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The Hidden World Beneath: How Factors Shape Rhizosphere Microbial Communities

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"The Hidden World Beneath: How Factors Shape Rhizosphere Microbial Communities"

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ABSTRACT

Rhizosphere is the region for microbial activity essential to soil fertility and plant health. It is the area of soil that is influenced by plant roots. This inhabits many types of microbes such as bacteria and fungi. They have essential roles for plant development and growth to produce simpler inorganic form of materials from complex matters. If physical conditions of soil become altered due to mixing of wastewater and material, excessive use of fertilizers and pesticides may cause a decline in microbial activity beneficial for plant health.

The aim of this study was to isolate bacteria from the rhizospheric soil of a healthy plant in order to determine the effects of changing factors such as pH, Temperature and salinity of soil.

For morphological identity and characterization of isolated bacteria, which led to identification of three distinctive isolates as Streptococcus spp., Staphylococcus spp. and Bacillus spp. Further, the growth of one isolate, Streptococcus spp., was optimized under various salinity, temperature, and pH by UV spectrophotometry. We found that alteration in these parameters significantly influenced bacterial proliferation. This suggested that they can sustain in an optimal range of these parameters to have proper functioning. These results highlight rhizospheric bacteria are sensitive to changes in the soil environment, which may have an impact on their advantageous functions in plant growth and ecosystem function. Developing measures to preserve soil health and promote sustainable agriculture requires an understanding of these interactions.

KEYWORDS: Soil health, pH, Temperature, Salinity, Plant Microbiome, Rhizospere, Microbial activity.

1. INTRODUCTION:

The soil is a vital component of the terrestrial ecosystem, a living, dynamic matrix. It is an essential resource for maintaining the majority of life processes as well as for agricultural production and food security.Soil biota functions play an important role in decomposition and nutrient cycling. Soil is thought to be a reservoir of microbial activity, despite the fact that living microbes occupy less than 5% of the total space. As a result, most microbial activity is limited to the 'hot-spot', or aggregates with accumulated organic matter, known as the rhizosphere. Plant and soil types both influence rhizosphere diversity and community patterns [1]. Soil fertility and plant health are determined by bacterial-plant interactions in the rhizosphere known as plant growth boosting rhizobacteria or plant health promoting rhizobacteria or nodule promoting rhizobacteria, these free-living soil bacteria that are good for plant growth can colonize the plant root and promote plant growth [2]. There are thousands of different types of bacteria linked to plant roots, which represents a huge diversity , also known as the plant's second genome, this intricate plant-associated microbial population is essential to plant health [3].

Microbes and plants have a dynamic interaction; plants do not exist in isolation. Microbes are allowed to proliferate throughout the tissue of plants, and this accumulation is known as the phytomicrobiome [4] [5] [6].The root microbiome is a complex microbial community that surrounds and inhabits the root system, which is the main organ for plants to absorb nutrients and water [7] [8]. Because of their critical roles in energy flow, organic matter conversion, and ecosystem material cycling, soil microorganisms may have a significant impact on soil carbon storage in response to climate change [9] [10]. Phylogenetic groups of bacteria found in soils are widely spread and plentiful in terms of the contributions made by each member of those groups to the overall bacterial communities in the soil [11] [12] [13].

Microbes belonging to the genera Bacillus, Serratia, Azotobacter, Azospirillum, Micrococcus, Arthrobacter, and Pseudomonas, as well as Rhizobia like Allorhizobium, Mesorhizobium, and Bradyrhizobium, use organic molecules from the rhizosphere, such as the sugars glutamine, betaine, and trehalose, to enhance their growth [14] [15].

Understanding soil microbial processes in relation to the elements impacting their environment is essential since soil is one of the most prevalent places on Earth where microbial processes occur. Temperature, water content, pH, and salinity are examples of environmental conditions that alter enzymatic activities, which in turn affect soil microbial processes, which are mediated by enzymes [16]. The pace at which organic matter breaks down and the mineralization of various organic compounds in the soil are both influenced by the soil's temperature [17] [18]. The biogeochemical cycle of soil is mostly driven by the microbial population, and alterations in the structure and characteristics of the soil can readily impact microbial activity [19]. Because of their relevance to food security, agriculture-based economies,

human health, and the quality of the air and water, soils are a valuable resource. However, soil health is at danger everywhere. Climate change, saltification, erosion, compaction, nutrient depletion, contamination with harmful heavy metals or pesticides, human-assisted soil-borne insect migration, and overgrazing are some of these risks [20].

2. MATERIAL AND METHODS:

2.1 **Soil Sample:** A healthy rooted plant's rhizospheric soil was used to get soil samples. The plant was collected from the Anurag Nursery in Dehradun and put in a bag right away to the lab. Additionally, the plant's root system was excavated in order to gather rhizospheric soil. Before being processed further, soil samples were placed in sterile plastic bags and kept at 4 ^{0}C [21].

2.2 **Isolation of bacterial strains from soil:** Bacterial strains were isolated from soil by dispersing and diluting a soil sample in 9 milliliters of distilled water that had been sterilized. It was well re-suspended. Using a glass spreader, 10µl of the soil sample was evenly spread across nutrient agar medium (NAM) plates. The plates were incubated at 37°C for the entire night. Three distinct live bacterial colonies were chosen at random from the plate, and their streaking was carried out on a another fresh NAM plate [22].

2.3 **Identification and Characterization of Bacterial Isolates**: Gram's Staining technique was used to identify the morphology of the bacterium.[23]. A number of biochemical tests were conducted in order to further characterize the isolates. Indole production, nitrate, citrate utilization, urease, catalase, motility, methyl red, Voges-Proskauer's, and carbohydrate tests were among these tests [24].

2.3.1 **Indole Production Test:** We assessed bacterial ability to produce indole through this test. For every isolated bacterium, indole media was made. Tryptone (10g) and NaCl (5g) dissolved in distilled water . Equal amounts of this autoclave-sterilized material were transferred to sterile test tubes. Each tube received an inoculation of media. At 37 °C, incubation lasted for 24 hours. When a few drops of Kovac's reagent were applied after incubation, a red ring formed on the media's surface, indicating that bacteria could generate indole. It was determined that these stains were indole-positive [25].

2.3.2 **Nitrate Reduction Test:** To ascertain whether bacteria could convert nitrate (NO_3^-) to nitrite (NO_2^-), bacteria were incubated at 37°C overnight after being introduced into nitrate reduction broth [26]. Two steps were taken to identify nitrite. By first introducing sulphanilic acid and then α-naphthylamine, we were able to identify whether nitrite was present in each culture. When the color didn't change, we added zinc dust to be sure nitrite was present. Unreduced nitrate would have been present if it had

been red. Since none of the strains changed color, they were all found to be nitrate positive [27].

2.3.3 **Urease Test:** For determination of hydrolyzing urea into carbon dioxide and ammonia, peptone, dextrose, NaCl, and K_2HPO_4 were dissolved in distilled water to create urease media. Additionally, phenol red was introduced as a pH indicator. Urea was added after the medium was autoclaved [28]. To enhance surface area, culture test tubes were tilted and capped. The bacterium tubes were streaked and then incubated at 37^0C for 24 hours. Bacterial strains that were urease positive shifted the colour from reddish-orange to bright pink, indicating that they could hydrolyze urea to produce CO_2 and ammonia [29].

2.3.4 **Citrate Test:** Agar and other salts are dissolved in distilled water to make citrate medium. As a pH indicator, bromophenol blue was added to the media. After streaking on the slanted test tubes were incubated at 37 ^{0}C for the entire night [30]. The media's pH dropped below 6.9 as a result of the bacteria's consumption, which causes acid formation. As a result, the color turned a deep blue, indicating a good citrate response. A negative reaction to citrate was indicated by no change in colour.

2.3.5 **Catalase Test:** Separately, the bacterial strains were placed on sterile, regular microscope glass slides.When a small amount of hydrogen peroxide was introduced to bacteria, no bubble formed, indicating that no O_2 had evolved. Since there was no catalase activity to convert H_2O_2 into H_2O and O_2, all strains were found to be catalase negative [31].

2.3.6 **Motility Test:** This test involved using a light microscope to examine a wet mount of bacterial culture on a sterile glass slide. A cover slip was applied over the sample, forming a thin film of fluid, and vaseline was used to fill the edges. The three strains were all found to be non-motile.

2.3.7 **H2S Test:** Agar, ferrous ammonium sulfate, sodium thio sulfate, peptone, and beef extract are dissolved in distilled water to prepare the media. The test tubes were slanted and the solid agar surface was streaked with bacteria. No colour change after an overnight incubation period suggested that bacteria were not producing H_2S gas. H_2S was not present in these microorganisms.

2.3.8 **Methyl Red Test:** To characterize bacteria whether they have the ability to produce acid from glucose or dextrose, sterile test tubes were filled with MR medium and a little amount of test bacteria was inoculated. Prior to incubation, methyl red drops were introduced as an indication. Before performing the process, the pH was 6.9. The media's color shifted to reddish-pink, indicating the generation of acid, which was MR positive. No color change was identified as an MR negative effect.

2.3.9. **Voges- Proskauer test:** The MR test is extended by the Voges-Proskauer test. This test determines whether bacteria are capable of producing acetoin. To do this, KOH and alpha-

naphthol were added to MR medium. When carbohydrates are broken down, they react with KOH and alpha naphthol, changing color. For this test, every bacterial sample tested positive [32]

2.3.10 **Test for Carbohydrate Fermentation:** The ability of the bacterial strains to ferment carbohydrates was tested with different sugars. Peptone, NaCl, beef extract, and certain carbohydrates—mannitol, sucrose, maltose, and dextrose—were used to make the media. As an indicator, phenol red was used. The ability to ferment these sugars was shown by a change in color [32].

2.3.11 **Amylase Test:** We added starch-containing media to a petri-plate and cooling down, bacteria were swabbed with a cotton swab. The plate was filled with iodine solution after being incubated at 37 ^{0}C for 24-48 hours. The presence of amylase where the starch had been broken down was revealed by the clear zone surrounding the growth. This test confirmed the ability of bacteria to produce amylase enzyme.

Table : Biochemical Tests performed for characterization of isolated bacterial starins

S.No.	Test Name	Isolated bacterial strains		
		Streptococcus spp.	***Staphylococcus spp.***	***Bacillus spp.***
1.	Indole production	+	+	+
2.	Nitrate reduction	+	+	+
3.	Urease test	-	+	+
4.	Citrate test	-	-	+
5.	Catalase test	-	-	-
6.	Motility test	-	-	-
7.	Methyl Red test	-	-	+
8.	Voges-Proskauer test	+	+	+
9.	H2S test	-	-	-
10.	Carbohydrate tests:			
	Dextrose test	+	-	+
	Maltose test	+	-	+
	Sucrose test	+	+	+
	Mannitol test	+	+	+
11.	Amylase test	+	+	+

3. OPTIMIZATION OF GROWTH OF BACTERIA ON DIFFERENT ENVIRIONMENTAL PARAMETERS:

In order to analyze the growth of bacterial strains isolated from plant rhizospheric soil samples under varying soil variables, including pH, temperature, and salinity, the strains were optimized in the laboratory.

3.1. **Effect of pH changes on bacterial growth:** The pH of the soil has a significant impact on the growth and population of microorganisms in the soil of plant roots. To examine the impact of pH variation on soil bacteria, we conducted tests using four growth media maintained at pH 3, 5 ,7 & 9. In each test tube, an isolated strain was inoculated. The tubes were incubated at 37°C for 24 hours to allow for optimal growth. After this period, we measured the bacterial population density using UV-spectrophotometry to assess how each pH level influenced the growth rates of the isolated strains. for 24 hours after being covered with cotton plugs. At a wavelength of 600 nm, UV spectroscopy was used to quantify the growth or density of bacteria in cultures under various pH conditions. Three milliliters of blank were placed in a quartz cuvette with the absorbance adjusted to zero for control and sample were tested (fig.1)

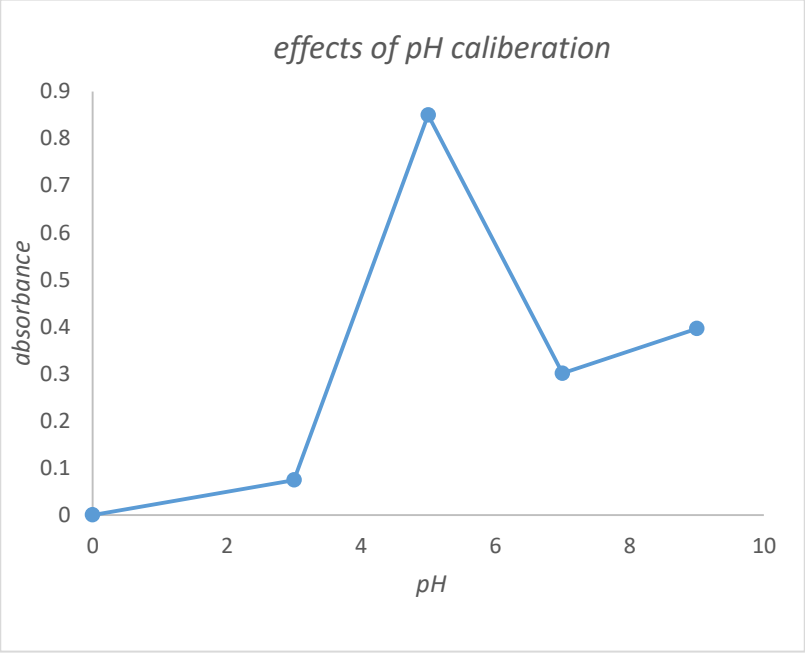

Fig. 1. Representaion of effects of pH changes on growth of *Streptococcus spp.* strain of soil-isolated bacteria. Absorbance was recorded for all cu;tures of this strain maintained at pH of 3, 5, 6 & 7. Absorbance found in range of pH 4 to 7 indicating more bacterial growth.

3.2 **Effects of temperature variations on bacterial growth :** We inoculated equal quantity of bacteria into nutrient broth and incubated them at three distinct temperature conditions—20°C, 37°C, and 42°C—for an entire night in order to observe the effects of temperature change on soil microbial isolates. The media became turbid due to good growth. UV spectrophotometry was used to evaluate how temperature changes affected the growth of bacteria. The results are shown in fig. 2.

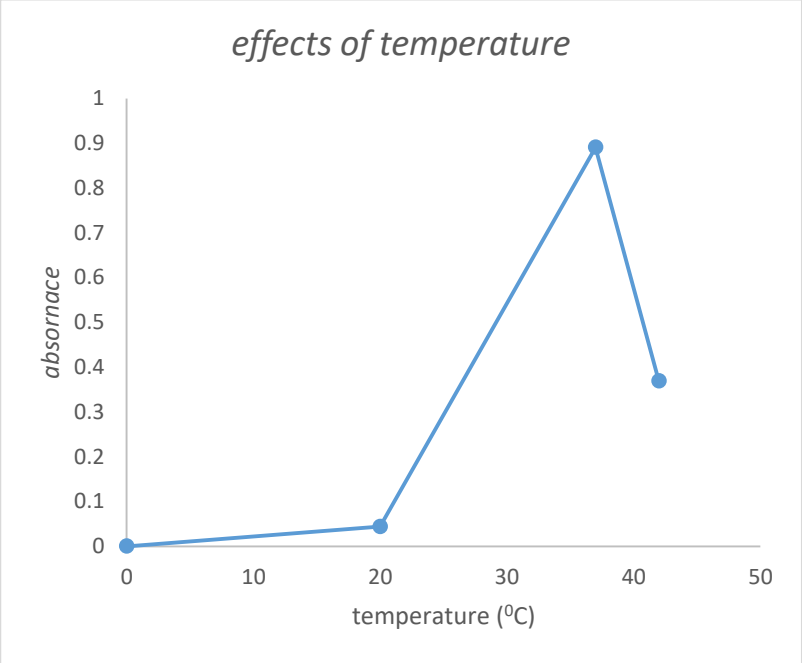

Fig. 2. Representation of effects of temperature changes on growth of *Streptococcus spp.* strain of soil-isolated bacteria. Absorbance was recorded for all cultures of this strain .High Absorbance found between 35^0C to 38^0C is optimal temperature. Below and above of this, growth become lessen.

3.3 **Effects of salinity on bacterial growth:** Bacterial strain was subjected to increasing salt concentration. Four types of nutrient broth were prepared having salinity of 10%, 20%, 30%, and 50%. Bacteria was inoculated in equal amounts of broth in sterilized test tubes. They were incubated at 37°C for 24 hours. Salinity effects were determined by measuring the absorbance at the wavelength 600nm with the help of a UV- spectrophotometer. We found results as shown by the graph.The graph illustrates a clear trend in bacterial growth as the salt concentration increased. Notably, there was a significant decline in absorbance readings at higher salinity levels, indicating that the higher salt concentrations adversely affected bacterial proliferation (Fig.3).

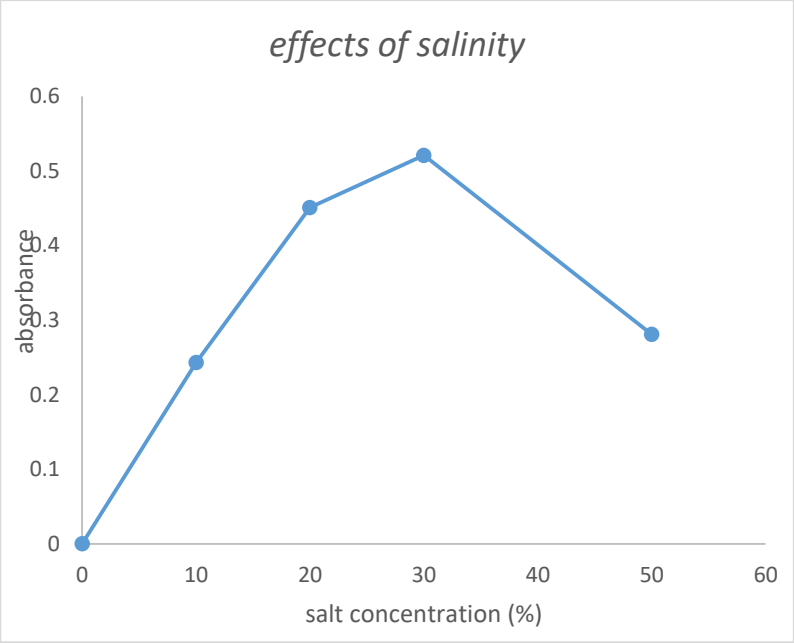

Fig. 3. Illustration of effects of altered salt concentration on *Streptococcus spp.* strain. Growth is increasing with increase in Salinity percentage upto 30% , above it their growth is declining.

4. RESULTS AND DISCUSSION:

Following the optimization of isolated bacterial growth under laboratory conditions, we examined the effects of modified soil conditions on rhizospheric soil bacterial growth. The absorbance readings were recorded for each culture broth, allowing for a comparison of bacterial growth across the different pH, temperature and salt concentration levels. This data was crucial in determining the optimal pH for the isolated strain's growth and understanding its physiological responses to environmental changes. Samples from each bacterial culture tube were estimated for their absorbance on the same wavelength. More bacterial density correlated with more absorbance. This relationship indicates that as the concentration of bacteria increases, the optical density of the culture also rises, allowing for better quantification of microbial growth.

5. CONCLUSIONS :

Based on the previously cited studies, we came to the conclusion that salinity, temperature, and soil pH are significant variables that impacts soil health which in turn affect microbial diversity. The population of microorganisms can be affected as the pH vary from optimal value. The growth of soil isolates was impacted by temperatures both above and below the typical incubation temperature. Bacteria can grow till a certain range of salinity

above which their growth would be hindered. These altered properties of soil impact on microbial activities which are beneficial for plant development and survival.

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