Conclusion

I started the present work in the spring of 2013. My prime idea was to study *Alnus nepalensis*, plant makes symbiosis association with *Frankia* and in turns increases the fertility of soil. I was particularly interested because, being grown in sub-Himalayan region of West Bengal, I have always noticed that *A. nepalensis* is one of the important species which visits early in the landslide regions. *Alnus* therefore is an excellent example of successional plant. My particular interest however the haemoglobin (Hb) management of *Alnus,* since beginning, I wondering how *Alnus-Frankia* symbiosis can manage to protect the nitrogenase from oxygen, as it is known that nitrogenase are oxygen labile and also thought whether Hb present in plant and bacteria may play any role or not in this regard.

Here I studied the Hb genes and proteins in respect to *Alnus-Frankia* symbiosis and the conclusions derived are given below:

I found that *A. nepalensis,* is distributed with a geographical boundary of 26°87′78″ to 27°34′71″N and 88°25′00″ to 88°61′76″E and an altitude ranging from 3616ft to 7598ft in sub Himalayan West Bengal and Sikkim. Each micro-ecological factors were found to be equally important for proper growth of this plant in fragile landslide area with specifically 19% to 31% relative humidity and acidic soil conditions. Study of *Alnus-Frankia* symbiosis showed that they can exist under extremely variable soil nutrient conditions but favors moderate to high soil carbon.

Population genetics study revealed that entire population of *A. nepalensis* in sub-Himalayan West Bengal and Sikkim were clusters depending on their geographical distribution. The species within selected area were found

to be genetically close to each other with minor exceptions. *A. nepalensis* collected from population I showed high and III showed low percentage of polymorphism amongst studies locations.

It has been also found that *A. nepalensis* collected from eastern part (population I) of Darjeeling hills were genetically more similar to western part (population II) of Darjeeling hills, while genetically distant from Kalimpong and Gangtok hills (population III), which situated other side of river Teesta. River Teesta bisects collection sites of population I and II with III, keeping population I and II on its right and population III on its left bank respectively, and may act as geographical barrier for dispersal of germplasm of *A. nepalensis* in studied region.

In-sillico analysis of the physiochemical properties and functionally active motif annotation revealed that class II non-symbiotic Hbs (nsHbs) showed a remarkable resemblance with class II symbiotic Hb (sHb)/legHbs (Lhbs) and those properties differ them from that of class I Hbs and ptHbs. Results also showed that actinorhizal Hbs (*Alnus firma, Casuarina. glauca, Myrica gale* and *Datisca glomerata*) showed general trend with other plant Hbs (pHbs), whereas nsHb *M. gale* had higher oxygen binding capacity compared to other actinorhizal class I nsHbs.

Sequence based analysis of actinoHbs (actinorhizal pHbs and actinobacterial Hbs (bHbs)) revealed that *Datisca* ptHb shared two common functionally active stretches with bHbs consisting of 50 and 21 amino acid sequences, containing “YjbI” like activity, which is responsible for inorganic ion transport and metabolism. This unique character found only in ptHbs similar to bHbs but totally absent in other pHbs. Sequence comparison also revealed that residue present in F7 position in actinoHbs, govern the stereochemistry of proximal histidine conserved in F8 position and moreover, this association revealed that the actinoHb proteins were might be structurally not related to the ferredoxin-NADP+ type reductase and ion transport mechanism but involve in a different mechanism to reduction of oxidized haem iron into ferrus form. This finding may suggest that actinorhizal pHbs are associated with the mechanism which is not related to inorganic ion transport and to

substantiate this property ptHbs may evolve into plant system from bacteria.

Sequence comparison of actinoHbs also revealed that single amino acid replacement of tyrosine-B10 by phenylalanine is responsible for higher O_2 dissociation constant in the actinorhizal nsHb and sHb than ptHbs and bHbs and replacement of phenylalanine in CD1 region lower the oxygen affinity in actinorhizal ptHb.

Codon usage properties revealed that *Frankia* Hbs are codon biased and expressed in a moderate to high manner and depending upon their 1) GC compositional constrains and 2) natural selection on their transitional efficiency. Whereas functional annotation revealed that the different functions associated within same genera of bHbs are depending on 1) their host specificity and 2) eco-geographical habitat.

Homology modeling revealed that the protein structure of actinorhizal Hbs (sHb, nsHb and ptHb) are homodimeric with heme cluster of hetero atom in the centre of each monomer. The arrangement of core region of actinorhizal sHb and nsHb proteins were made by the polar serine and glutamate residues, and covered by the apolar sidechain residues of isoleucine-46, valine-120 and phenylalanine-123, which made possible the electrostatic interaction within core region. However this type of cover-up arrangement by apolar residues were found to be absent in *Datisca* ptHb, similar to *Frankia* bHb, which subsequently reflect the packaging of protein structure in *Datisca* ptHb was not good enough as like other actinorhizal pHbs (sHb, nsHbs).

Structure based analysis revealed a common nest amongst modeled nsHb proteins, i.e. Ala87(A), Gly88(A), Lys89(A), which substantiated their functionality and made the protein stable. Whereas conformation dynamics revealed that *Casuarina* sHb and *Datisca* ptHb shows dissimilarities with actinorhizal nsHb proteins. *Datisca* ptHb showed deformation energy subsequently lowest than other actinorhizal Hb proteins and signify that protein can deformed very easily, and therefore confirms the result obtained from physiochemical data that *Datisca* ptHb protein is unstable than that of other actinorhizal Hbs.

Phylogenetic analysis based on structural elucidation along with motif analysis and functional divergent analysis pointed that plant truncated

Hbs (ptHbs) showed lower divergence rate with bHbs than that of other pHbs. The evolution of ptHbs might have taken place to overcome the inorganic ion transport and related metabolisom into plant system.

A partial mRNA Hb gene from *A. nepalensis* was identified, which showed 96% similarities with class I nsHb of *A. firma*. The expression study of Hb genes depicted that Hb expresses in an elevated manner in nodules, when inoculated with *Frankia*, but the expression level is significantly high in untreated plant root region. This might be due to its search to find out their microsymbiont *Frankia* for interaction.

Therefore from this study I may conclude that by improving the efficiency of Hb genes in both host plant and its microsymbiont, the global nitrogen balance may be improved, this will in turn benefits the society and ecological wellbeing.