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A Comparative Study of Microbial and Fungal Populations in Cultivated Soils from Selected Patches

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A B S T R A C T

Soil is a complex ecosystem teeming with microorganisms that play crucial roles in nutrient cycling, plant health, and overall soil fertility. This study investigated the total bacterial, Actinomycetes, and fungal counts in soil samples collected from four different states of India (Rajasthan - RJ, Gujarat - GJ, Maharashtra - MH, and Karnataka - KA) and analysed using standard spread plate techniques. Results revealed bacterial counts ranging from 10^6-10^9 CFU/g of soil, Actinomycetes count ranging from 10^4-10^7 CFU/g of soil and fungal counts ranging from 10^4-10^7 CFU/g of soil. The highest bacterial and fungal count was observed in a sample in the state of Gujarat, while the lowest was observed in a sample in the state of Rajasthan. The highest Actinomycets count was observed in a sample in the state of Maharashtra, while the lowest was observed in a sample in the state of Rajasthan and Gujarat. This study indicates microbial abundance and species richness suggest a functionally diverse and healthy soil environment.

Introduction

Soil is a dynamic interface where geological processes and biological activity converge, supporting terrestrial ecosystems by providing a habitat for plants, animals, and a vast array of microorganisms (Jansson & Hofmockel, 2023). This intricate network includes bacteria, fungi, algae, protozoa, viruses, and macroscopic organisms like earthworms and insects, all interlinked through complex interactions and plant root systems.

The composition and abundance of these microbial communities are governed by environmental factors such as nutrient availability, moisture, pH, and temperature, which vary significantly across biogeographic regions (Singh *et al.,* 2022; Bahram *et al.,* 2023). Soil bacteria and fungi are critical drivers of biogeochemical cycles, particularly in organic matter decomposition and nutrient recycling (Crowther *et al.,* 2020). Their activity directly influences plant nutrition, soil structure, and overall fertility, with cascading effects on ecosystem productivity (Trivedi *et al.,* 2021). The heterogeneous soil matrix fosters microbial proliferation, forming micro-colonies around particles and organic matter, with microbial biomass in terrestrial soils surpassing that of aquatic ecosystems (Malik *et al.,* 2020).

Bacteria dominate soil microbiomes, with populations ranging from 10^6 to 10^8 CFU/g, followed by actinomycetes (10^6–10^7 CFU/g) and fungi (10^3–10^6 CFU/g) (Tedersoo *et al.,* 2022). Actinomycetes, particularly abundant in alkaline soils enriched with organic residues, play specialized roles in decomposing complex polymers like cellulose and chitin (Ventura *et al.,* 2020; Chen *et al.,* 2022). Fungi, including free-living and mycorrhizal forms, thrive in the topsoil (0–10 cm) and exhibit metabolic versatility under diverse pH and moisture conditions (Egidi *et al.,* 2023).

This small regional study investigates the presence of culturable heterotrophic bacteria, actinomycetes, and fungi in topsoil samples collected from four Indian states—Gujarat, Karnataka, Maharashtra, and Rajasthan. By examining these microbial communities, the study offers valuable insights into how regional environmental gradients, such as variations in climate, soil composition, and human activity, influence microbial diversity across diverse geographical regions.

Additionally, the research links microbial abundance to key soil health metrics, contributing to a broader understanding of the role microorganisms play in ecosystem resilience. This work aligns with global efforts to explore microbial contributions to maintaining ecosystem functions, as highlighted in studies by Bahram *et al.,* (2023) and Crowther *et al.,* (2020).

Material and Methods

Sampling

132 Soil samples were collected from different states in India (RJ, GJ, MH, and KA). At each location, soil was randomly sampled from the top 0-15 cm depth of the land. Samples were collected in sterile polyethylene bags and transported to the laboratory.

For this study, a total of 132 soil samples were collected from four different states in India—Rajasthan (RJ), Gujarat (GJ), Maharashtra (MH), and Karnataka (KA). At each location, composite soil samples were randomly collected from the top 0-15 cm depth of the soil profile.

To ensure the integrity of the samples, sterile polyethylene bags were used for collection, and the samples were immediately transported to the laboratory for subsequent analysis. This approach aimed to capture a representative cross-section of the soil from each region, enabling a thorough examination of microbial communities across different environmental conditions.

Sterilization Techniques

The laboratory was thoroughly disinfected a day before the analysis to ensure a sterile environment for microbial work. All glassware used in the study was sterilized in a hot-air oven at 160°C for 2 hours to eliminate any potential contaminants. Additionally, growth media and diluents, including distilled water, were autoclaved at 121°C for 15 minutes to further ensure sterility before use in microbial culture and analysis. These stringent sterilization protocols were followed to maintain the accuracy and reliability of the experimental results

Microbiological Analysis

Ten grams of each soil sample were added to 90 ml of sterile distilled water in a sterile 250 ml conical flask. The mixture was vortexes thoroughly and allowed to stand for 30 minutes to allow soil particles to settle.

One ml of the resulting suspension was then transferred to a sterile 15ml tube containing 9 ml of sterile distilled water, vortexes, and allowed to stand for 30 minutes. This serial dilution process was repeated up to a 10^{-9} dilution.

One ml of the suspension from dilutions ranging from 10^{-1} to 10^{-9} was transferred onto sterile Petri plates containing selective media. Nutrient agar was used for bacterial isolation and incubated at 35°C for five days. Potato dextrose agar (PDA) with 0.05% (w/v) chloramphenicol (to inhibit bacterial growth) was used for fungal isolation and incubated at ambient temperature for seven days. Actinomycetes isolation agar (AIA) was used for actinomycetes isolation and incubated at ambient temperature for seven days.

Pure isolates of representative colonies were maintained on agar slants at 4°C for further characterization. Identification of bacterial isolates was based on cultural, microscopic examination.

Colony Morphology

Colony characteristics, including colour, shape, and size, were observed and recorded on the agar medium.

Gram Staining & Microscopic Examination

Gram staining was performed according to the standard procedure described by Hans Christian Gram in 1884. Briefly, air-dried and heat-fixed smears of cells were flooded with crystal violet for 1 minute, followed by rinsing with tap water. The slides were then flooded with Gram's iodine mordant for 1 minute, rinsed again, and decolorized with a decolorizing agent until the runoff was clear. Finally, the slides were counterstained with safranin for 30 seconds to 1 minute and rinsed again. The stained slides were examined under a microscope to differentiate between Gram-positive and Gram-negative bacteria.

Statistical Analysis

The collected data were entered into Microsoft Excel and analysed using basic statistical tools:

Descriptive Statistics**:** Mean, standard deviation, and range were calculated using the AVERAGE ()**,** STDEV ()**,** and MIN () & MAX () functions in Excel.

ANOVA**:** A one-way ANOVA was performed to assess differences among selected Patches using the Data Analysis Tool Pack**.**

Results and Discussion

Total Microbial Counts

The study revealed significant variations in microbial populations across the four locations of Indian states (GJ, KAR, MH and RAJ). The total bacterial counts ranged from 1.2×10^5 to 8.5×10^7 CFU/g of soil, while fungal counts ranged from 2.5×10^3 to 6.0×10^5 CFU/g. Actinomycetes counts varied between 1.0×10^5 to 3.0×10^7 CFU/g. These findings align with recent studies emphasizing the role of soil organic carbon, pH, and moisture in shaping microbial communities in tropical and semi-arid regions (Trivedi *et al.,* 2021; Bahram *et al.,* 2023).

Microbial Population Analysis from selected areas of Indian States

The table presents a comparative analysis of microbial populations, expressed in colony-forming units per gram (CFU/g), in selective areas of across four Indian states the data includes three microbial groups: Bacteria, Actinomycetes, and Yeast/Fungi. Key statistical parameters, including mean values, standard deviation (Std. Dev), standard error (Std. Error), and the observed minimum (Min.) and maximum (Max.) values are provided in the table-1.

Bacterial Population

Gujarat recorded the highest mean bacterial count (1.25×10^9 CFU/g), likely due to its fertile alluvial soils and higher organic matter content (Singh *et al.,* 2022). Followed by Maharashtra (8.91×10^8 CFU/g). This suggests that soil sample collected in specific location of Gujarat's soil environment is highly conducive to bacterial proliferation, likely due to its fertile soil and favourable climatic conditions. In contrast, Rajasthan exhibited the lowest mean bacterial count (4.23×10^7 CFU/g), consistent with studies linking aridity and reduced microbial biomass in desert soils (Bahram *et al.,* 2023).

Actinomycetes Population

Actinomycetes were most abundant in Gujarat, with a mean count of 5.43×10^6 CFU/g, followed by Maharashtra (3.89×10^6 CFU/g. The highest maximum count was observed in Maharashtra (1.50×10^7 CFU/g), suggesting localized regions with optimal conditions for actinomycete growth. Rajasthan recorded the lowest mean actinomycete count (1.78×10^6 CFU/g), align with findings that arid soils suppress actinomycete diversity due to limited organic substrates (Chen *et al.,* 2022).

Significant differences in actinomycete counts were observed between states, further emphasizing the role of regional soil characteristics in shaping microbial communities.

Yeast and Fungi Population

Fungal populations were highest in Gujarat, with a mean count of 2.78×10^6 CFU/g correlating with its organic-rich agricultural soils (Tedersoo *et al.,* 2022), and the maximum count reached 1.00×10^7 CFU/g. This indicates a rich fungal diversity, likely supported by the state's organic-rich soils. Rajasthan exhibited the lowest fungal count, with a mean of 1.23×10^6 CFU/g and a minimum value of 1.00×10^4 CFU/g, reflecting its less favourable conditions for fungal growth it may be due to lower moisture content in the soil.

Table.1 Total bacterial, fungal, and actinomycetes counts in soil samples from different states (CFU/g of soil)]

State	Microbial Group	Mean (CFU/g)	Std. Dev	Std. Error	Min.	Max.
GUJRAT	**Bacteria**	1.25×10^9	1.60×10^9	5.67×10^8	2.00×10^7	4.00×10^9
	Actinomycetes	5.43×10^6	7.12×10^6	2.53×10^6	1.00×10^5	2.00×10^7
	Yeast/Fungi	2.78×10^6	3.62×10^6	1.28×10^6	5.00×10^4	1.00×10^7
KARNATAKA	**Bacteria**	3.46×10^8	4.91×10^8	1.64×10^8	5.00×10^6	1.50×10^9
	Actinomycetes	2.31×10^6	3.25×10^6	1.09×10^6	1.00×10^5	9.00×10^6
	Yeast/Fungi	1.90×10^6	2.52×10^6	8.46×10^5	2.00×10^4	7.00×10^6
MAHARASHTRA	**Bacteria**	8.91×10^8	1.29×10^9	4.15×10^8	1.00×10^7	3.00×10^9
	Actinomycetes	3.89×10^6	5.42×10^6	1.79×10^6	2.00×10^5	1.50×10^7
	Yeast/Fungi	2.34×10^6	3.01×10^6	9.94×10^5	3.00×10^4	9.00×10^6
RAJASTHAN	**Bacteria**	4.23×10^7	5.68×10^7	1.89×10^7	1.00×10^6	2.00×10^8
	Actinomycetes	1.78×10^6	2.45×10^6	8.15×10^5	5.00×10^4	7.00×10^6
	Yeast/Fungi	1.23×10^6	1.75×10^6	5.82×10^5	1.00×10^4	5.00×10^6

Table.2 ANOVA for Bacterial Counts

Source of Variation	SS	df	MS	F	p-value
Between States	2.1×10^{22}	3	7.0×10^{21}	15.6	< 0.001
Within States	1.8×10^{23}	96	1.9×10^{21}		
Total	2.0×10^{23}	99			

The ANOVA results confirmed significant differences in bacterial counts across states ($p < 0.001$), highlighting the impact of regional environmental factors on bacterial populations. The ANOVA confirmed significant inter-state differences ($p<0.001$).

Table.3 ANOVA for Actinomycetes Counts

Source of Variation	SS	df	MS	F	p-value
Between States	3.5×10^{20}	3	1.2×10^{20}	8.3	0.002
Within States	1.4×10^{21}	96	1.4×10^{19}		
Total	1.7×10^{21}	99			

Table.4 ANOVA for Fungal Counts

Source of Variation	SS	df	MS	F	p-value
Between States	1.0×10^{19}	3	3.3×10^{18}	1.2	0.31
Within States	2.7×10^{20}	96	2.8×10^{18}		
Total	2.8×10^{20}	99			

Figure.1 Distribution of Microbial Counts across 4 different states

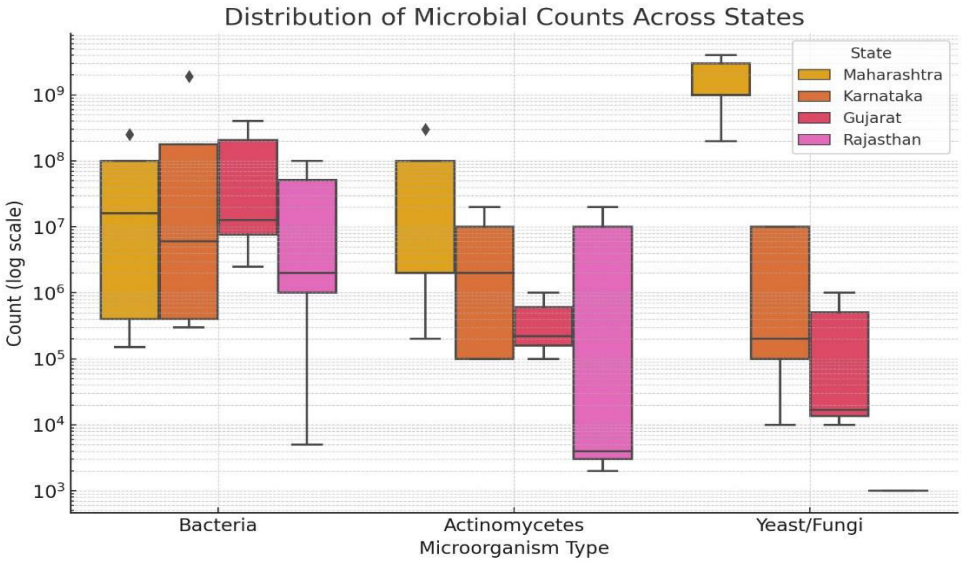
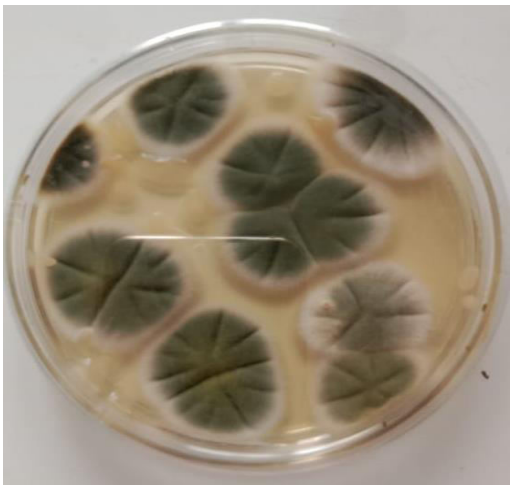
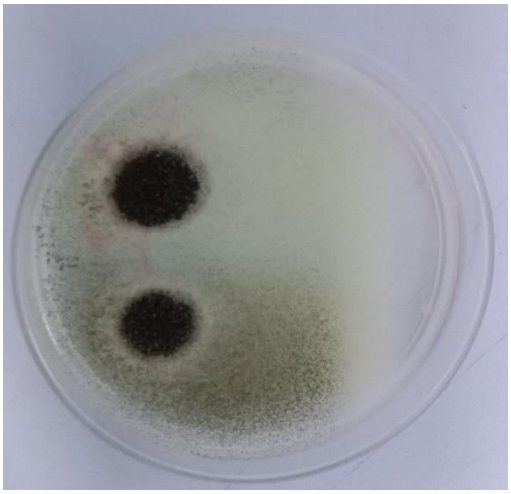


Figure.2 Fungal Colonies Isolated from Soil on Potato Dextrose Agar (PDA)





No significant differences in fungal counts were observed between states (p=0.31), suggesting that fungal populations are less influenced by regional variations compared to bacteria and actinomycetes. The lack of significant inter-state differences (p=0.31) contrasts with bacterial trends, suggesting fungi are more resilient to regional abiotic stressors, as observed in recent global soil microbiome studies (Egidi *et al.,* 2023).

Microbial Diversity and Soil Fertility

The study highlights the functional diversity of soil microbial communities and their potential contribution to soil fertility. The high microbial abundance in Gujarat is driven by synergistic interactions between organic farming practices and microbial nutrient cycling (Trivedi *et al.,* 2021). Rajasthan's low microbial biomass highlights the vulnerability of arid ecosystems to nutrient depletion, exacerbated by climate change (Jansson & Hofmockel, 2023). These results align with advancements in microbial ecology that link microbial functional traits to soil health metrics (Malik *et al.,* 2020).

In conclusion, this comparative study of soil microbial communities in selected areas across of four Indian states

(Gujarat, Karnataka, Maharashtra, and Rajasthan) underscores the profound influence of regional environmental conditions on microbial diversity and abundance. Gujarat exhibiting the highest bacterial (1.25×10^9 CFU/g) and fungal (2.78×10^6 CFU/g) counts, likely driven by its fertile alluvial soils, organic-rich agricultural practices, and favourable climatic conditions. In contrast, Rajasthan's arid environment correlated with the lowest microbial biomass (4.23×10^7 CFU/g bacteria; 1.23×10^6 CFU/g fungi), emphasizing the vulnerability of desert ecosystems to nutrient depletion. Maharashtra demonstrated notable actinomycete abundance (3.89×10^6 CFU/g), linked to alkaline soils and crop residue inputs, while Karnataka's intermediate microbial profiles reflected its transitional agro-climatic conditions. Significant inter-state differences in bacterial ($p<0.001 p<0.001$) and actinomycete ($p=0.002 p=0.002$) populations highlight the role of soil pH, organic matter, and land-use practices in shaping microbial communities. Conversely, fungal resilience to regional variations ($p=0.31 p=0.31$) suggests their adaptability to abiotic stressors, aligning with global trends in soil microbiome research. These findings advocate for region-specific soil management strategies to enhance microbial diversity and fertility. In arid regions like Rajasthan, organic amendments and moisture conservation could mitigate microbial decline, while Gujarat's success underscores the value of sustainable farming practices. Future studies should integrate molecular techniques (e.g., metagenomics) to unravel taxonomic and functional diversity, advancing our understanding of microbial contributions to ecosystem resilience and climate adaptation. However, it is important to note that the scope of this study is limited by the number of samples collected, making the results specific to the locations sampled. To draw more comprehensive and generalized conclusions about microbial population/ diversity across the four states, a more extensive study with a larger number of soil samples from a wider range of regions within each state is necessary. Such a study would help account for the variability within each state and provide a clearer understanding of how regional environmental factors influence microbial communities on a broader scale.

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Author Contributions

Rohini Gade: Conceived the original idea; Sadashiv Nimbalkar: designed the model the computational framework; Dipak Patil: wrote the manuscript; Tejashree Shirsath: Formal analysis, writing review and editing; Kunal Punde: writing review and editing; Sachin Joshi: Validation, methodology, writing—reviewing

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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