Emergence in mapping microbial diversity in tea (Camellia sinensis (L.) O. Kuntze) soil of Assam, North-East India: A novel approach

PN Bhattacharyya, SR Sarmah, P Dutta, AJ Tanti

Mycology and Microbiology Department, Tocklai Tea Research Institute, Tea Research Association, Jorhat, Assam, India.

Abstract
Tea [Camellia sinensis (L.) O. Kuntze], Theaceae, is an economically important non-alcoholic, aromatic beverage yielding perennial crop, extensively cultivated in Assam, North-East India. Exploitation and conservation of tea soil microflora is important in minimizing the use of toxic chemicals since the later is known for its adverse effect on natural environment. Isolation and exploitation of microbial resources of native tea growing soil are important mainly due to their immense potentiality in maintenance of biogeochemical cycles, promotion of plant growth, inhibition of destructive pests and pathogens etc. Quantitative as well as qualitative distribution of microbes in tea plantations of Assam, N. E. India, in the context of their geographical position and geological and geochemical habitats are need to be worked out, since there is scarce information concurrently on the spatial and temporal patterns of microbial diversity and distribution patterns in tea ecosystem by constructing maps. Geographic information system (GIS) is useful in this regard to strengthen the microbial resource management strategies in tea.

Keywords: geographic information system, mapping microbial biodiversity, microbial resources, pest and disease suppression, tea

1. Introduction
India is one of the important mega biodiverse countries. North-east India is the bio geographical gateway of greater India that can be considered as one of the richest biodiversity hot-spot zone [1] and is known for its potential genetic resources. Being one of the important parts of biodiversity hot-spot region, the state of Assam has been regarded as an active centre of evolution of many novel gene pools. Gneissic (metamorphic rock) complex of Assam, which is in continuity with the geological set up of Meghalaya, comprised the peninsular crystalline rocks and thereby shows the evidences of development of deep-seated fracture lineaments trending along with E-W and NE-SW axis [2].

Tea [Camellia sinensis (L.) O. Kunze] is one of the oldest, non-alcoholic, beverage yielding perennial crop, widely consumed all over the world. The evergreen and long-lived (over 100 years of cultivation) tea plantations are genetically diverse [3], that usually provides a steady microclimate and food supply for various plant pests and pathogens. The microbial diversity of tea soil is extremely diverse [4]. There are approximately 1.1 x 10^{10} prokaryotes residing in tea soil, which can thrive in a variety of soil habitats including those with extremes of temperature, pH, water and salt stress [5]. The fact that microbes are essential for the entire tea ecosystem since they perform numerous important functions like maintenance of biogeochemical cycles, promotion of plant growth [6, 7], inhibition of destructive tea pest and pathogens etc. [8-12] has spurred keen interest in scientists for the exploration of tea soil microbial diversity. Growth promoting substances are produced in large quantities by the beneficial microorganisms that influence indirectly on the overall morphology of the plants. However, unfortunately, due to the inaccessibility of some tough terrains, tea soil microflora of this geologically important region has not so far been explored properly, which if explored properly would substantially provide valuable information about the soil microbial ecology and the energetics of microbial metabolism in that particular soil habitat. Integration of microbiological data with environmental parameters, ecological data and geographical location could improve our understanding on the spatial and temporal patterns of microbial diversity, population structure and function in tea soil [13]. Mapping microbial biodiversity on the basis of their geographical position and geological and geochemical habitats is, thus, required since there are almost no investigations concurrently on both the tea soil microbial population numbers as well as their distribution patterns.

The present research highlights the emergence of mapping of microbial-biogeography to understand the soil microbial diversity better, since the later can directly affect wide range of ecosystem processes relating to the quality of our environment. Exploitation and conservation of microbial diversity in tea soil and their suitable appliance is also essential in minimizing the use of toxic chemicals [14, 15] that are responsible for deterioration in soil quality as well as product toxicity. Besides, proper microbial exploitation and putting microorganisms in maps is needed to ensure the representative of a particular microbial population in an area.

2. Microbial world: the unique landscape
The microbial world is the largest unexplored reservoir of biodiversity on earth [16] (up to the last century, the nature and identity of only a tiny fraction (just < 10%) of microscopic landscape is known), due to which research based on microbial ecology becomes an important frontier in present day biological science. The most recent estimate suggests that only approximately 5% of fungal species and 0.1% of total bacterial diversity are known of which a small fraction has been examined for their metabolic profile [17]. Microorganisms are much more diverse both in their life forms as well as in their distribution patterns. Microbial diversity describes the
dissimilar types of microorganisms and their relative abundance in a natural habitat [18]. Soil microorganisms are reported to influence the physical, chemical and biological properties of soil either directly or indirectly [19]. Critical analysis on microbial communities and complexity of microbial diversity in tea plantations of different geographical variations caused due to the heterogeneity of soil physico-chemical properties, as well as utilization of extra doses of diverse agrochemicals is, therefore, considered as one of the challenging areas of tea soil microbiological research.

3. Ecology of tea soil microflora

Tea plantations usually resemble to “single species forest” [20, 21]. Although tea soil microbiology was initially explored in 1901, while the studies on occurrence and functionality of mycorrhizae on tea roots was reported later [22, 23]. Since then, several bacterial and fungal strains like Aspergillus, Azotobacter, Azospirillum, Fusarium, Penicillium, Trichoderma and phosphate solubilizers like Bacillus and Pseudomonas have been reported in tea soil [24, 25]. Tea rhizosphere microflora have also important plant promoting abilities as a result of which they may be exploited for their beneficial role in crop enhancement and pest and disease suppression in tea [6, 7, 26]. Potentiality of microbials such as Aspergillus niger, Azotobacter chroococcum, Azospirillum brasilense, Bacillus subtilis, Pseudomonas corrugata, Rhizobium sp., and Streptomyces nojiriensis in enhanced growth of tea along with pest and disease suppression are reported. In rhizosphere, bacteria are comparatively competing more for nutrients and ecological space, as a consequence of which they have devised various offensive tools for intra and interspecies competition, such as antibiotic substances, bacteriolytic enzymes and bacteriocins [27]. The rhizosphere of established tea bushes have several specific characteristics such as negative rhizospheric affect, lowering of soil pH, antagonistic activities among microbial communities and dominance of certain species [27], which are strongly associated with this perennial tea plant. Fig. 1 represents the habit of the tea canopy along with a close view towards its rhizosphere.

In 10-year tea plantations, both the diversity index and the richness index were maximum in normal soils. The evenness index gradually increased as the soil fertility increased. The domination index was, however, highest in poor soils, lower in rich soils and lowest in normal soils. In 20-year tea plantations, the diversity index and the evenness index were highest in normal soils and lowest in poor soils. The richness indexes in rich and normal soils were similar, and both were higher than that in poor soils. The results, thus, showed that soil fertility and cultivation age along with changing climate have significant impact [28, 29] on microbial distribution in the plant rhizosphere. The overall interactions among the tea roots, microbes and environmental conditions prevailing in tea rhizosphere seems to favor the growth of antagonistic microbes [27, 30, 31] which are known to produce strong antibiotics with potential biocontrol activities. The antagonistic behaviour of Bacillus subtilis against Corticium invisum, the causal agent of black rot disease of tea was established in vitro [9]. Trichoderma viride and T. harzianum showed their efficiency in controlling charcoal stump rot, brown root rot and Poria branch canker diseases of tea. Beauveria bassiana, a native tea soil entomopathogen recorded up to 50% control of Helopeltis theivora, the major sucking pest of tea [10]. Antagonistic actinomycetes, native to tea soil, were also found effective in controlling certain tea pathogens [11]. Scientific investigations on the properties of diverse categories of microbes involved in tea ecosystem, in particular with the interaction between the tea plants, pests and other natural enemies as well as the linkage between the entire microbial consortium [32, 33] is necessary to understand the tea ecosystem more precisely.

4. Mapping microbial diversity

Biodiversity is a fundamental component of life on earth creating complex ecosystems that could never be created by humans [34]. Biodiversity can be defined as the hereditarily based variation at all the levels of organizations ranging from the genes within a single species to the species composing of a local community and finally to the communities of the multiparous ecosystems of the world [35]. There is significance of conservation strategies related with global biological diversity [36]. The value of biodiversity, both intrinsically and to humans, is immeasurable and, therefore, it should be protected. The diversity patterns of many macroorganisms are known [37]. For instances, the global ranges of almost all kinds of mammals, birds and amphibians are available at a resolution of approximately 100 km. Maps of biodiversity play a pivotal role
in understanding the ecology, evolutionary biology and conservation biology. Diversity maps offer insight into the action of ecological and evolutionary processes [38] and thereby can contribute significantly towards the diversity and distribution of life at large scales of space and time. The proliferation of biological databases and the easy access enabled by the internet has an added advantage in this regard [39].

The microbial world is vast, with $10^{30}$ organisms present on the Earth [40]. There is a proverb stating “everything is everywhere, but, the environment selects” [41]. Thus, he initiates the studies on microbial biodiversity. Efforts aimed at the conservation of higher organisms can be correlated and modelled in microorganisms for understanding the evolutionary processes and biological interactions that sustain natural ecosystems [42]. Despite the key role of microorganisms in a wide range of biogeochemical cycles, development of pharmaceuticals, synthesis of new enzymes and chemicals and even in carrying out novel processes [43], the patterns of distribution of many microorganisms including bacteria, fungi and actinomycetes within and between the planet’s major habitat types have remain entirely unknown [40] as they are not documented properly. The documentation and bioprospection of microorganisms carry enormous significance considering their potential for human welfare. There are reports on global distributions of microbial biodiversity patterns [44]. The investigation indicated the significance of exploitation of the vast microbial resources that have enormous potentiality for remarkable scientific, social and economic impact. The motivation in understanding the microbial biogeography extends beyond drawing and interprets a map of microbial diversity.

North-east India, located in the Eastern Himalaya region, one of the mega biodiversity hotspots of the world, has pristine natural ecosystems that offer tremendous scope for the bioprospection of novel organisms, hitherto-unexplored to scientific world [25, 45]. Tea is grown in India in a wide variety of climatic variables, at latitudes from 8° 12’ to 32° 13’ N. The story and history of Indian tea industry are as fascinating as the legends associated with the origin and spread of the tea plant itself [46]. Assam tea soils represent as one of the largest reservoirs of microbial diversity [24, 47, 48]. The strategic goal of research is to develop a database that has application to fundamental research in microbial biodiversity and biogeochemistry, the discovery of new biological products and resource management, to accommodate location-specific information based on the characterization and documentation of microorganisms from this pristine soil. Geographic Position System (GPS) is used to coordinate the sampling locations, data, time, type of sample collected e.g., soil, water, mud or roots with characterized microbial taxa. Bioinformatics is an asset to trace the phylogeny of the untapped microbiota [49].

5. Methodology

Random sampling [50] is followed while collecting the soil samples from rhizosphere. Randomly collected soil samples (Fig. 2) can be mixed thoroughly and the composite sample is used in the laboratory for the isolation of microbes like bacteria, fungi and actinomycetes. The soil samples are normally kept in a refrigerator at 4±1 °C till isolation and estimation procedures were completed.

Standard protocols such as Serial dilution plate method [51], Direct count method, Warcup’s method [52] was followed to isolate the microbial populations. Different nutritional media may be employed to isolate specific groups of microbes like phosphate solubilizers, cellulose decomposers, nitrogen fixers and sulphate reducers etc. Bergey’s Manual of Systematic Bacteriology [53] and Cappuccino and Sherman [54] can be used for identification purpose of microbial isolates. Different taxonomic monographs [55, 56] are useful for fungal identification. Molecular identification of microbial cultures can be made for phylogeny construction. Physico-chemical properties of tea soil will be determined using standard protocols.

Development of microbial database that “maps” microbial biodiversity in tea growing areas of Assam can be constructed using GIS and internet accessibility system using microbiological, geochemical and geographic data and maps from standard sources. The final component of the geospatial database development can be incorporated in simple spatial analysis using already available tools of the ArcGIS platform.

6. Future prospects and challenges

Microbial species typically have higher densities and shorter generation times than macroorganisms [57]. Thus, the microorganisms can undergo in a process of rapid genetic divergence. Diversification leads to microbial speciation. The unseen microbial resources, encompassing a diverse spectrum of microorganisms, deserve immediate attention in terms of documentation and bioprospection.

The microbial diversity can be of immense value to biotechnology for the exploration and documentation of new genes [42]. In the present decade, successful assessment of microbial diversity in rhizosphere soil is possible with the consensus approach of utilizing the molecular genetic techniques along with the conventional methods. The ribosomal database, which now contains more than 10,000 complete and partial nucleotide sequences of collection cultures and clones, is being constantly enlarged in the coming decades that would allow more adequate interpretation of 16S rRNA sequences of the total DNA extracted from rhizospheric soil [58]. The metagenome analysis involves the cloning of the large

Fig. 2: Random soil sampling.
fragments of soil bacterial DNA [59] and express cloning may be the further progress in this line. Knowledge on microbial diversity in terms of their geographical distribution as well as mapping model is, however, a novel concept. Exploration of tea soil microbial diversity from diverse ecological niches of Assam would hold promise for the isolation of biotechnologically significant microbial strains and even novel species since it is typical to retrieve hundreds to thousands of microbial taxa from a single gram of soil [60]. Thus, there is a perceived need to develop databases with emphasis on the microbial diversity of the largest untapped reservoirs in the biosphere. Mapping microbial biodiversity has important implications for the stability and functioning of overall ecosystem processes. The continual and dynamical development of faster sequencing techniques, together with the advancement of methods to deal with the exponentially increasing amount of data generated, are expanding gradually for the analysis of microbial communities from an unlimited variety of habitats and environments.

7. Conclusion
As microorganisms are rapidly disappearing from the earth due to anthropogenic disturbances [61], an approach for proper documentation of microbes would be instrumental in protection and bioprospection of microbial biodiversity [62]. The need to incorporate microbial indicators into existing indexes is now unquestionable. The reliability of microbial indicators depends on how well one can capture the variety of responses which characterize the inherent diversity of microbial communities. Knowledge on relationship between microbial population numbers and their overall function is required [63] to fulfil the aim of basic and applied scientific research and natural resource management. As the potentiality of beneficial microbes in agriculture is steadily increased since it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements [16, 64]. The present work is also aimed in exploit the diversity of region specific beneficial plant growth promoting microorganisms (PGPMs), research on which would better facilitate their application as a reliable biological component in sustainable tea plantation system. Carefully annotated conservatories of microorganisms located in strategic geographical regions would facilitate rapid technology transfers in future.

8. Acknowledgements
The authors are thankful to the Director, Tocklai Tea Research Institute (TTRI), Tea Research Association (TRA), Jorhat, Assam, India for providing the necessary facilities to undertake the study.

9. References