

# Conclusion

*Live your life as an exclamation rather than explanation.*

Sir Issac Newton

The present work was initiated in the year 2018 with a focus to study the diversity of the actinobacterial population from selected biotopes of our country. We envisaged both *in vivo* and *in silico* approaches to tap the unexploited microflora of these biotopes and the identification of bioactive metabolites which can be further explored for novel drugs, bio-fertilizers, etc. From the social perspective, we also documented the health status of tea garden workers of the Darjeeling and Dooars regions. The main aim was to understand the correlation between microbial flora and

its impact on the health of the workers. The soil microbiome from biotopes such as tea gardens, paddy fields, vegetable gardens, rubber plantations and mangroves were considered for the diversity studies using metabarcoding studies. The isolation and characterisation of actinobacteria was mainly focused on *Streptomyces*. It was isolated and characterized by morphological and biochemical methods. Selected isolates were characterized up to molecular level by 16S amplification. Out of the total 65 isolates, five of the isolates were sequenced to the genome level.

The biochemical studies of these strains revealed several potential properties such as melanin production, cellulase production, lipase production, etc. Apart from this, the selected isolates were employed for PGPR studies in the mung bean plant. Among these, one of the isolates VRA-16 significantly exhibited PGPR properties. This particular isolate could be a good agent for biofertilizer production.

Furthermore, the culture extracts of two strains were sent for GC-MS analysis which had helped us to detect several bioactive compounds. Some of the selected metabolites were subjected to molecular docking with tuberculosis proteins 1USL,1BES,2VVO,3BSQ and 2NRJ,3PYD.5V8E (enteric toxins of *Bacillus cereus*). The compounds Celidoniol, Pentacosane showed a docking score of more than -7 can be explored further for novel drugs. The study of the bacterial gene clusters by antiSMASH software further elucidated the potential of the newly isolated strains and showed the presence of various antibiotics, pigments etc.

Bioinformatic tools have become

indispensable nowadays because of the surge in genomic data. We have done the whole genome sequencing of five of the characterized isolates. Codon usage analysis of the selected genomes along with the sequenced isolates has been done. It has revealed the preference of GC-rich codons which is the characteristic attribute of the actinobacteria. The lower effective number of codons showed higher GC, GC<sub>3</sub> as well as Fop and it is evident that GC richness was influencing codon usage in *Streptomyces*. RSCU analysis showed the optimal use of G/C ending codons and C ending codons were more frequent than G ending codons. Codon usage heat map displayed the use of GC rich codon GCC, GAC, GGC. The amino acid usage analysis showcased the use of Alanine as most-used amino acid followed by Leucine, Glycine, Valine. GC3-NC plot of the protein-coding genes of the strains displayed the deposition of PHX and PLX separately and the PHX genes were more towards the extreme GC-rich region as expected. The correlation between CAI and GC<sub>3</sub> as well as Fop underlines the fact that gene expression

was affecting codon usage variation and its frequencies in the genomes.

Phylogenetic analysis by 16S and MLSA tree exhibited similar grouping of the isolates in both cases. BLAST matrix is used to compare the similarities and differences among the genomes. The Pan Core genome plot studies revealed vast differences between Pan-genome and Core-genome which underlines the diversity among the genomes.

The 16S amplicon sequencing-based on V3-V4 region was carried out for the soil samples from different biotopes. The soil microflora exhibited distinct patterns for all biotopes. The rhizosphere and non-rhizosphere of the actinorhizal plant *Alnus nepalensis* provided an interesting observation which showed the prevalence of diazotrophs in the rhizosphere. This might be due to the phyto signals which attract the selective microbial community.

Another very important and significant findings from one of the metagenomic analyses was that chemical fertilizers are influencing the pathogenic population in the tea garden. Contrastingly, organic manure favors

the growth of beneficial microflora in the tea garden. This observation emphasizes the benefit of organic manure thereby influencing microbial communities which may indirectly affect the health of the human population. The amplicon sequencing studies of other biotopes highlight the diverse population of the microbiome in the respective biotopes. These studies indicate that rubber plantation is displaying unique microbial population, whereas paddy field and vegetable field were exhibiting more or less similar micro biome .The tea garden and mangroves were showing analogous behavior in microbial distribution, this may be due to the extremophilic nature of the habitat.

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.■