Abstract

Actinobacteria which were previously known as actinomycetes or ray fungi form an important constituent of the microbial biome. It comes next to proteobacteria in terms of number and distribution. Actinobacteria are mostly aerobic, gram-positive to gram variable with high G+C content and occupy a diverse microbial niche. They share some characteristics with fungi, such as colony morphology, mycelial growth and musty smell, on the other hand, peptidoglycan cell wall structure is common with bacteria. A phylogenetic study based on 16SrRNA classifies actinobacteria into six classes i.e., Acidimicrobia, Coriobacteriia, Nitriliruptoria, Rubrobacteria, Thermoleophilia and Actinobacteria.

Actinobacteria form an integral part of the microbial community which is involved in antibiotic and secondary metabolite production, bioremediation, nitrogen fixation, etc. They are in constant interaction with other organisms as symbiotic or pathogenic microbes altering the immediate environment. Bioactive compounds produced by microbes exhibit pharmacological or toxicological effects in other organisms. Actinobacteria have been considered as one of the significant groups of microorganisms as they represent a broad range of valuable and prominent sources of pharmaceutically active metabolites. These metabolites are detected by GC-MS technique and are utilized as antimicrobial and anti- cancerous agents. In nature, actinobacteria play a pivotal role in the cycling of organic compounds and have also been associated with soil organic matter production, owing to their black pigments called melanin, which are related in

respect to soil humic acid.

Among actinobacteria, *Streptomyces* is so far the largest genus with approximately 900 species. Hence, this genus is a valuable resource for novel secondary metabolites (SMs). The most widely distributed genus of actinobacteria, Streptomyces has been the focus of research by biologists because of the commercial applicability of the substances produced. S*treptomyces*, a soil-dwelling, high GC content, gram-positive,actinobacteria continue to be a promising microorganism for biosynthesizing several clinically important secondary metabolites including antibiotics, antiviral, anti-fungal, anti-tumoral, anti-parasitic and immune-suppressors.

Human activities are creating new compounds and the microbial community is constantly evolving to cope up with this stress. The mechanism of action of the microbial community *in vitro* and *in vivo* conditions may deduce the remedy for degradation of the environment as well as the drug resistance in organisms. Agrochemicals such as pesticides and fertilizers are essential inputs to improve the productivity of the farmer in terms of farm produce, but their use is also associated with chemically induced injuries to health. In order to overcome this problem Integrated pest management utilizing microbial pesticides is gathering momentum. This is because they are more specific, have low relative cost and are more eco-friendly.

The ongoing proliferation of whole -genome sequences is a stepping stone for systems biology which aims to study the integrated network constituted by the complete repertoire of genes (genome), the population of transcripts (transcriptome), the population of proteins (proteome), the population of metabolites (metabolome), and fluxes of an organism or cell, in relation to intrinsic and environmental stimuli.Codon usage patterns and preferences vary significantly within and between organisms.

Recent advances in genome sequencing-based approaches have revealed that most of the secondary metabolite gene clusters (SMGCs) of actinobacteria are condition-dependent and are inactive under normal laboratory conditions. Thus, the SMs producing capacity of actinobacteria has been under-estimated. The proportion of uncultivable microbes to cultivable microbes is still very high. To circumvent such cultivation limitations, strategies have been developed based on the extraction of microbial DNA directly from an environmental sample and its subsequent analysis or exploitation (for biotechnological purposes) independent of its original host. This approach, which is based on the recovery of a sample's microbial metagenome (the sum of all microbial genomes), has, in theory, great potential for biotechnological and ecological studies of the system under investigation, for example soil. Such a metagenomics-based view of the community will aid in enhancing our understanding of microbial functioning and interactions in a soil ecosystem.

Bioremediation and bio-augmentation are the techniques for the improvement of the capacity of contaminated soil or water to remove pollution by the introduction of specific capable strains of microorganisms. In this context, it is vital to identify and characterize the microbial consortia mainly consisting of actinobacteria from native as well as pristine biotopes with promising qualities for bioaugmentation. This will help to improve the production and quality of tea and increase its demand in the international market.

Hence, the objectives of this present study were to identify and characterize the actinobacteria from different biotopes. Molecular documentation of different actinobacterial strains using16S rRNA primers and Whole-genome sequencing of a few potential actinobacteria such as *Streptomyces* were carried out. Comparative analysis of different genomes through bioinformatic tools was also part of this research. Apart from this, Bioassays of selected strains and the GC MS analysis of microbial cultures for their metabolites were also done. Another aspect of this current study was the Metagenomic analysis of soil microbiome from different biotopes such as tea gardens, mangroves, rubber plantations, paddy fields, forests and vegetable fields. Moreover, a survey of the health condition of tea garden workers in the Terai and Dooars region was carried out with the help of resident tea garden physicians and other concerned authorities through interviews and literature surveys.

Characterization of the isolated microbes by slide culture technique and growth in different media was studied along with Gram's staining and spore chain morphology studies using standard protocols. Biochemical studies were done for, nitrate reduction, melanin production, starch hydrolysis, etc. Molecular Identification of Actinobacterial Isolates by 16S rDNA amplification using actinospecific primers was executed. Sequence similarity studies and comparative genomics were carried out using the available algorithms.

We could isolate several actinobacteria from various biotopes but the further characterization was limited to *Streptomyces* isolates. There were 25 *Streptomyces* isolates from soil samples of mangroves, 10 from tea garden, 8 from the vegetable field, and 2 each from Rubber estate and forest soil which was isolated on selective media- Streptomyces media and International

Streptomyces Project (ISP 4). The isolate VRA 1 showed maximum inhibition to all of the test pathogens and the antagonistic activity of the newly isolated strains proved their potential to be utilized as antimicrobial agents in the future. The ability of the isolates to produce industrial enzymes like lipase, gelatinase, amylase and cellulase makes them industrially important. The isolated strains were found to have PGPR activities such as Phosphate solubilization, IAA, Ammonia production. The ability of the strains to produce extracellular enzymes cellulase further adds to their PGPR potential as cellulase is known to contribute to their antagonism against phytopathogens. The isolates with PGPR were found to improve shoot length, root length, fresh weight of the mung bean plant. It is promising because the actinobacterial strains with PGPR reported in this study can be exploited for biofertilizer production. GC-MS analysis of the extract produced by the selected strains, it was found that there were many bioactive compounds with antimicrobial, antifungal and antioxidant activities. Thus, the isolate with the potential to produce antimicrobial compounds has been further explored for its bioactivity against pathogens that cause various diseases. Further, the antimicrobial compounds were then assessed by in silico molecular docking approach for Tuberculosis and enteric disorders that were prevalent among the tea garden workers. We found that compounds Celidoniol,Pentacosane, Deoxyspergualin, Nonalactone showed the highest binding affinity with tuberculosis proteins (1USL) and enteric toxin(2NRJ).The potency of these compounds can be further validated by *in vivo* studies and could be utilized by the pharmaceutical industry.

The five isolates were sent for the

whole genome sequencing. The sequencing was done by MicrobesNG at the University of Birmingham, the UK using Illumina sequencing technology. The codon usage of the four sequenced isolates (VRA 1, VRA 16, TEA 02 and TEA 10) and the selected *Streptomyces* strains along with an out-group *Kitasatospora* indicated towards the biased use of GC rich codon. This shows that GC richness is an important factor that determines codon usage. The effective number of codon (Nc) of the genomes suggests that these high GC-rich genomes exhibited considerable heterogeneity in codon usage. A strong positive correlation between CAI and Fop was observed in all the cases revealing the utilization of the majority of optimal codons by PHX rather than PLX ($p<0.01$). CAI values also showed a significant positive correlation with GC3 and Nc values attributed to the strong compositional constraints among the genes. RSCU was also found to correlate strongly with Fop, GC3 and CAI This correlation pointed towards the preferred usage of GC3 rich optimal codons and a certain level of translational selection on these genes. Hence, we may hypothesize that GC compositional constraints along with the translational selection have an influence on the codon usage as well as expression pattern. The pan- core plot has been given in a total of 18759 genes were accumulated in pan-genome. However, the core gene count was reduced to 152. The core genes were found to be the housekeeping genes involved in some major metabolic and signal transduction pathways.

The antiSMASH studies of strains showed that they harbor several secondary metabolite biosynthetic gene clusters (BGCs). These findings may help to explore and utilize the secondary metabolites in agriculture, the pharmaceutical industry, etc.

The metagenomic study of the soil samples from different biotopes provided us with valuable insights into the soil microbiome. The study of soil samples from different altitudes of Darjeeling hills along with the rhizospheric soil from an actinorhizal plant *Alnus nepalensis* showed the abundance of diazotrophs in the rhizosphere.

We also targeted two very popular tea gardens of Darjeeling hills- Makaibari (Mak) and Casselton (Cas) to study the soil community through a metagenomic approach. Our studies showed that the abundance profile of pathogenic microbes was much higher in Cas than in Mak. This also underlined our survey results which has shown the prevalence of tuberculosis and gastric disorders among the tea garden workers.

Based on the OTU number, Chao1 bacterial species abundance index, and Shannon microbial diversity index, α-diversity analysis was conducted on the microbial diversity of various samples. The metagenomic analysis of paddy field, tea garden, and other selected biotopes showed the abundance of *Candidatus* solibacter of *Acidobacterium*. The actinobacterial population was much reduced in the studied biotopes. Among the bacterial population, the genus *Isophaera* showed high density, followed by *Gemmatimonas*, *Bfidobacterium*, *Frankia*, *Streptomyces* etc.

It is apparent from the current work that actinobacteria has the potential to deliver innumerable metabolites which could solve the problem of emerging antibiotic resistance, help in novel drug production. The quality of tea can be improved by the use of organic manure and biofertilizers which will reduce the MRL. This will in turn boost the tea industry which aids in the development of the tea garden community. The metagenomic studies indicate the reduced species diversity in the agricultural fields and plantation areas. It is an alarming situation, where the microbial population is affected by manmade activities and this may pose threat to our own existence. However, it has also shown that use of organic manure favours the microbial population which is promising.■