Conclusion and future aspects

R. solanacearum is one of the most extensively studied phytopathogenic bacterium. I was introduced to *R. solanacearum* during my MSc project in the laboratory in January 2013, in which I was assigned to characterize a potential adhesion function in this bacterium. After joining in the same laboratory for my PhD research in July 2013, I got involved in standardization process of *R. solanacearum* pathogenicity study in seedling stage of tomato host plant along with other senior colleagues in the laboratory. The success of the leaf clip inoculation as well as the root inoculation methods in tomato seedlings remained very useful to characterize several virulence functions such as *hrpB, hrpG* and *phcA* in *R. solanacearum* F1C1. While creating these insertion mutants in *R. solanacearum* F1C1 back ground (chapter II in this thesis), we understood that natural transformation method in GMI1000 has to be modified for this strain. To our surprise this strain grows at a high concentration of glycerol (10 – 15 %) under which the transformation is possible.

Like tomato, eggplant is an important vegetable in India. This crop is severely affected by bacterial wilt caused by *R. solanacearum*. Though a lot of study has been carried out to understand *R. solanacearum* pathogenicity functions in tomato including studies from our laboratory described above, no characterization of any pathogenicity function has been reported in eggplant. *R. solanacearum* pathogenicity in different host is a challenge as the disease magnitude is highly variable among different hosts. Considering both economical as well as pathogenicity understanding point of view, *R. solanacearum* pathogenicity study in eggplant is very important. Therefore, I had the excitement to take this project. Though initially we thought it will be easy to do the work in eggplant considering that it has already been done in tomato; the first difficulty we realized when we started germinating the seeds. We tried different ways to standardize the germination process with different cultivars as it was less efficient and delayed in compared to tomato seeds. After successful standardization of the germination process, we inoculated *R. solanacearum* in 14-15 days old eggplant seedlings by the leaf clip inoculation method and we found out that the pathogen is highly aggressive in eggplant seedlings. The significantly higher virulence of *phcA* mutant in eggplant seedlings in comparison to tomato seedlings was a surprising observation in our comparative pathogenicity study between the two

hosts. Though almost similar pathogen load was deposited at the inoculated sites of both hosts, the bacterial saturation within the seedlings was different between both eggplant and tomato. How the bacterium adapts to eggplant host's environments, alters the niche environments for its better survival and causes faster disease appearance is likely to be an important future research emerged from this study. Transcriptome studies of the pathogen isolated from infected seedlings of both the hosts might give an important clue in this regard. Our work suggests that eggplant seedlings can be used as a model host to study *R. solanacearum* pathogenicity at molecular level.

During our pathogenicity studies in both tomato and eggplant hosts, we regularly observed that some of the inoculated tomato seedlings escaped the disease unlike the eggplant seedlings. It is pertinent to note here that *R. solanacearum* exhibits an exciting behavior as it can reside in some of its host without any visible disease symptom. They are referred to as tolerant hosts. However, in our case, tomato and eggplant were susceptible hosts and hence, the above observation instigated us to understand this enigmatic behavior of latent infection in susceptible hosts and hence took the second project. We had done thousands of inoculations in tomato seedlings with different concentration of the pathogen and with two different modes of inoculations. We are still not in a state to propose a solid hypothesis underlying this disease behavior or mechanism of escapees. However, our study confirmed that the number of escapees is dependent upon the bacterial load at the site of inoculation as well as the number of inoculated sites. Presence of high pathogen population is well reported in tolerant hosts which is also the case in susceptible host latent infection (escapees), found out in our study. Investigating and comparing the pathogen *in planta* growth rate both in tolerant hosts as well as in escapees will be very interesting. Comparison of pathogen load between wilted and healthy looking inoculated seedlings revealed that the pathogen load is similar in both the categories of the seedlings. This focused study to understand pathogen population inside the escapees suggested that pathogen load alone may not be sufficient to cause disease in the hosts. Whether plant defense against the pathogen is a localized event in the plant and/or an event involving the entire host plant is an open question.

Though the role of polyphosphate metabolism homologues such as *ppk1*, *ppk2, ppnk* and *ppx* occur in all the *R. solanacearum* phylotypes, the role of these

genes in its virulence is not known. The mutagenesis study in these genes indicated that *ppk1* is required for the optimal virulence of the bacterium in tomato seedlings. In future quantification of the polyphosphate in the wild type as well as in these mutants and unveiling their exact virulence mechanism will be an important future study. In case of *ppnk* mutant we observed virulence deficiency; however complementation did not happen. Hence, generation of new mutants at multiple sites of the gene by different mutation strategies and phenotyping of those mutant strains for their motility and virulence will be required to check if the phenotype (reduced swimming motility and virulence) is phenocopied.

Overall, during the research carried out in this thesis on the above aspects we understood that *R. solanacearum* pathogenicity in seedlings is a very complex process. Some of the features are mentioned as follows: (i) after inoculation into hosts, disease appearance time among the inoculated seedlings varies. In some seedlings disease appears after two days of inoculation whereas in some plants disease appears after six days of inoculation; (ii) some of the susceptible inoculated seedlings do not exhibit disease symptoms during the period of study; (iii) seedlings having high bacterial load do not exhibit disease but seedlings with lesser bacterial load have disease; (iv) mutants like *phcA* grows, migrates and multiplies within the host like the wild type but disease score is lower.

R. solanacearum adaptation inside the host plant likely to involve tissue specific differential gene expression, which will give an insight about its vivid pathogenicity attributes.