CHAPTER 5

SUMMARY AND FUTURE DIRECTION

Delving into the wide gamut of plant-microbe interactions has been a major research thrust over the years. Among others, endophytic microbes are known for their colonization potential inside plants' internal tissues, without pathogenic consequences. The bio-molecular and physiological ploy, adopted by the endophytes to establish an association with the host plant; discerning of the endophyte as non-pathogenic by the host; the crucial factors of endophytic localization and maintenance of microbial load in the plant's internal tissues; interaction of endophytic species with other microbial communities inside the host; precise correspondence of microbial load and the host's developmental stage as well as the response of the endophytes to biotic and abiotic stress, experienced by the host plant, are pertinent investigatory facets in plant-health research. Among others, the proposition that various plants carry endophytes through seeds from generation to generation to protect themselves from pathogens is a recent hypothesis. These above intricacies have made endophytes an exciting topic of research. Plants also encounter different pathogenic microbes that cause different diseases by various modes of infection. Some of these pathogens can enter the internal tissues of the host plant, but unlike the endophytes, these grow rapidly and hijack the plant to cause severe diseases. *Ralstonia solanacearum* is one such bacterial phytopathogen that causes a lethal bacterial wit disease in various plants including some economically important plants like tomatoes, potatoes, brinjal, chili, etc. Albeit, different methods have been applied to control the disease caused by the pathogen, none of them are found to be adequate yet. *R. solanacearum* F1C1 strain was originally isolated from a wilted chilly plant in Tezpur, Assam, India. Previously, different methods were developed to study the pathogenicity of the F1C1 strain in tomato and brinjal seedlings. The strain has been found to cause disease in the seedling stages of tomato and brinjal in gnotobiotic conditions. The seedling model has been recently studied by different research groups for plant-microbe interaction and bio-control efficacy of microbes against plant pathogens like *R. solanacearum*. Based on the outline, this study has been carried out to investigate the role of bacterial endophytes present in the tomato seedlings grown in a controlled environment against the pathogenic F1C1 strain, and to study the efficacy of the isolates in controlling the disease caused by the pathogen.

In this Ph.D. research, different bacterial endophytes were isolated from healthy tomato seedlings germinated under gnotobiotic conditions. The bacterial isolates were tested against the F1C1 strain using *in vitro* approach. Out of a total of twenty-one isolates, five were exhibiting F1C1 antagonistic activity. These five isolates were then studied for their interaction with each other and it was found that some of the isolates were able to inhibit each other apart from inhibiting F1C1. All the twenty-one bacterial isolates were characterized for twitching motility, and swimming motility, and molecular characterization was done by 16S rDNA sequencing. All five isolates were studied for bio-control efficacy in tomato seedlings by mixing them in increasing volume with F1C1 individually and mixing all isolates as consortia and inoculating them in the seedlings by root dip and leaf clip inoculation. All the isolates were found to have an impact on reducing the disease caused by F1C1.

One strain N4T belonging to *Pseudomonas putida* was studied for bio-control potential against F1C1 infection in tomato seedlings by pre-inoculating through the root dip method. It was observed when N4T was pre-inoculated; the disease caused by F1C1 was reduced. Apart from N4T pre-inoculation water was also found to play a role in reducing the disease.

P. putida N4T was studied for its colonization in tomato seedlings, brinjal seedlings, and grown-up tomato plants. The N4T strain was found to be able to colonize in vascular tissues of tomatoes and also was able to colonize in brinjal seedlings. Thus, N4T was confirmed to be an endophyte. Then, it was found that N4T was effective in reducing the disease caused by F1C1 in grown-up tomato plants upon inoculation by the soil drench method. Also, N4T was able to protect brinjal seedlings from F1C1 infection upon mix-inoculating through the leaf clip method. Furthermore, the role of a gene *gacA* of *P. putida* N4T in the inhibition of F1C1 and C10 was studied. An insertion mutation in the *gacA* homolog of N4T was created by $CaCl_2$ treatment followed by the heat shock method. The ability of *gacA* mutant in inhibition of F1C1 and C10 was compared by agar well diffusion assay. It was observed that the mutant strain was deficient in inhibiting C10 but it was proficient in inhibiting the pathogen F1C1. This indicates that the gene *gacA* gene has a role in the inhibition of C10 but not in the inhibition of F1C1.

In addition, this work has also tried to analyze the genome of one *R. solanacearum* F1C1 antagonistic bacterium *P. putida* N4T. The genome was sequenced

using the Illumina platform and then after annotation, the genome was analyzed for different traits. An *in-silico* study was performed to find out different secondary metabolite gene clusters present in the genome. Then using other tool different genes was predicted for their plausible role in plant growth-promoting traits. And further different genes for plant colonization and virulence were predicted using *in silico* tool. The *P. putida* N4T genome was compared with five other *Pseudomonas* genomes in a comparative phylogenomics study.

In conclusion, this study focuses on exploring bacterial endophytes isolated from healthy-grown tomato seedlings in their role against a bacterial phytopathogen F1C1. Isolating bacterial endophytes from tomato seedlings and testing them for the protection of the same host against bacterial phytopathogen is an intriguing concept. These study further advocates for the future use of seedlings as a model for studying plant protection assay. But the detailed study in the greenhouse using grown-up plants and rigorous field experiments will further validate the efficacy of these endophytes to establish them as successful bio-control agents. Also, it will be very important to study the mechanisms involved in the antagonism of F1C1. It will be interesting to isolate compounds/metabolites responsible for the antagonism. As different combinations of bacteria are now a day's been tested against phytopathogens for the development of consortia, it will be important to study if the isolates can inhibit the pathogen when inoculated together in the field. Also, as the bacteria were isolated from healthy tomato seedlings grown under the controlled condition it is hypothesized that they are carried by the host plant. It will be interesting to authenticate if the bacteria are carried by the host from generation to generation using different experimental work.