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Enumeration, isolation and characterization of bacteria of Gopalpur soil series of Bangladesh

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ABSTRACT

For enumeration, isolation and characterization of bacteria soil sample from Gopalpur soil series was collected. The counted bacterial population was as 4.5×10^7 CFU/g soil. Moreover, colony characteristics as well as morphological characteristics were studied with an intensive care. Well-isolated colonies of nutrient agar plates were evaluated in size, pigmentation, form, margin and elevation. Staining characteristics were determined by Gram stain, capsule stain, spore stain and acid fast stain methods. Most of the bacteria of this soil were found as rod shaped. All types of bacteria were available as non-acid fast. Gram- positive bacteria were prominent where Gram-negative, spore forming, non-spore forming; capsulated and non- capsulated bacteria were observed.

Keywords: soil series, enumeration, isolation, colony characteristic, morphological characteristics

1. INTRODUCTION

The most successful as well as ancient form of life is bacteria. Without distinct nucleus we get them as very small, single celled and prokaryotic. It is very simple to study the morphology of bacteria. Most bacteria are found only 0.5 to 2.0 micrometers in diameter. They are with a few basic shapes: spherical, bacillus and spiral are found 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 having a vital role both in productivity and as decomposers [2]. Among the microorganisms, it is difficult to classify and hardest to identify the bacteria though they are the most widely distributed, the simplest in morphology, the smallest in size. To identify bacteria from microbial culture is very much important for its classification. The various activities of different bacteria in our environment can be known and what types of

bacteria are available in nature can be understood with the classification [3]. It is reported that the presence of some 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 eradicate heavy metal contamination, remove pathogenic microbes and maintain the acidic soils [9]. Unknown bacterial species are examined and compared with the known species by identification of bacteria being a comparative process. It is urgent in order to isolate 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 limited, development of this branch compared with other branches of soil science is negligible. Series wise identification of soil bacteria will ensure an important way to know the dynamic soil environment. Thus, the emergence of some works in soil microbiology is being felt. The main objectives of this study were to isolate soil bacteria, determine their number, and examine their colony and morphological characteristics (shape, arrangement, staining characteristics).

2. METHOD AND MATERIAL

2.1. Sample collection

Soil sample of Gopalpur series (N: 22° 55.040′, E: 89° 31.704′) was collected from Bakuria village of Khulna district from. (0-15 cm) soil sample weas collected from the field and taken into laboratory using thermo flask.

2.2. Isolation of bacteria

Sample was prepared by soil and physiological water (dw + 0.9% NaCl solution) as described by Dubey and Maheshwari (1999) [10] and serial dilution of sample was performed.

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.

2.3. Enumeration

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.

$$T = \frac{(\text{no. of colonies} \times \text{dilution factor})}{(\text{volume of sample(ml)})}$$

Where;

T=Total bacteria per gram soil

2.4. Characterization

Colony characteristics and morphological characteristics of isolates 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 [13]. Staining characteristics were determined by Gram stain, capsule stain, spore stain and acid fast stain [12].

2.4.1. 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.

2.4.2. Negative staining

A drop of nigrosin was placed close to one end of a clean slide. A loopful of inoculum 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.

2.4.3. 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. The smear was flooded with Gram's iodine and kept for 1 minute and washed with tap water. Ethyl alcohol (95%) was added drop by drop till crystal violate failed to wash from smear and 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.

2.4.4. 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. The smear was washed with 20% copper sulfate solution. After air drying, the slide was examined under oil immersion.

2.4.5. 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 slide was removed from hot plate, cooled and washed with tap water. The smear was counterstained with safranin for 30 seconds and washed with tap water. After air drying, the slide was examined under oil immersion.

2.4.6. 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 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 and washed with tap water. Then the smear was counterstained with methylene blue for 2 minutes and washed with tap water. After air drying, the slide was examined under oil immersion.

2.5. Data Analysis

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

3. RESULTS AND DISCUSSION

The study found distinct colorful colonies of bacteria. The total bacterial number was 4.5×10^7 CFU/g soil.

There were the successful isolation, purification and characterization of bacteria from Gopalpur soil series. Nine types of distinct colorful colonies of bacteria were observed by spread

Table 1. Colony characteristics of isolated bacteria

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

Table 2. 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	Capsulated	Non acid fast
2	Rod	Single	Gram-negative	Spore forming	Capsulated	Non acid fast
3	Rod	Chain	Gram-positive	Spore forming	Capsulated	Non acid fast
4	Rod	Chain	Gram-negative	Non spore forming	Capsulated	Non acid fast
5	Rod	Chain	Gram-positive	Spore forming	Capsulated	Non acid fast
6	Round	Single	Gram-positive	Non spore forming	Non capsulated	Non acid fast
7	Round	Chain	Gram-positive	Non spore forming	Non capsulated	Non acid fast
8	Rod	Chain	Gram-positive	Spore forming	Non capsulated	Non acid fast
9	Round	Chain	Gram-positive	Non spore forming	Non capsulated	Non acid fast

plate technique as well as streak plate technique.

Table 1 presents the colony characteristics of isolated bacteria. The result shows variables in bacterial colonies in soil sample. The colonies were moderate and large in size; irregular, circular and rhizoid in form; serrate, filamentous, undulate, entire and lobate in margin; white, pink and yellow in color; and flat, raised and umbonate in elevation (Table 1). The another observation was to examine the morphological characteristics where rod shaped, chain, spore forming, gram positive and non-acid fast bacteria were prominent (Table 2). Khan and Rashid (2008) also identified bacterial colonies having those morphological characteristics [14]. The observation shows that rod shaped bacteria is dominantly contained in the soils of different regions of Bangladesh [15]. Many reports stated that different rod shaped bacteria having spore forming morphology is abundantly present in Bangladesh soils [16-19].

4. CONCLUSION

As very limited works done on isolation and identification of soil microbes from Bangladesh soil, Ii 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.

5. SOURCE/S OF FUNDING

NA

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