Summary and Conclusion

- Diversity of total endophytic community and culturable nitrogen fixing endophyte community of five *Poaceae* plants (Rice, Wheat, Sorghum, Pearl millet and Maize) by DGGE analysis and band sequencing showed diazotrophic community to be similar in different plant parts but different than total endophytes.
- Diazotrophic endophytic bacteria (31 isolates) were obtained from different parts of five *Poaceae* family plants by repeated growth on Nitrogen free media.
- Identification of diazotrophic endophytic bacteria showed the affiliated to *Actinobacteria*, *Proteobacteria* and *Firmicutes* representing 14 genera were isolated, where *Arthrobacter*, *Rhizobium* and *Bacillus* spp. were more cosmopolitan.
- Diazotrophic endophytic bacterial isolates showed presence of *nifH* gene and sequences of selected *nifH* genes showed similarity with the nitrogenase reductase of *Pseudomonas stutzeri*
- Diazotrophic endophytic bacterial isolates showed plant growth promoting traits such as the production of siderophores, indole acetic acid, phosphate solubilization and antagonistic activity towards fungal pathogen and secretion of hydrolytic enzymes. A number of traits in each organism varied; siderophore production was seen in all isolates while antifungal activity in the least number of isolates.
- Cross-colonization of total thirteen gram-negative endophytic bacterial isolates in wheat plants was observed by tagging the strains with constitutively *gfp* expressing pHC60 plasmid and confocal laser scanning microscopy (CLSM) technique. Re-isolation of bacteria from inoculated plant parts showed maximum colonization in root and least in leaves.
- Pot inoculation experiments conducted using diazotrophic endophytic bacterial isolates on wheat plants for 30 d of growth period showed good growth promotion by most isolates and correlated with siderophore and IAA production traits of the endophytes.
- Diazotrophic endophytic *Streptomyces* spp. showed the biocontrol activity against the cereal pathogen *Rhizoctonia solani* and rice pathogen *Magnaporthe oryzae* B157.
- GC-MS analysis of the antifungal extracts of diazotrophic endophytic *Streptomyces* spp. showed the presence of 2-(chloromethyl)-2-cyclopropyloxiran, 2, 4- ditert-Butylphenol, and 1□ethylthio□3□methyl□1, 3□butadiene in different strains.

- The EGFP (pIJ8660) tagging of *Streptomyces* spp. showed their ability to enter and colonize wheat and sorghum plants using CLSM technique. Colony counts showed colonization of rice plants.
- Plant growth promotion was recorded in pot inoculation experiments for rice, wheat and sorghum by inoculation with diazotrophic endophytic *Streptomyces* spp. Strain SS8 was most efficient. The root:shoot ratio indicated they promote the growth of aerial parts of the plant.
- Disease suppression was found for rice against *M. oryzae* B157 and for wheat and sorghum against *Rhizoctonia solani* upon colonization with diazotrophic endophytic *Streptomyces* spp.
- *Streptomyces* spp. inoculated rice plants did not show significant up-regulation of defence related gene expression under healthy conditions. Upon challenge with the *M. oryzae* B157 enhanced gene expression was seen for *NPR1*, *PR10a* and *LOX2* to different extents by the different strains.
- Diazotrophic endophytic isolate *Pseudomonas* sp. WS5 was genetically modified by cloning the 2,4-Diacetylphloroglucinol (2,4-DAPG) gene cluster (*phlDACB*) individually from *Pseudomonas protegens* Pf-5 and *Pseudomonas* sp. G22.
- 2,4-DAPG production by recombinant strains as analyzed by HPLC showed it to be 8.24 µg ml^{-1} and 6.32 µg ml^{-1} whereas the native strains *P. protegens* Pf-5 and *Pseudomonas* sp. G22 produced 1.28 µg ml^{-1} and 1.53 µg ml^{-1} respectively after 120 h.
- The antifungal extract of the recombinant strains of 2,4-DAPG producing *Pseudomonas* sp. WS5 showed an antagonistic effect against *M. oryzae* B157 and *R. solani*.
- Endophytic colonization by recombinant strains of 2,4-DAPG producing *Pseudomonas* sp. WS5 protected the rice, sorghum, and wheat plants from fungal pathogens.
- Endophytic colonization of rice plants with recombinant strains of 2,4-DAPG producing *Pseudomonas* sp. WS5 showed upregulation of *NPR1* under healthy conditions and further upregulation of *NPR1* along with *PR10a* upon infection with *M. oryzae* B157.

In conclusion, diazotrophic endophytic bacteria colonizing *Poaceae* plants showed rich diversity, possessed many other plant beneficial traits and was able to colonize multiple plant species and promote the growth of plants. Antibiotic production was rare among the diazotrophic endophytic

bacteria. Siderophore production was ubiquitous among the strains which might provide a competitive advantage for niche colonization. Novel endophytic *Streptomyces* strains possessing nitrogen fixation and biocontrol properties are reported and show potential as an effective biofertilizer and biocontrol agent for economically important members *Poaceae* plants. Genetic modification of endophytes to produce 2,4- DAPG proved to be a successful strategy for protection of plants from aerial invasion by fungal pathogens through two way mechanism that of direct antagonism and induction of ISR response. The work sheds light on basic aspects of diazotrophic endophytic bacteria and has also provides novel and effective organisms that can be explored further to develop as a successful tool in agriculture for plant growth promotion and protection.