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Preliminary Study on Isolation and Identification of Different Microbes from the Soil of Bago River Near the Village of National Races, Yangon Region in Myanmar

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Abstract: - The different soil samples are collected from some selected regions along the bank of Bago River near the Village for National Races. The collected samples are tested for presence of bacteria and fungi. The microorganisms existed in the soil samples are screened in the Petri dishes by using serial dilution agar plating techniques. The observed colonies of microorganisms are isolated into pure culture and kept in refrigerator. The observed microbes are determined by their morphological characters and staining characters.

Keywords:- Bacteria; Fungi; Colonies.

I. INTRODUCTION

Soil is the product of weathered rock. However, a mass of weathered rock does not constitute a soil without the intervention of living processes. Soil has been subjected to the action of air and water which have altered and removed some of the original components so that the proportions of the various substances are not the same as in the parent rock. The mineral particles constitute the basis foundation of soil but not the whole of it. [6]

Soils are excellent culture media for the growth of many kinds of organisms. The microscopic life of soils includes bacteria, yeasts, molds, algae, diatoms, and protozoa. Most soil organisms are found in the surface layers. The numbers and kinds of organisms found in soil depend upon the nature of the soil, depth, season of the years, state of cultivation, reaction, amount of organic matter, temperature, moisture, etc. [3]

Methods are available for counting the organisms in soil as well as for isolating various species in pure culture. Since the organisms may vary considerably in their growth requirements, many types of culture media must be employed. The organisms may be aerobic, anaerobic, or facultative types. Soil structure has a significant influence on erosion, water intake, and crop growth. A stable granulated soil will permit rapid water uptake, drainage, aeration, and beneficial microbe activity, whereas a dispersed or compact soil has a low infiltration rate. Crops grow more luxuriantly in fertilized, well-aerated, drained, and granulated soils. The most important factors in maintaining or improving the granulation of soils are the presence of microorganisms and their decomposition products. [1]

Good soil structure (1) increases water intake and drainage; (2) has a direct effect on plant growth; (3) influences biological activity such as nitrogen fixation, nitrification, and decay of organic matter; (4) holds to a minimum the activities of anaerobic organisms which reduce sulfates and nitrates to products unavailable or toxic to plants. Nitrogen is the cornerstone of the structural requirements of all living cells. It is absolutely necessary for the growth of all organisms. Without an available supply of this element, life could not endure. The biological fixation of nitrogen far exceeds all other cycles in magnitude. [1]

Among various types of soil biota, many kings of fungi can be founding soils, but the specific genera will vary with types of soil and with its physical and chemical properties. [4]

The different soil samples are collected from along the bank of Bago River near the village for National Races. The present work is conducted with the following objectives.

- to study isolation and cultural method for microbes
- to observe the different kind of microbes
- to observe the activity of microbes in soil

II. MATERIALS AND METHODS

➢ *Collection of soil samples*

The different soil samples are collected from along the bank of Bago River near the village for National Races.

➢ *Media and chemicals*

Chemical reagents employed during the course of this investigation were the products of Oxide limited London, British Drug House, England or pure forms of E.Merck, Germany were used throughout the present investigation.

➢ *Cultivation of bacteria from soil samples*

The sterilized glass wares were used throughout the research. Potato-Dextrose-Agar (PDA) medium for cultivation of soil bacteria. YDC Agar (Yeast Extract Dextrose Calcium Carbonate Agar), culture medium for bacteria species on agar slants. Cultivation of Fungi from Soil Samples; Czapek-agar-medium for cultivation of soil fungi. Serial dilution method for the cultivation of Bacteria and Fungi. Test for the characterization of bacteria. The hydrolyzed starch was tested with iodine solution. The appearance of clear zones is assumed as the positive result for the present of amylase enzyme activity.

The pure isolated fungi species were mounted on the clear glass slides and stained with lactophenol cotton blue by Needle-mounted method. The phenol crystals were dissolved in the liquid by gently warming and the dye was added into solution. [5] [2]

III. RESULTS

The collected soil samples from the bank of Bago River consisting largely of mixed species of soil organisms were isolated into pure culture. Isolated was carried out by serial dilution plate methods.

A. Isolation of bacteria from muddy soil samples

The different soil samples were collected and screened for soil organisms remaining bacteria and fungi. A few numbers of bacteria colonies were developed after 24 hours' incubation and fungal colonies were appeared after 72 to 96 hours' incubation. The resulting plates were sub cultural for 3 to 4 times to get pure cultural isolated. Five numbers of bacteria isolates and three number of fungal isolated were recovered from the investigation area of Bago River.

Soil serial dilution of 10^0 and 10^{-5} produced must of the isolated. In this study, *Pseudomonas* sp. was recovered from 10^{-9} dilution and *Bacillus* sp. was obtained from 10^{-3} cultures.

B. Characterization and Identification of Bacterial isolates

Cultural staining characteristics of each bacterial isolates were given in (Table 1).

The biochemical test for *Pseudomonas* sp. was listed in (Table 3). The test showed that all isolated were able to grow in aerobic condition. Four out of three were isolated aerobic negative long, rod shaped bacilli. One was aerobic gram positive and was aerobic, gram positive cocci. Gram negative rod noted as isolate No.3 were aerobic, motile rod, catalase positive, fermented especially cellobiose and fructose. It utilized citrate, reduced nitrate and hydrolyzed starch. Some of these characters were the genus *Pseudomonas* sp. and that isolate No.3 may be considered as *Pseudomonas* sp.

According to the cultural characteristics and micrograph, the isolate No.1 was aerobic, gram positive, longer rods. It may be assumed as the genus *Bacillus* sp. Similarly, isolate No.2 was aerobic, gram positive and spherical shape. It may be considered as *Staphylococcus* sp. Isolate No.4 was aerobic, gram negative spherical rod shape (cocco or coccobacilli). Isolate No.5 was aerobic, gram negative, rod shape which are forming as filamentous (filamentous bacteria). These two isolates were not yet clarified in this study.

C. Characterization and Identification of fungal isolates

Cultural characteristics of fungal isolate obtain from the bank of Bago River were given in (Table 2). All isolates were able to grown in both anaerobic conditions. Three isolates were black smoky and velvety, blue green velvety, white with blue green discrete colony were recovered.

According to the cultural characteristics and micrograph isolate No.1 was showed the presence black colored colonies. Colonies have a powdery appearance, branched and septate. The hyphae enlarge at the apices to form unbranched conidiophores, conidia 1-celled, globose often black colored in mass. These characters were the same as the genus *Aspergillus niger* and thus isolate. No.1 may be assumed as *Aspergillus niger*. Similarly, whitish blue green, forming as discrete colonies colony noted as isolate No.2 was showed the presence of colorless smooth conidiophores vesicles globose, ovate or elliptical, with radiate stigmata. Conidial heads hemispherical to globose, usually green or blue green, conidia globose to sub globose. These characters were resembled as the genus *Aspergillus* sp. and thus isolate No.2 may be assumed as *Aspergillus* sp.

In addition, the isolate No.3 was showed blue green velvety colonies. Conidiophores upright, simple, terminating in a globose. Conidia 1-called, globose blue green colored in mass. These characters were the same as the genus *Aspergillus* and thus isolate No.3 may be assumed as *Aspergillus* sp.

Sr. No.	Isolate No.	Cell structure	Number of bacteria	Color of colony	Gram staining reaction	Locality
1	1	Long rod	*Bacillus* sp.	Cream	Positive	10 ft from bank of Bago River
2	2	Spherical	*Staphylo-coccus* sp.	White	Positive	10 ft from bank of Bago River
3	3	Short rod	*Pseudom-onas*sp.	Cream	Negative	10 ft from bank of Bago River
4	4	Spherical rod (Coccobacilli)	Not identified	Cream	Positive	10 ft from bank of Bago River
5	5	Filament-ous bacteria	Not identified	Cream	Negative	10 ft from bank of Bago River

Table 1:- Cultural and staining characteristics of Bacterial isolates from soil samples

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Sr. No.	Isolate number	Name of colony	Colour of colony	Septate hypha	Conidia and Conidio-phore	Locality
1	1	*Aspergillus niger*	Black	Present	Smooth and distinct	10 ft from bank of Bago River
2	2	*Aspergillus* sp.	White with blue green	Present	Smooth and distinct	10 ft from bank of Bago River
3	3	*Aspergillus* sp.	Blue green	Present	Smooth and distinct	10 ft from bank of Bago River

Table 2:- Cultural characteristics of Fungal isolates from soil samples

No.	Biochemical Test	*Pseudomonas sp.*
1	Growth at 41˚C	+
2	Starch Agar Medium	+
3	Catalase Test	+
4	Carbon sources for growth; Cellobiose Fructose	 + +
5	Nitrate Reduction	+
6	Citrate Utilization	+

Table 3:- Biochemical test for identification of bacteria(*Pseudomonas sp.*) from soil samples in National Races Village

pH Soil: Water (1:2:5)	EC Soil: Water (1:5)	Text-ure	Total N_2	Exchangeable cations			Available Nutrients	
				Ca	Mg	Na	P	K_2O
1	2	3	4	5	6	7	8	9
Slightly acid	High	Silty clay loam	Very low	High	Low	Very high	Very high	Very high

Table 4:- Myanmar Agriculture Service (Land Use) Interpretation of Results

IV. DISCUSSION AND CONCLUSION

In the present studies, the muddy soil samples were collected at equal distance of (10ft) from each other at the bank of Bago River near the National Races village. In this study, the observation was made on the morphological and biochemical studies of soil organisms distributed in the selected areas of Bago River. Eight isolates, of which five bacterial isolates and three fungal isolates were obtained. These isolates were considered as *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Aspergillus niger* and *Aspergillus* sp..

Among these only one isolate of *Pseudomonas* sp. was carried out on their biochemical tests. Biochemical tests were enzyme activity of *Pseudomonas* sp. These tests were growth of 41ºC, Starch agar media, Catalase test, Carbon source of cellobiose and fructose, Nitrate reduction and Citrate utilization. All tests were original colors of reagent change each respective color.

According to the cultural staining characteristics and biochemical examination, the genus of *Pseudomonas* sp. studied were aerobic, motile, catalse positive, fermented carbohydrate, especially cellobiose and fructose. It hydrolyzed starch, utilized citrate, reduce nitrate. According to the cultural, characteristics one isolate of black colony may be assumed as *Aspergillus niger*. It has simple, unbranched conidiophores, smoky, the colony of black colors, conidia 1 celled and globose. The soil types from Bago River were classified in Myanmar Agriculture Service (Land Use) depending on their results, the soil pH- 6.1.

According to the results Ca^{++} (11.26), Mg^{++} (0.80), mill mole/100 gm, K^{+} (0.96) meq / 100 gm, P (42.44) and K_2O (45.15) mg/100 gm. The soil texture showed silty clay loam. Among the bacterial culture isolates existed in muddy soil from Bago River area, five bacterial isolates have been considered in genus level. Other bacterial isolates not yet clarified have been studied. It was due to the bacterial isolates show similarities in their morphology.

Bago River near the Village of National Races Bago River is untouched area for the microbial research work. So from this muddy soil can provide desirable microorganisms, useful for industrial, horticultural, medicinal and enzymatically purpose. In this study, Microorganism especially bacteria and fungi which occur in soil are collected, isolated and reported as research paper.

Therefore, the young scientists who specializing in soil microbiology carry out more research works which can benefit the production of useful materials for the development of Myanmar.

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