are only 0.5 to 2.0 micrometers in diameter. They have a few basic shapes: spherical, bacillus and spiral are found in the environment as either individual cells or aggregated together as clumps (Brady and Weil, 2002). The maintenance of life and the ecosystem, both on land and in the water, depends on bacteria, since they play a vital role both in productivity and as decomposers (Stevenson, 1986). Among the microorganisms the bacteria are the most widely distributed, the simplest in morphology, the smallest in size, the most difficult to classify and the hardest to identify. Identification of bacteria from microbial culture is very much important for its classification. From the classification the various roles of different bacteria in our environment can be known and what types of bacteria are present in nature can be understood (Joklik *et al.*, 1992).

Identification of bacteria is a comparative process by which unknown bacterial species are examined, and compared with the known species. Before proceeding to the identification of an unknown bacterial species, it is necessary to isolate the pure culture by different techniques. Research in soil microbiology in Bangladesh is unfortunately very limited. Development of this branch compared with other branches of soil science is therefore negligible. Series wise identification of soil bacteria will go a long way to understand the dynamic soil environment. Thus, the urge of some works in soil microbiology has been felt. Besides many limitations, it was the aim to gather some information relating soil bacteria. The main objectives of this study were isolate soil bacteria, estimate their number, determining this colony & morphological characteristics (shape, arrangement, staining characteristics).

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Materials and methods

Sample collection: Soil samples of Sara and Mirpur series were collected from two different locations of Kotwali thana of Jessore district. The general description of the locations is discussed in Table 1.

Table 1: General description of the studied soil collected from different locations

Sample No.	GPS Reading	Location	Physiography	Soil Series
1	N: 23º 12.085′ E: 89º 13.009′	Village: Bahadurpur Upazilla: Jessore	Ganges meander floodplain	Sara
2	N: 23º 07.669′ E: 89º 15.903′	Village: Shabati Upazilla: Jessore	Ganges meander floodplain	Mirpur

Soil preparation: Top (0-15 cm) soil samples were collected from the field and taken into laboratory using thermo flask. Fresh soil samples were used for this study.

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). Serial dilution of sample was performed as described by Joklik *et al*. (1992). Then nutrient agar media was prepared as described by Prescott and Harley (2002). Nutrient agar media and equipments used in bacterial culture were sterilized by exposure to steam at 121^0C temperature and 15 lbs (pound per square inch) of pressure as described by Prescott and Harley (2002). Then petriplate for bacterial culture was prepared as described by Joklik *et al*. (1992). From isolated colony obtained by spread plate technique pure culture was prepared by streak plate technique as described by Prescott and Harley (2002). Three replications were adopted for spread plate technique and streak plate technique.

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

$$\text{Total bacteria per gram soil} = \frac{\text{no of colonies} \times \text{dilution factor}}{\text{volume of sample (ml)}}$$

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

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.

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 30^0 angle and placed in front of bacterial suspension to form a thin smear. After air drying, the slide was examined under oil immersion.

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 violate 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.

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.

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.

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.

Results

Sara soil: Distinct colorful colonies of different species of bacteria were found. Total bacteria in soil were 9.8×10^{7} CFU/g soil.

Colony characteristics of bacteria of Sara soil: Six types of distinct colorful colonies of different species of bacteria were found by spread plate technique and well observed by streak plate technique. The colony characteristics of isolated bacteria are presented in Table 2.

Table 2: Colony characteristics of isolated bacteria of *Sara* soil

Colony no.	Size	Pigmentation	Form	Margin	Elevation
1	Large	White	Rhizoid	Filamentous	Flat
2	Moderate	White	Irregular	Lobate	Raised
3	Large	Pink	Circular	Entire	Convex
4	Large	White	Irregular	Lobate	Convex
5	Moderate	Pink	Circular	Undulate	Raised
6	Small	Pink	Circular	Entire	Umbonate

Morphological characteristics of bacteria of Sara soil: The morphological characteristics of isolated bacteria of Sara soil are presented in Table 3.

Table 3: Morphological characteristics of isolated bacteria of *Sara* soil.

Colony no.	Shape	Arrangement	Gram stain	Spore stain	Capsule stain	Acid fast stain
1	Rod	Chain	Gram-positive	Spore forming	Capsule absent	Non acid fast
2	Rod	Cluster	Gram-negative	Spore forming	Capsule absent	Non acid fast
3	Rod	Chain	Gram-positive	Spore forming	Capsule absent	Non acid fast
4	Rod	Chain	Gram-positive	Spore forming	Capsule absent	Non acid fast
5	Neither rod nor round	Chain	Gram-negative	Non spore forming	Capsule absent	Non acid fast
6	Rod	Chain	Gram-negative	Non spore forming	Capsule absent	Non acid fast

The shape and arrangement of isolated bacteria were determined by negative staining and are shown in Fig. 1.

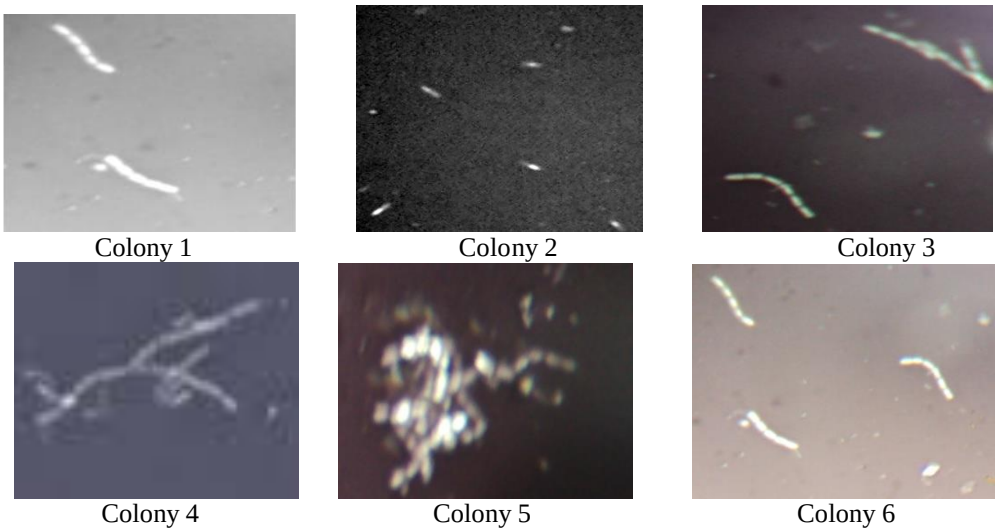


Fig. 1. Shape and arrangement of bacteria of Sara soil by negative staining.

Mirpur soil: Distinct colorful colonies of different species of bacteria were found. Total bacteria in soil were 8.2×10^{7} CFU/g soil.

Colony characteristics of bacteria of Mirpur soil: Four types of distinct colorful colonies of different species of bacteria were observed by spread plate technique and well observed by streak

plate technique. The colony characteristics of isolated bacteria were studied carefully and are presented in Table 4.

Table 4: Colony characteristics of isolated bacteria of Mirpur soil.

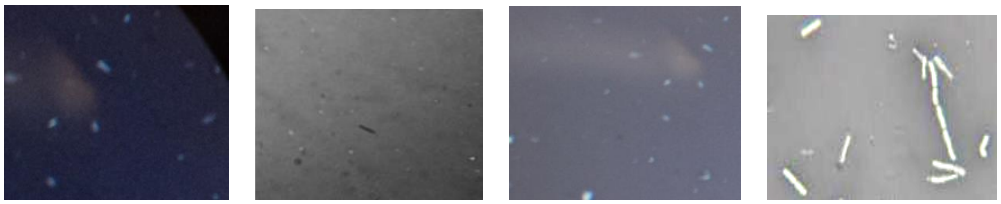
Colony no.	Size	Pigmentation	Form	Margin	Elevation
1	Large	White	Irregular	Undulate	Raised
2	Moderate	Pink	Circular	Entire	Raised
3	Moderate	White	Irregular	Serrate	Flat
4	Moderate	White	Circular	Entire	Umbonate

Morphological characteristics of bacteria of Mirpur soil: The morphological characteristics of isolated bacteria were studied under microscope and are presented in Table 5.

Table 5: Morphological characteristics of isolated bacteria of Mirpur soil.

Colon no.	Shape	Arrangement	Gram stain	Spore stain	Capsule stain	Acid fast stain
1	Rod	Cluster	Gram-positive	Spore forming	Capsule present	Non acid fast
2	Round	Cluster	Gram-negative	Spore forming	Capsule absent	Non acid fast
3	Rod	Cluster	Gram-positive	Spore forming	Capsule present	Non acid fast
4	Rod	Chain	Gram-negative	Spore forming	Capsule absent	Non acid fast

The shape and arrangement of isolated bacteria were determined by negative staining and are shown in Fig. 2.



Colony 1 Colony 2 Colony 3 Colony 4

Fig. 2. Shape and arrangement of bacteria of Mirpur soil by negative staining.

Discussion

Gram- positive, Gram- negative, spore forming, non acid fast bacteria are available in Mirpur and Sara soil. Both types bacteria of either with capsule or without capsule were found. Mishra (2000) described bacilli as most numerous bacteria in soil. From this study, it is obvious that bacilli of different arrangements are numerous bacteria in soil.

Conclusion

Sara soil bore more bacterial population than Mirpur soil. Most of the bacteria of both soils were bacilli, Grampositive, Gramnegative and spore forming. Bacteria of Sara soil had no capsule. All

of the bacteria of both soils were non acid fast.

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