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Isolation and study of Soil microbes

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Abstract: **Soil provides substratum for plants and animal life, it is a favourable habitat for various microorganism, and the production of the crops also depends on the quality of the soil. Soil and microorganisms are interdependent, microbes add nutrients to soil. So the present study was undertaken to isolate and identify certain microbes from the soil and to study their characterization.**

Keywords: **-- Soil, microbes**

I. INTRODUCTION

The soil is considered as the land surface of the earth which provides the substratum for plant and animal life. The soil represents a favourable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa(Wielgosz E.etal,2010) . Depending on the amount of organic matter present in the soil, the biological activity eliminates pathogenic agents, decomposes organic matter and other pollutants into simpler components (frequently less noxious), and contributes to maintaining the physical and biochemical properties required for soil fertility and structure. . All soils contain both bacteria and fungi, but they may have different relative amounts depending on soil conditions. The microbial communities of the soil perform a fundamental role in cycling nutrients, in the volume of organic matter in the soil and in maintaining plant productivity (Van der Heijden M.G.A, etal.2008). The role of soil microbes is of high interest, because they are responsible for most biological transformations. Microorganisms are an integral part of soil and take part in the cycle of matter (Allison S.D, et .al.2010; Castro H.F.et.al, 2010), thus also affecting soil fertility, which determines achieving an appropriate quality and quantity of the harvest. Activity of the microorganisms in soil are frequently dependent on environmental factors such as temperature, moisture, vegetation structure and nutrient availability, all of which are probably to be affected by climate change (IPCC, 2007). The activity of soil organisms can be divided into four functions: 1. Regulation of the turnover & nutrient cycling 2. Biological degradation 3. Maintenance of soil structure and 4. Interaction with plants. Soil organisms can act as bio-filters by cleaning up soil pollutants. Many agrochemicals are broken down by soil biota. Their effectiveness will be modified by the soil environment. Herbicide degradation is faster in soils with high microbial activity. Based on the above facts the present study was designed to observe and analyse and it is framed with the following objectives: To isolate micro-organisms; to identify the bacteria ;to analyse bio chemical characterization and to study optimization parameters.

II. MATERIALS AND METHODS

Sample collection: Garden soil samples were collected and stored in sterile container and aseptically transferred to the laboratory for further analysis.

Isolation of microorganisms:1gram of the above soil sample was mixed in 9 ml sterile distilled water and shaken vigorously for 10 mins. After that 0.1ml of the resulting liquid was spread on the surface of nutrient agar, then incubated at 30oC for 18-24 hours and microorganism grown were pickup and maintained in petri plate for characterization.

Identification of the bacteria: The microorganisms found in the soil sample were identified by study of morphological and bio-chemical characterization. Morphological characteristics such as abundance of growth, pigmentation, optical characteristics, size and shape were studied on different agar plates using Grams' staining and Endospore staining Bio chemical characterization methods were done using Indole production test, Methyl red test,Voges -prokauer test ,citrate utilization test,Triple sugar iron test,Nitrate reduction test

Carbohydrate fermentation test ,in addition Gelatin hydrolysis and Starch hydrolysis were done.pH and temperature effects were studied under Optimization parameters

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III. RESULTS

Isolation of microorganisms from soil samples

Subculture of pH tolerance microorganisms

Pure culture of the efficient pH tolerance bacterial isolates on Nutrient agar

Table :1Table showing Morphological and Biochemical Characterization of the pH tolerant microorganisms

S. No	Biochemical tests	Bacillus cereus(S1)	Bacillus subtilis(S2)
1	Gram staining	Positive Rod shape	Positive Rod shape
2	Endospore staining	Positive	Positive
3	Indole production	Negative	Negative
4	Methyl red test	Positive	Negative.
5	Voges Proskauer test	Negative	Negative
6	Citrate utilization	Positive	Negative
7	Triple sugar iron	Alkaline slant	Alkaline slant
8	Nitrate reduction	Positive	Positive
9	Gelatin hydrolysis	Positive	Positive
10	Starch hydrolysis	Positive	Positive
11	Utilization of sugars a) Glucose b) Sucrose	Acid formation Negative	Acid formation Acid formation

Table:2 Table showing the PH effect on soil microbes

S.No	pH	Strain S1	Strain S2
1	5	0.54	0.46
2	6	0.59	0.66
3	7	0.75	0.87
4	8	1.07	1.01
5	9	0.59	0.65

Table :3 Table showing temperature effect on soil microbes

S. No	Temperature	Strain S1	Strain S2
1	30	0.60	0.51
2	35	0.70	0.72
3	40	1.03	1.01

IV. DISCUSSION

Microorganism in soil are important because they affect soil structure and fertility, microbes can make nutrients and minerals in the soil available to theplants, produce hormones that spur growth, stimulate the plant immune system and trigger stress response. One among the soil microbe is bacteria, up to 10 billion bacterial cells inhabit each gram of soil in and around plant roots, a region known as the rhizosphere. In the present study two bacteria namelyBacillus cereus and Bacillus subtilis were isolated, and the effect of pH and temperature was studied. The maximum activity was obtained at pH 8 for Bacillus subtilis. Below and above this optimum pH, the activity was found to be decreased.

Similarly, Bacillus cereus pH 7 and 7.5. The activity was found to be maximum at neutral pH 7. Then, the growth was decreased with the increase in alkaline pH to pH 8 and 9.

It is reported that the microbial growth activity of Bacillus subtilis, growth activity was slightly increased at 30oC, it was found to be maximum at 37oC. The enzyme production reduced gradually with further increase in incubation temperature to 40oC.This is due to the denaturation of microbial strain at higher temperatures(Davidson E.A and Janssens I.A 2006).

Similarly, the effect of incubation temperature on microbial growth activity was studied for Bacillus cereus. The results revealed that the Bacillus cereus produced least activity at 37oC. Then the activity was maximally increased at 40oC.reports says that variation in pH on either side of optimum value lead to decline in microbial growth

ultimately lowering production. Most of bacterial cultures require neutral pH for optimum growth. Bacteria are favored by neutral pH conditions . In our study, pH values of soil were at alkali levels and thus support the growth of bacteria. The microbial growth activity was for Bacillus subtilis, at 30oC, was slightly increased and it was found to be the maximum at 37oC. The enzyme production reduced gradually with further increase in incubation temperature to 40oC. This is due to the denaturation of microbial strain at higher temperatures.

Similarly, the effect of incubation temperature on microbial growth activity was studied for Bacillus cereus. The results revealed that Bacillus cereus produced least activity at 37oC. Then the activity was maximally increased at 40oC.,which indicates that the temperature influences both the growth of microorganisms and biological activities. Range of temperature for maximum amylase production usually varies from one organism to another but generally optimum temperature and stability was observed between 30-50℃.This study is an initial work and found there is relationship between temperature and pH and thus from the study it may be concluded that , if the soil pH and temperature is maintained properly the soil quality will improve in turn the productivity of the field.

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