

DIVERSITY OF AEROBIC BACTERIAL GROUPS IN CHANDUR FOREST AREAS OF WEST BENGAL, INDIA

²BAISAKHI SAHA, ¹SOUVIK BAG, ¹ABHIJIT MANDAL AND ¹SOUMENDRANATH CHATTERJEE*

¹Parasitology and Microbiology Research Laboratory, Department of Zoology, The University of Burdwan, Burdwan 713 104, West Bengal, India

²Department of Zoology, Rabindra Mahavidyalaya, Champadanga, Hooghly 712 401, West Bengal, India

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Abstract– Ancient landscapes safeguarded by local communities, harbour small forest patches, and the Chandur Forest stands out as a crucial one in the Hooghly district, West Bengal. This tropical dry forest, characterized by numerous trees, is surrounded by urbanization. The soil in these areas represents a complex ecosystem hosting various microorganisms, including bacteria. This study aimed to assess the bacterial diversity and physiochemical properties of soil samples collected from different zones within the Chandur Forest. In this investigation, the bacterial diversity in soil samples from ten specific zones within the Chandur forest of the Hooghly district, West Bengal, labeled as Region 1 to 10, was scrutinized. The populations of various aerobic bacterial groups were quantified using the serial dilution method. The soil samples underwent dilution up to a 10⁻⁵ dilution. The populations of aerobic heterotrophic, Gram-negative, spore-forming, phosphate-solubilizing, nitrifying, asymbiotic N₂-fixing, and nitrate-reducing bacteria ranged from 35 to 11 × 10⁷, 29 to 10 × 10⁴, 39 to 20 × 10⁶, 31 to 15 × 10⁵, 39 to 19 × 10⁵, 41 to 22 × 10⁵, and 42 to 25 × 10⁵ cfu/g dry soil, respectively. The physical properties of soil samples from the study sites were also assessed, revealing organic carbon content, available nitrogen, phosphate, and potassium levels ranging from 0.19% to 0.63%, 17 to 37 kg/ha, 41 to 71 kg/ha, and 166 to 302 kg/ha, respectively. Hierarchical classical clustering was employed to elucidate similarities between different groups of soil bacteria. This emphasizes the complex interaction among microbial communities, environmental factors, and the plant ecosystem within the Chandur forest. Consequently, the soil takes center stage as a vital element in the Chandur forest ecosystem, where microorganisms play pivotal roles in governing soil fertility, nutrient mobilization, soil health, and exerting significant influences on plant development. The present study underscores the notably abundant bacterial populations thriving in the soil of the Chandur forest.

INTRODUCTION

Amidst the alarming pace of environmental degradation and deforestation driven by rapid urbanization, there are pockets of nature where small forests endure, preserving natural plant and animal communities in nearly pristine conditions. These remnants of nature not only contribute to environmental well-being but also serve as sanctuaries for a diverse array of indigenous and medicinal plants, playing a crucial role in generating medicinal, insecticidal and pesticidal effects (Chatterjee *et al.*, 2023a; Chatterjee *et al.*, 2023b). Deforestation is intricately intertwined with human culture, contrasting with natural events. Forests offer a range of concealed yet crucial soil

services, including humus formation, carbon sequestration, atmospheric gas stability, climatic change stabilization, nutrient cycling, facilitation of community assembly and succession, water and air prefiltration, flash flooding control, water supply regulation, and aesthetic significance. The soil's microbial load plays a pivotal role in sustaining terrestrial and aquatic ecosystems, as highlighted by studies conducted by Azmi *et al.*, 2016; Bag *et al.*, 2022a; Bag *et al.*, 2022b and Mandal *et al.*, 2023. The exploration of microbial diversity is essential for comprehending microbial ecology in soil and various ecosystems, as emphasized by Atlas, (1998). Microorganisms are omnipresent in nature and exert influence over all known ecosystems on Earth (Atlas, 1998). The widespread presence of

microorganisms can be mainly attributed to their small size, ease of dispersal, adaptation to diverse habitats, and the ability to utilize a wide variety of substrates as a nutrient source (Pandey *et al.*, 2007). Soil-dwelling microbes can be aptly referred to as the “Biological engine” of the Earth, given their pivotal role in fundamental nutrient cycling processes, dynamics of soil structure, degradation of pollutants, and regulation of different plant communities (Breure, 2004). Additionally, microbes contribute significantly to soil aeration and fertility, two critical aspects of soil function. The generation of soil organic matter enhances the soil’s ability to uphold its functional structure once established. Soil organic matter is a direct product of the collective biological activities of plants, microbes, animals, and abiotic factors. The microbial community primarily comprises five major groups: bacteria, actinomycetes, fungi, algae, and protozoa (Holt, 1986; Azmi *et al.*, 2016). Among these groups, bacterial populations generally surpass others (Alexander, 1978). In order to comprehend the intricacies of interactions facilitated by soil microbes, it is crucial to assess soil microbial diversity. Several studies have explored soil microbial diversity in various regions of India (Das and Dangar, 2007; Chatterjee *et al.*, 2014; Bag *et al.*, 2022a; Bag *et al.*, 2022b and Mandal *et al.*, 2023). However, there is currently no research on bacterial diversity specifically conducted in the Chandur forest areas of Hooghly, West Bengal, India, Latitude 22.8864303 and Longitude 87.7049383. The Chandur Forest, situated near Arambagh town in the Hooghly district, is a reserved forest located on the banks of the Dwarkeswar River. The district of Hooghly, named after the town of Hooghly on the west bank of the Hugli River, is characterized by its flat terrain, with no area having an elevation exceeding 200 meters. The district has a rich history, spanning thousands of years, and was first visited by the Portuguese sailor Vasco da Gama. In light of the ecological context, the current study aims to investigate the diversity of soil bacteria and the physicochemical properties of the soil in different locations within the Chandur reserved forest, West Bengal, India.

MATERIALS AND METHODS

Collection of soil samples

Between January and March 2023, a systematic soil sampling effort was undertaken for analysis. The

process involved extracting the top layer of soil from each designated study area, with 100 g collected at a depth of 1 cm. To maintain the samples’ sterility, they were securely wrapped with elastic bands in sterile polythene bags. Subsequently, these soil samples were transported to the Parasitology and Microbiology Research Laboratory within the Department of Zoology at The University of Burdwan.

In the laboratory, the collected soil samples underwent thorough physicochemical and microbiological analyses as part of the study. This analytical approach aimed to unravel insights into the diverse aspects of soil composition and microbial communities within the Chandur reserved forest areas of Hooghly, West Bengal, India.

Analysis of soil samples

Bacterial strains were isolated from soil samples using the serial dilution method. The soil samples were diluted up to a 10^{-5} dilution. To determine the population of aerobic heterotrophic bacteria, 10 μ l of the serially diluted soil suspension (10^{-5}) was mixed with 100 ml of sterilized nutrient agar medium (containing beef extract 3g/l, peptone 5g/l, NaCl 3g/l, agar 2g/l, pH 7) and poured onto sterilized plates. These plates were then incubated for 24-48 hours in a BOD incubator at $30 \pm 1^\circ\text{C}$. For enumerating various bacterial populations, 10 μ l of the soil suspension (10^{-4} dilution) was mixed with 100 ml of different specialized agar media. The mixtures were incubated for 24-48 hours at $30 \pm 1^\circ\text{C}$ in a BOD incubator to identify distinct bacterial groups individually. To determine the spore-forming bacteria, the soil suspension underwent pasteurization for 30 minutes at 60°C . Subsequently, serial dilutions were prepared from the pasteurized soil samples and poured onto sterilized nutrient agar (NA) plates. The soil samples were cultured on starch agar medium for 24 hours, followed by treatment with Gram’s iodine to identify starch-hydrolyzing bacteria. Counting and identification of bacterial colonies producing a halo zone (indicating positive results) were conducted. Gram-negative bacteria were observed using MacConkey agar media. The formation of a halo zone around the colonies on insoluble phosphate-containing media ($\text{Ca}_3(\text{PO}_4)_2$) depicted inorganic phosphate-solubilizing bacteria. The nitrifying bacterial population was quantified on Winogradsky’s medium containing $(\text{NH}_4)_2\text{SO}_4$ (1.0 g/l), and colonies were visualized (pink color) by saturating

the plates with sulphanic acid reagent. Nitrifying bacteria were counted at 5–30 day intervals after incubation. The colony-forming unit (CFU) per gram of dry soil was estimated (Cappuccino and Sherman, 1992; Benson, 1967). Standard procedures, as outlined by Sumner and Miller, (1996), were employed to assess the physicochemical properties such as organic carbon, nitrate nitrogen, organic phosphate, and potassium in the soil samples.

RESULTS

The soil samples collected from various regions within the Chandur reserved forest areas of Hooghly, West Bengal, India, exhibited diverse microbial populations. The aerobic heterotrophic, Gram-negative, spore-forming, phosphate-solubilizing, nitrifying, asymbiotic nitrogen-fixing, and nitrate-reducing bacterial populations varied across the sampled regions, ranging from 35 to 11×10^7 , 29 to 10×10^4 , 39 to 20×10^6 , 31 to 15×10^5 , 39 to 19×10^5 , 41 to 22×10^5 , and 42 to 25×10^5 cfu/g dry soil, respectively (see Table 1). Notably, in comparison to other locations, Region 3 displayed higher counts for aerobic heterotrophic, Gram-negative, phosphate-solubilizing, nitrifying, asymbiotic nitrogen-fixing, and nitrate-reducing bacterial populations (Table 1). Additionally, the spore-forming bacterial population reached its peak in Region 5 (Table 1).

The soil samples collected from the study areas exhibited a range of organic carbon content, spanning from 0.19% to 0.63%. Notably, Region 3 demonstrated the highest organic carbon content at 0.60% (refer to Table 2). In terms of accessible nitrogen and phosphate content, the samples showed variations, ranging from 17 to 37 kg/ha and 41 to 71 kg/ha, respectively. Region 3 recorded the highest levels for both accessible nitrogen and phosphate (refer to Table 2). The potassium levels in the soil samples ranged from 166 to 302 kg/ha, with Region 5 registering the highest value (refer to Table 2). The pH of the surveyed soil in the study area varied from 6.0 in Region 4 to 7 in Region 2 (refer to Table 2).

Bacteria of different types, including Heterotrophic (AHB), Gram-negative (GNB), spore-forming (SFB), phosphate-solubilizing (PSB), nitrifying (NB), asymbiotic nitrogen-fixing (ANFB), and nitrate-reducing (NRB), were grouped based on their distribution patterns through hierarchical cluster analysis of soil samples from various study

Table 1. Bacterial population (cfu/g dry soil) of different region of Chandur forest of Hooghly District, West Bengal, India.

Groups of Bacteria	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10
Aerobic heterotrophic bacteria ($\times 10^7$)	16 \pm 0.02	11 \pm 0.62	35 \pm 0.45	21 \pm 0.22	31 \pm 0.01	19 \pm 0.03	16 \pm 0.36	22 \pm 0.12	23 \pm 0.22	18 \pm 0.26
Gram negative bacteria ($\times 10^4$)	14 \pm 0.33	10 \pm 0.7	29 \pm 0.51	17 \pm 0.09	26 \pm 0.06	12 \pm 0.21	13 \pm 0.21	18 \pm 0.31	15 \pm 0.04	13 \pm 0.27
Spore forming bacteria ($\times 10^6$)	21 \pm 0.01	20 \pm 0.08	36 \pm 0.61	29 \pm 0.81	39 \pm 0.03	32 \pm 0.33	23 \pm 0.22	26 \pm 0.42	26 \pm 0.07	22 \pm 0.33
Phosphate solubilising bacteria ($\times 10^5$)	19 \pm 0.05	15 \pm 0.01	31 \pm 0.06	25 \pm 0.77	28 \pm 0.65	26 \pm 0.41	22 \pm 0.54	24 \pm 0.11	18 \pm 0.66	21 \pm 0.07
Nitrifying bacteria ($\times 10^5$)	23 \pm 0.04	19 \pm 0.02	39 \pm 0.31	27 \pm 0.07	34 \pm 0.14	20 \pm 0.61	25 \pm 0.62	21 \pm 0.08	24 \pm 0.36	22 \pm 0.61
Asymbiotic N ₂ fixing bacteria ($\times 10^5$)	28 \pm 0.52	22 \pm 0.61	41 \pm 0.21	31 \pm 0.66	38 \pm 0.32	29 \pm 0.44	28 \pm 0.52	24 \pm 0.31	27 \pm 0.11	30 \pm 0.29
Nitrate reducing bacteria ($\times 10^5$)	32 \pm 0.06	25 \pm 0.36	42 \pm 0.44	33 \pm 0.52	39 \pm 0.22	29 \pm 0.32	30 \pm 0.05	27 \pm 0.11	26 \pm 0.44	32 \pm 0.51

areas (Figure 1). The spore-forming (SFB) and phosphate-solubilizing (PSB) bacterial groups shared similar zone, demonstrating 62% similarity. This cluster was linked to the nitrifying bacterial (NB) group with 69% similarity. Additionally, this same cluster showed an association with the asymbiotic nitrogen-fixing (ANFB) and nitrate-reducing (NRB) bacterial group share with 66% similar zone (refer to Figure 1). On the other hand,

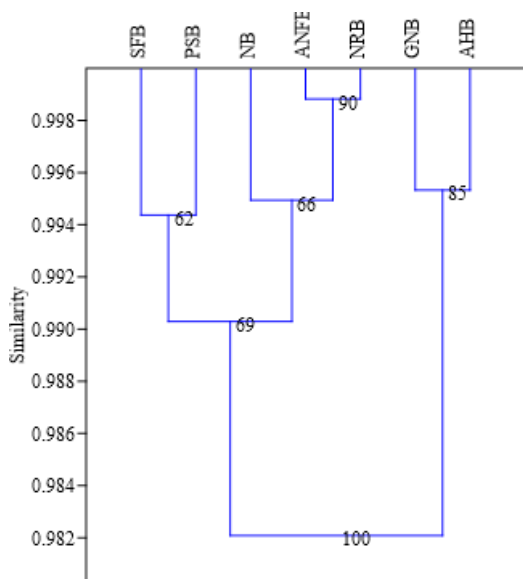


Fig. 1. Agglomerative hierarchical cluster analysis (AHC) of the soil bacterial groups of different Region of Chandur forest of Hooghly District, West Bengal, India. (AHB - aerobic heterotrophic bacteria; GNB - Gram-negative bacteria; SFB – spore-forming bacteria; PSB – phosphate solubilising bacteria; ANFB- asymbiotic nitrogen fixing bacteria; NB- nitrifying bacteria; NRB - nitrate-reducing bacteria.)

the aerobic heterotopic bacterial (AHB) and Gram-negative (GNB) bacteria formed a separate cluster with an 85% similarity. This cluster was linked to those cluster with 100% similarity (refer to Figure 1).

DISCUSSION

Soil microorganisms play a pivotal role in decomposing organic matter, facilitating the release of minerals that subsequently enhance soil nutrient content. Their active involvement in nutrient cycling is crucial for influencing soil fertility (Yao *et al.*, 2000). With the aid of modern genetic tools, these microorganisms can be identified. Despite gaps in our knowledge regarding their other ecological characteristics, their behavior can be controlled or managed to enhance ecosystem services and plant productivity (Sathya *et al.*, 2017). While numerous studies have explored the microbial diversity of soil in various regions, notable contributions include works by Das *et al.* (2013), Radhakrishnan and Varadharajan, (2016); Sharma *et al.* (2019); Dhakar and Pandey, (2020); Kavitha *et al.* (2020); Bag *et al.* (2022a); Bag *et al.* (2022b) and Mandal *et al.* (2023). Microbial communities in a given environment establish intricate relationships with each other, engaging in either competition for limited resources or collaboration through the exchange of metabolites and chemical signals (Nadell *et al.*, 2008). Beyond influencing below-ground biodiversity, soil-dwelling microorganisms also significantly impact above-ground biodiversity by contributing to the formation of soil structures (Dodd *et al.*, 2000), promoting plant health (Smith and Goodman, 1999), and influencing plant nutrition (George *et al.*, 1995). Consequently, the

Table 2. Physicochemical parameters of soil samples collected from different region of Chandur forest of Hooghly District, West Bengal, India.

Region	% of Carbon (C)	Phosphate (P)(kg per hectare)	Potassium (K) (Kg per hectare)	Nitrogen (N) (Kg per hectare)	pH
Region 1	0.21	44	179	21	6.7
Region 2	0.19	41	166	17	7.0
Region 3	0.63	71	242	37	6.6
Region 4	0.34	39	177	21	6.0
Region 5	0.60	69	302	31	6.3
Region 6	0.26	56	180	19	6.2
Region 7	0.29	46	196	22	6.3
Region 8	0.41	54	230	24	6.5
Region 9	0.18	39	210	27	6.5
Region 10	0.70	63	245	20	6.4

characterization of soil samples relies heavily on the qualitative and quantitative assessment of soil bacteria (Arunachalam and Arunachalam, 2000). The composition of the soil microbial population is intricately linked to the physico-chemical parameters of the soil and variations in microhabitats (Sathe *et al.*, 2018).

The abundance of aerobic heterotrophic bacteria in various regions of the Chandur reserved forest areas in Hooghly indicated a substantial presence of organic matter in the soil. As noted by Kaur and Singh (2000), sandy soils typically harbor a lower population of heterotrophic bacteria due to their limited organic content compared to clay or humus soils. It's crucial to acknowledge that soil comprises micro-niches with diverse physicochemical properties across different scales when attempting to correlate bacterial occurrence or activity with soil parameters. In Region 3, where the organic carbon content was highest (0.63%), the aerobic heterotrophic bacterial population was also elevated, surpassing other regions within the forest. This aligns with the findings of Dinesh *et al.* (2004), who emphasized the impact of organic carbon on the development of soil microbiota. Hecker *et al.* (2007) suggested that Gram-positive bacteria can dominate over Gram-negative bacteria due to their stress tolerance and the ability to produce endospores. Interestingly, Region 5, characterized by sandy soil from the river belt of Dwarkeswar, exhibited the highest population of spore-forming bacteria. These bacteria play a significant role in decomposing organic matter such as hemicelluloses, celluloses, and pectin, contributing to the breakdown of plant materials and humic substances. The soil microbiota, as highlighted by Bever, (1994), is influenced by various abiotic factors. Understanding these complex interactions provides valuable insights into the dynamics of soil microbial communities in the Chandur reserved forest areas. Increased levels of available phosphate and potassium can support bacterial growth, with the higher concentration likely stemming from increased turnover rates influenced by microbial activity in the soil (Bever, 1994). These microorganisms play a pivotal role in mobilizing various nutrients, such as sodium, potassium, and iron, thereby enhancing their bioavailability (Rashid *et al.*, 2016). These nutrients, in turn, assist plants in adapting to different forms of stress and fending off attacks by pathogenic organisms (Wang *et al.*, 2020), significantly influencing soil fertility and promoting

plant growth. Among the various sites in the Chandur forest, the soil in Region 3 exhibited the highest phosphate content, providing an explanation for the elevated population of phosphate-solubilizing bacteria in that area. Nitrogen, a commonly limiting nutrient in soils, enters the ecosystem largely through fixation. The higher nitrogen content in the soil of Region 3 is attributed to the accumulation of nitrate-reducing bacteria in higher quantities. These findings align with the perspectives of previous researchers such as Azmi and Chatterjee, (2016), Rashid *et al.* (2016), and Bag *et al.* (2022a). The cluster analysis conducted in the study demonstrated correlations between bacterial groups based on their relative density.

A comprehensive assessment of a microbial ecosystem requires the integration of both biotic and abiotic factors to accurately evaluate community structure and biodiversity. While various soil physicochemical characteristics contribute to this variation, it's important to recognize that the predominant vegetation in the Chandur forest may also exert an influence on the bacterial community (Osborne *et al.*, 2011). The soil bacterial community is dynamic, subject to regular changes driven by different environmental factors. It may undergo shifts, giving way to another community better suited for survival in the evolving environment (Lupatini *et al.*, 2013). This underscores the intricate interplay between microbial communities, environmental conditions, and the plant ecosystem in the Chandur forest, highlighting the need for a holistic understanding of these factors for a more accurate evaluation of the microbial ecosystem.

CONCLUSION

Small forested areas host a diverse array of flora and fauna, surpassing the richness found in the surrounding regions. Within these forests, the soil exhibits notable vertical stratification, shaped by the decomposition of organic matter from litter and the weathering of the mineral matrix. As a result, the soil emerges as a crucial component of the Chandur forest ecosystem, with microorganisms playing pivotal roles in overseeing soil fertility, nutrient mobilization, soil health, and exerting profound influences on plant development. The current place of work underscores the remarkably abundant bacterial populations within the soil of Chandur forest. This discovery opens avenues to establish databases and conduct comparative studies across

diverse locations, thereby enriching our comprehension of microbial diversity. Consequently, the soil in the Chandur forest represents a valuable reservoir of diverse bacterial resources. Further exploration of these resources holds the potential to unveil valuable insights and applications across various fields.

Conflict of Interest- None

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