Chapter 5
CONCLUSION

Before I conclude my work, I must make a summary of it. So, I summarize my whole work in a point wise fashion, which is as followes :

•*Frankia* strains from different parts of West Bengal were isolated from the nodules of *C. equisetifolia* and *A. nepalensis* by a newly standardized isolation technique.

- The culture characteristics and Field Emission Scanning Electron microscopy of the bacterium was performed.
- Effect of different media and hormone concentrations on the germination of seed of *A. nepalensis* were studied.
- The characteristic root hair deformation and visible nodule formation in *A. nepalensis* and *C. equisetifolia* seedlings authenticate the presence of *Frankia* in the medium.
- Physiological characterization of *Frankia* strains were performed.
- Heavy metal resistance profiling of *Frankia* strains were performed and heavy metal induced pigment production of *Frankia* strains was reported.

•Codon usage and codon preferences, G+C composition, effective number of codon (Nc) and codon adaptation index of heavy metal resistance genes of *Frankia* strain EAN1pec, ACN14a and CcI3 were calculated. From these study the life cycle pattern of *Frankia* strains were predicted.

- A new pair of PCR primers specific for distal part of 16s rDNA region of *Frankia* were developed and subsequently, a 520 bp long portion of *Frankia* genome were amplified with those primers followed by PCR-RFLP profiling revealed the diversity of *Frankia* in West Bengal.

At the time of starting of my work, a simple but basic question was in my mind, that was the problem of studying the diversity of *Frankia* and I set my mind to work on it. After starting the work, I found that isolation of *Frankia* was a big problem and there was no

easy technique for isolation of the endophyte from the nodule. Ironically the solution of this question opens up many avenues. I choose a bidirectional approach to find the answer of the diversity related questions. One was strain based work and other was metagenomic work. At this point of study, I decided that I should stick to common parameters only for studying the diversity of both *Alnus* based *Frankia* and *Casuarina* based *Frankia.* I selected standard biochemical and metal resistance parameters for them. On the basis of these results it was found that *Frankia* population of this region was divided in to two major clades. The first clade included the bacterium isolated from *C. equisetifolia* and the second clade included bacterium isolated from *A. nepalensis*. Each clade was again sub divided into two minor clades. This pattern supported the presence of two physiological groups among the bacteria isolated from each host. The results of the metal resistance work were also interesting. *Frankia* strains isolated from *A. nepalensis* were less resistant to the heavy metal salts than the strains isolated from *C. equisetifolia* . It was hypothesized that *Frankia* isolated from *A. nepalensis* facing less heavy metal stress than the *Frankia* isolated from *C. equisetifolia.*

In the year 2007, three *Frankia* whole genome sequences were available in public domain. I took this opportunity to start studying the metal resistance genes of *Frankia* with bioinformatics tools like CodonW, ClustalW, CAI calculator, etc. The metal resistance genes of *Frankia* had high CAI values which was indicating better expression levels. These genes also showed strong codon bias, the result obtained in this study put an additional support to the hypothesis that *Frankia* strain CcI3 is more symbiotic than saprophytic, since they remain largely in the nodules (a protective place for them) and hardly grow in the soil. This kind of work was first of its kind in India.

For metagenomic work, it was found that there was no PCR primer that had the ability to amplify the portion of 16s r-DNA of both *Alnus* based and *Casuarina* based *Frankia.* So, we had developed a new PCR primer for that and found positive results. To study the diversity, the amplicons were digested with restriction enzymes and PCR-RFLP profiles were obtained. Based on those PCR-RFLP profiles, it was found that a

more or less distinguished pattern emerged in case of *Alnus* based *Frankia.* The *Casuarina* based *Frankia* were places in different clades. The probable reason of *Casuarina* based *Frankia* behaving differently from *Alnus* based *Frankia* could be that all the *Casuarina* plantations in West Bengal are exotic and were perhaps brought from different places.

Now, it is the time to answer the question which I had asked at the time of staring of my work. I shall answer it in a positive mood. I have successfully detected the diversity of both *Alnus* based and *Casuarina* based *Frankia* sticking on the common parameters. Although their diversity was totally intrapopulation in origin and do not cross the line of speciation.