

# Conclusion

Integration of ecological and genetic approaches in molecular studies along with geographic distributions of organisms is becoming more common (Johnson and Cicero, 2002; Lapointe and Rissler, 2005). This is partly because of increasing amount and accuracy in the process of collecting socioeconomic and natural data, housing fine-scaled climate data and new modelling techniques (Graham *et al.*, 2004; Elith *et al.*, 2006). The use of such kind of database in environmental niche modelling (Soberon and Peterson, 2005) with relation to the species is proving to be a powerful means in understanding how abiotic factors like temperature, precipitation, seasonality (Graham *et al.*, 2004; Wiens and Graham, 2005), aspect, elevation, slope, etc. influences the geographic limits of lineages and species. To address the idea I have made an approach to use remote

sensing satellite FCC data along with Survey of India maps (both 1:50000 scale) in generating an environmental map (land use & land cover, slope, elevation and aspect) to study the environmental factors of *H. salicifolia* growing area. Land use and land cover data proves especially valuable for predicting the distribution of both individual species (Jennings, 2000) and assembly of different species (Kerr *et al.*, 2001) across broad areas that could not otherwise be surveyed because of the tough and some inaccessible terrains of the study area.

Variations in environmental factors like nutrient concentration, nutrient availability, temperature, pH, etc. have been found to affect the kinetics of soil microbes directly. The environmental factors act as a gene controlling enzyme system (McArthur *et al.*, 1988). Therefore, there must be a

relationship between genetic diversity of *Frankia* populations with their environmental conditions. Studies have also been conducted on microbial populations to show close relation between the genetic diversity and environmental changes of a particular ecosystem (Nevo *et al.*, 1984) and some evidences have proved positive relation between genetic polymorphism and environmental heterogeneity (Hedrick *et al.*, 1976). The *Frankia* population diversity has been shown to be affected by ecological factors determined by elevation, like temperature, precipitation, etc. and soil composition parameters like pH, OC, moisture, type, etc. (Chen *et al.*, 2008).

As has already been discussed in previous chapters, *H. salicifolia* are good sources of dietary supplement, natural antioxidants including carotenoids, vitamins, phenols, flavonoids, and a high level of antioxidative property against free radical species. The total antioxidant activity can vary from plants growing in different environmental conditions, and these may affect overall protective benefits of human health, which needs to be further investigated. Antioxidant study from the leaf and bark of male and female plants of *H. salicifolia* is

the first report from Sikkim Himalayas but there may not be any report on antioxidant (phenolics, flavonoids, etc.) of the host plant affecting genetic diversity of *Frankia* in *Hippophae*.

It has been understood that during biotic interactions, plant secondary metabolites and phenolics give positive signals, during plant-environment interactions. One such interaction is during the early stage of nodule formation and establishment of symbiotic relationship, phenolic based reorganisation mechanism between the host and bacterial symbiont is involved (Gould and Lister, 2006). These signal interaction mechanism is well studied in mycorrhiza (Steinkellner *et al.*, 2007) and legumes (Cooper, 2004) but very little is known about the role of phenolics in the symbiosis between actinorhizal plants and *Frankia*, and no attempts have even been made to study this type of signal interactions in *Hippophae salicifolia*. There has been a report on the responsiveness of *Frankia* strains to *Myrica gale* fruit exudates phenolics with their symbiotic compatibility by Popovici *et al.* (2010). However, my study apart from providing the antioxidative property of *H. salicifolia* of Sikkim has opened up a new scope for using these compounds

to correlate the symbiotic interaction of host-microorganism and genetic diversity of *Frankia* to get a better cultivar of this ecologically and economically important plant species.

Quantification of root nodules from *H. salicifolia* plants growing in different locations of the study area can be attributed to assessing the population size of *Frankia* in the study area. Jeong and Myrold (2003) have also stated that counting of nodule from different actinorhizal plants and can be the indirect means of measurement of population size of host infective *Frankia* population in the soil.

Vegetative propagation of *H. salicifolia* using different means like cuttings using different combinations and proportions of growth regulators and layering in different soil combinations was studied. Further propagation through seeds in combination with different chemicals to study breaking of dormancy was carried out in this study. The species showed its survival and adaptability in a different condition from its natural habitat. The outcome of this study may not be directly involved in the study of genetic diversity but the outcome of this can be useful in planning, getting desired selected plant accession and

faster propagation compared to natural condition. The root nodules out of these propagated plants could have given an interesting result, if *Frankia* could have been isolated from them in pure culture. Insufficient nodules per plant for genomic DNA isolation hindered study of genetic diversity due to environmental and time factor to complete this research work, which will be taken up in future.

During the study of genetic diversity of *Frankia* associated with *Hippophae* of Lachen, I took up the environmental parameters of the study area, which can also reflect the plant-environment relationship on the genetic diversity of *Frankia* through the host. Intense studies have revealed that host plant controls the root nodulation and nitrogen fixation processes in actinorhizal plants (Dobritsa and Novik, 1992; Wall and Huss-Danell, 1997; Valverde and Wall, 1999). Environmental factors like light, water, soil nutrients availability, soil pH, etc. and bacterial factors such as physiologic state, population and ability to fix nitrogen are also thought to effect nodule development, growth and function in actinorhizal plants (Huss-Danell 1997). In my study also it was revealed that the roots of *H.*

*salicifolia* growing in the riverine areas possessed more nodulations compared to non riverine areas. However, the difference in diversity observed could be understood by the characteristics of the niches occupied by the plant, which in turn controls its microsymbiont in the root. Therefore we can conclude that environmental factors control the genetic diversity of *Frankia* in the root nodules of the actinorhizal plant and *H. salicifolia* cannot be an exception.

Earnest beginning in the study of microbial aspect of *Frankia* species started only 15 years ago when first strain was announced. It was also found that *Frankia* were slow growing organisms and much of intervening time has been spent on establishment of different techniques of growing *Frankia* and pursuing hypothesis related to the nature of organism, its vesicle, spores and physiology evolved from the past studies. If we see from the global perspective, fair amount is known about *Frankia* strains on phylogenetic position, structure and function of spores and vesicles, physiology of carbon and nitrogen metabolism, ecology in the soil and nodule. Recently some details have started pouring in about the genetics of this unique microorganism. In my

study, I also isolated *Frankia* strains of *H. salicifolia* in pure culture, which may also be the first isolation from the Hills of Sikkim. The same has been reflected in the results and discussion chapter (chapter IV). This has led to the understanding of the structure of this isolated strain and also helped in conducting plant infectivity test. The amount of *Frankia* colonies could not suffice the isolation of genomic DNA from pure culture, hence genomic DNA for its genetic diversity was isolated from the root nodules directly, as has been done in many cases of such studies in various actinorhizal plants.

During the infectivity test *in vitro* and *in situ* the *Hippophae* plants showed positive results by getting infected with the *Frankia* inoculums. This has not only showed that *Hippophae-Frankia* strains are spore+ but has also opened up an area to carry out more work to study communication mechanism between the actinomycetes and the host actinorhizal plants in the rhizosphere.

Phylogenetic comparisons of *Frankia* and its close lineages are promising approach to understand the evolution of the organisms. Out of 21 actinorhizal genera described (Benson and Silvester, 1993), 11 genera fulfilling Koch's postulate could have

their 16S rDNA sequence determined and compared for its diversity study (Normand *et al.*, 1996).

For the presence or absence of actinorhizal plants, climatic and soil conditions are the factor that always influences *Frankia* (Dawson *et al.*, 1989; Huguet *et al.*, 2004; Oakley *et al.*, 2004). This study focuses on single *Frankia*-nodulating species growing in variable microclimatic region at different elevations, aspects and slopes of Lachen valley. Total of 11 DNA isolates gave positive results when subjected to PCR. Study of genetic diversity using *H. salicifolia*-*Frankia* genome and comparing it with other genome sequences having 16S rRNA, and also PCR-RFLP study, provided some interesting facts related to genetic diversity of *Frankia* strains of *H. salicifolia* occupying this niche with physically untraceable climatic conditions. It is understood that distribution of this *Frankia* strain is also basically affected by altitudes or the host plant growing habitat. Reports by Dai *et al.* (2004); Igual *et al.* (2006); Jeong and Myrold (1999); Oakley *et al.* (2004) and few others supports this findings that topography and altitude plays a vital role in composition of *Frankia* community. Soil in the study

area was almost the same throughout, so not much difference was observed due to soil conditions or their compositions. Although some authors like Burleigh and Dawson (1994); Crannell *et al.* (1994) have co-related that soil pH, organic matters, etc. may influence *Frankia* genotypes but from various studies and results expressed in chapter IV, especially with reference to *Frankia*, even soil pH or organic matters, had no major role to play in *Frankia* population and distribution in Lachen valley. Some ecological factors like altitude, aspect, slope and microclimatic conditions like temperature and moisture in the *H. salicifolia* growing area have shown the genetic diversity among its *Frankia* strains, perhaps through host or direct impact, and also may be the major determinants of *Frankia* population in the study area. Similar work based on *Frankia* population diversity on *H. rhamnoides* taking few ecological parameters focussing on elevation and plant cover by Chen *et al.* (2008) supports my findings.

With the advent of many innovative and informative researches throughout the world on evolution and genetic diversity of *Frankia* (in the soil or in different actinorhizal hosts or in

different ecological conditions involving different ecological and environmental parameters), many opportunities to study this organism are coming up. It is felt that there are still more potential working areas in the days to come, which include studies on genetics and molecular genetics of the host plant infection process, symbiotic interaction, the phylogeny and taxonomy of those strains that have yet to yield infective strains and physiological studies in relation to symbiosis. Above all, there is a great need to understand integrated phylogenetic and taxonomic studies of *Frankia* strains to understand its actual phylogeny and taxonomy, which will lead to universal identity of this microorganism. Presently, out of many phylogenetic studies carried out by researchers globally, the outcome is based either on a particular host, region or environmental factor. Very few comparisons have been found, which is not sufficient to present the actual status of *Frankia* strains on a global basis. The work is difficult and time consuming too, but such genetic studies would help to lay a base with a

unifying hypothesis to answer questions like why actinorhizal nodules form on certain distantly related plants but not on other plants of the same family. This may share co-evolution theory of both the symbionts and their diversity.

Broadly, actinorhizal plants play an important role in different environmental conditions. *Hippophae salicifolia* growing in the Lachen valley of Sikkim too has been found to occupy an important and influential position in the existing ecosystem, especially in the context of climatic changes, which are proposed to occur in the next few decades. Since these species can survive and ameliorate the effect of vegetation losses because of nutritional deficiency in the soil, they can be a pioneering species to keep the vegetation integrity of the area but its practical issue of actinorhizal symbiosis needs to be addressed along with genetic diversity, which will not only explain its phylogeny and evolution but shall also help in understanding better variant of *Frankia* strain for future planning of better eco-friendly cultivar.